PROCEEDINGS AND PAPERS

of the

Forty-second Annual Conference of the California Mosquito Control Association, Inc.

and the

Thirtieth Annual Meeting of the American Mosquito Control Association, Inc.

February 24 - 27, 1974

held at the

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OBITUARY

Arthur F. Geib
1911 – 1974
Manager
Kern Mosquito Abatement District

Arthur F. Geib, well known among his associates for his patronage of research and development and for his intense interest in all aspects of mosquito control, here examines the antique customized brass microscope which was used by John B. Smith of New Jersey in his world-renowned research with mosquitoes from 1900-1910.

Art was born in Riverside, California, on February 24, 1911. His schools were Riverside High School, Riverside Junior College, University of California College of Agriculture at Davis, School of Public Health at Berkeley, and University of Arizona at Tucson.

His experience included service as a Sanitarian in San Luis Obispo, Kern, Tulare, and Riverside Counties, and for the State Health Department in Tulare County. During World War II he supervised mosquito control about military establishments as an agent of the USPHS and for the California State Department of Public Health.

He became manager of the Kern Mosquito Abatement District in 1945, served for 27½ years except for a 1½ year leave and a short leave to engage in research for the control of the mosquitoes involved in the transmission of filariasis (elephantiasis) in Pago Pago, Samoa.

Throughout his long service to the Kern District, he was noted for progressive mechanical developments, including both aircraft and ground insecticide application equipment, and the efficient use of chemicals.

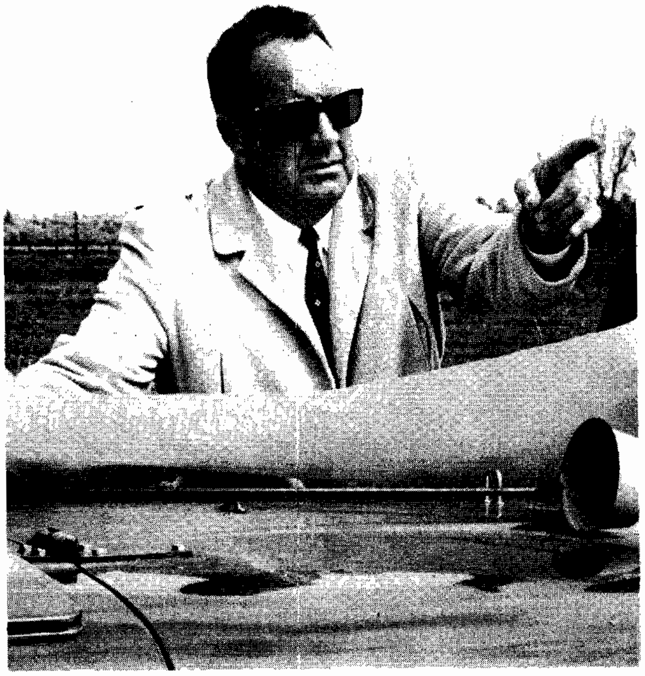
Always active in the committee work of many organizations, he was President of CMCA in 1947 and of AMCA in 1962. He served variously as a member of the Biological and Agricultural Advisory Committee of the University of California, the Vector Advisory Committee of the State Department of Public Health, the Comprehensive Health Planning Association of Kern County, and of Rotary.

He married Marguerite McHenry in Riverside on October 14, 1939. They had two children, Douglas and Jo Ann, both of Bakersfield.

He died suddenly on February 16, 1974 after having announced plans for retirement.

RETIRED

Edward D. Davis
Fresno Mosquito Abatement District
Manager to 1974



Edward D. Davis was born on October 8, 1908 in Roanoke, Virginia, but came to live in Redlands, California when he was only three years old. After finishing high school, he attended San Bernardino Junior College in 1927 and 1928, going on to University of California, Davis as an "Aggie", majoring in horticulture, with a minor in entomology. He completed his training in 1932, then worked as a specialist for Cal-Spray Corporation in Southern California and in the San Joaquin Valley for several years, then in 1946 became the first manager of the Consolidated Mosquito Abatement District. On January 1, 1949, he moved to Fresno to manage the Fresno Mosquito Abatement District, staying until his retirement in March of 1974.

Mr. Davis and his wife Charlotte live in Fresno and often spend weekends in Carmel, Palm Springs, or other resorts. Ed has always loved the out-of-doors, sports, travel, and people. In return, he was always a popular delegate at the CMCA, AMCA, and other meetings among operational, technical, and research people, where his hearty laugh and colorful expressions often highlighted the discussions.

In retirement, Ed spends much of the time on the Davis vineyards in the Caruthers area of Fresno County, which are largely devoted to producing grapes for raisins, but still somehow manages to find time to pursue his hobbies.

Jack H. Kimball
Orange County Mosquito Abatement District
Manager to 1974

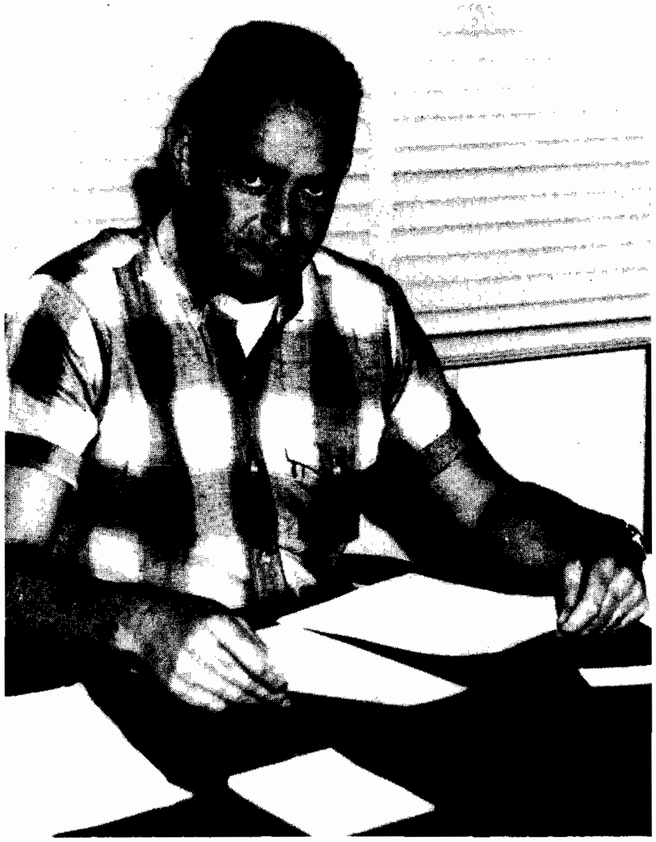
Jack H. Kimball served as the first and only manager of the Orange County Mosquito Abatement District for 26 years from October 20, 1947, to his retirement on March 29, 1974.

Born in Burlingame in 1913, graduated from Burlingame High School in 1930, he majored in Sanitary Engineering and was granted a B. S. Degree in Civil Engineering in 1935 by the University of California, Berkeley. While there he met his wife, Flora. They now live in Newport Beach and have a daughter, Nancy in Salinas; and two sons, Bill in Berkeley, and Jim in San Francisco.

He was President of the CMCA in 1950 and was Chairman of the Local Arrangements Committees for the joint AMCA-CMCA Meetings at Disneyland in 1961 and 1974. Jack served on the Board of Directors of CMCA as Representative of the Southern California Region in 1957, 1960, 1965 and 1973. Routinely he was an active member of many CMCA Committees.

Retirement finds him living the gracious life, in a beautiful home in "Fabulous Orange County", busily indulging in his hobbies of fishing, bird watching, gardening, and entertaining, with no lessening of his interest in mosquito control, although freed of the arduous day-to-day tasks.

RETIRED



Dennis Ramke
Tulare Mosquito Abatement District
Manager to 1973

Dennis Ramke started mosquito control work at the age of 19 when he joined the Tulare Mosquito Abatement District as a Laborer. Because of his natural abilities and intelligent approach to the problems of the District, he worked his way up to Operator in 1948, Division Foreman in 1950, General Foreman in 1951, Source Reduction Specialist and Assistant Manager in 1955, and Manager in 1961.

Denny worked vigorously in CMCA activities, twice hosting Equipment Shows, serving as Representative of the Southern San Joaquin Valley Region in 1967 and 1968, and he was elected Vice President and Conference Program Chairman in 1973.

Denny was active in hunting and fishing, which hobbies gave his body a chance to recuperate from the trials and frustrations of fighting mosquito problems. He retired from the District in December, 1973.

William L. Rusconi
Napa Mosquito Abatement District
Manager to 1973

William L. Rusconi was born in California November 29, 1910, and spent most of his life in Napa Valley. During World War II he was employed at the Mare Island Naval Shipyard, and joined the Napa Mosquito Abatement District in 1947, becoming manager in 1950. He retired on December 28, 1973, after having provided excellent mosquito control throughout the District.

Bill served on the Board of Directors of CMCA as Representative for the Coastal Region in 1960, as Vice President in 1963, President Elect in 1964, President in 1965, Past President in 1966, and again as Representative for the Coastal Region in 1970 and 1972.

In his retirement, he finally has time to indulge in his hobbies of fishing and hunting. His wife, Mary, an equally pleasant person, attends most of the CMCA meetings with him and is considered an "unofficial member" and a great favorite among the other ladies who attend the social functions. They have three grown children, all married: Vicki, Shirley, and Mary Frances. Bill and Mary will continue to live in Napa.

California Mosquito Control Association

Volume 42 February 24 - 27, 1974

FACTS, FIGURES, I REMEMBER: "THEN" AND "IN THE NOW"

John R. Waples

Orange County Mosquito Abatement District

Post Office Box 87, Santa Ana, California 92702

Orange County will be celebrating its eighty-fifth birthday this March. The county encompasses 782 square miles, has an altitude range from 0 to over 5,000 feet, and has 42 miles of beautiful Pacific Ocean shoreline. It has an average yearly rainfall of about 12 inches and a yearly mean temperature in the 60°F range.

In 1947 the principal industry was agriculture. There were approximately 350 poultry ranches, 200 dairies, and citrus growing was the number one monetary crop. The county produced one third of the lima beans in the world. Since the tremendous population explosion commencing in the early fifties, agriculture has given way to homes and other industries. One now has to know where to look or you will not see an orange grove, a lima bean field, a poultry ranch, or a dairy. Other food crops such as celery, carrots, lettuce and the exotic strawberry are being produced, but in diminished acreage. Tourist attractions such as Disneyland, Knotts Berry Farm, Lion County Safari and the alluring Pacific Ocean are attracting visitors in the millions.

When the Orange County Mosquito Abatement District was formed in 1947, there were 11 cities that joined the District. Now, all 26 cities are in the District, an increase of 127%. The population was approximately 200,000 persons. Now there are over 1,600,000 persons, an increase of 600%. The assessed valuation of the County was $352,000,000. Now the value is approximately $4,250,000,000, an increase of 800%. The permanent budgeted positions to carry out the District functions in 1947 were 13. The present budgeted positions are 21, an increase of 54%. The service requests have increased 1130%.

I remember when the property we are assembled on today was an orange grove. I remember when there were 200,000 persons in Orange County and there were probably that many mosquitoes per person. I remember when the responsibility for mosquito control was charged to the Orange County Health Department and the budget consisted of a part-time man, a hand sprayer and a drum or two of diesel oil.

I remember when Richard F. Peters (at that time the Mosquito Control Specialist, Bureau of Vector Control, California State Department of Public Health) made a survey of Orange County relative to the formation of a mosquito abatement district and wrote a report on that survey. I propose to quote some of the findings of that survey and make personal observations about each one.

> "Mosquitoes in Orange County in the past have constituted an important barrier to the full enjoyment of undisturbed living."

Prior to the District formation, the unscreened outdoor patio living was nonexistent. Now that type of living is taken for granted. Anyone not experiencing those early days probably questions if there was really ever a mosquito problem.

> "The benefits to be derived from organized mosquito control are numerous, touching upon every part and parcel of activity throughout the County. The benefits are as important to the agriculturalist as to the public health and to the public comfort."

Although Orange County's agriculture community has diminished, other commercial activities have increased. Can you visualize 50,000 persons at Disneyland on a beautiful summer evening, all battling mosquitoes?

> "The cost of a Mosquito Abatement District is relatively little in view of the relief obtained. The individual cost per person per year would be less than one admission to the local Cinema."

This observation is probably the understatement of the survey. Have you ever stood in line a block long to buy a ticket at the local movie house and heard anyone complain about paying a minimum of two dollars per person per ticket?

> "A County wide mosquito abatement district for Orange County is sound and desireable. Its formation would represent a worthy investment in the future of the County."

Can you imagine the sunny beaches, Disneyland, Knotts Berry Farm, Lion Country Safari, California Angels, etc., becoming a large part of Orange County's economy without organized mosquito control?

I remember after the survey by Richard F. Peters that the then Orange County Health Officer, Edward Lee Russell, M.D. (retired) provided administrative and moral support for the District formation.

I remember all the many hours of work, both mental and physical, performed by the (late) E. E. Frisby, at that time Director of Environmental Sanitation, Orange County Health Department, toward getting the District approved by Orange County citizens. Mr. Frisby became the Charter President of the Board of Trustees and held that office until his death in 1963.

I remember the day Mr. Frisby and Mr. "Bill" Obarr (Charter Member and still a member of the Board of Trustees), representing the city of Santa Ana, signed a personal note which provided the funds for the District's first year of operation.

I remember that after signing the note, Mr. Frisby said: "Now if I can get the man to manage the District that I know will do the job, my work will not have been in vain." That man was and is Jack H. Kimball, the original and only Manager of the Orange County Mosquito Abatement District.

In The Now.--The Mosquito is still a potential nuisance in Orange County. The District has a 25 year plan adopted 5 years ago. The District is well staffed administratively, technically and professionally.

The members of the District's Board of Trustees are an intelligent, positive thinking, people oriented group.

Not too many months from now, it appears that all vector control in Orange County will be the responsibility of the District.

In Summary.–The past 28 years Orange County has been blessed with an efficient, progressive, positive thinking administration of the Mosquito Abatement District.

Jack Kimball, I am sure, would say it was because of the District's Board of Trustees.

Having had knowledge of the District since its inception and been a member of the Board of Trustees for 11 years, I can honestly say it has been because of Jack and his excellent staff.

I sincerely hope that the citizens of Orange County affected by both Management and Trustee cooperation will say we have been a good "team".

Finally, I will say, there are penalties for doing an excellent job, that is, if there is no mosquito annoyance, who needs a mosquito abatement district?

OUR CHANGING SCENE

H. D. Jackson

American Airlines

Fort Worth, Texas

[This keynote talk was an inspirational, humorous and informative presentation stressing the concepts of "innovation" and "communication".]

THE PRE-DDT ERA OF MOSQUITO CONTROL IN THE UNITED STATES

Kenneth L. Knight, Ph.D.
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Department of Entomology, Raleigh, North Carolina 27607

Pest management, as a philosophy of control embracing total integration spatially and temporally of applicable population-regulating mechanisms in an economically and environmentally sound manner, has wide acceptance today. Use of the individual elements of a pest management program is not new. What is recent is the effort, through population monitoring techniques, computor technology, and systems modeling, to apply these elements in a complementary manner.

From its beginning at the turn of the century, the control of mosquitoes appears to have been somewhat more advanced than that of other insects. Possibly this is true because good mosquito control requires an area-wide approach. Also, unlike in agricultural pest control, destruction of the breeding area can be practiced in mosquito control. These factors presumably forced not only an early development of a varied and versatile technology but also one with early overtones of integrated control.

Out of a curiosity to learn how the art of mosquito control developed and with the thought that the information gained might provide helpful guidance for the future, I recently examined much of the U. S. literature on the subject that was published prior to the release of DDT during World War II. A resume of what I found follows.

Before 1900, a philosophy seemed to prevail that the misery caused by mosquito bites was a manifestation of divine will exercised to strengthen man's character. However, tolerance of co-existance with mosquitoes was abruptly ended by Ross's 1899 confirmation that malaria was transmitted by "dapple-wing" mosquitoes and Reed's 1901 report that yellow fever was carried by the "stegomyia" mosquito. Not only was worldwide interest immediately aroused in the possibility of controlling these diseases through the destruction of mosquitoes, but in the United States, at least, a serious interest in improving man's comfort through mosquito control quickly surfaced.

L. O. Howard had experimented with mosquito control as early as 1892 and in the time since then had published several treatments dealing with mosquitoes and their control (Howard 1892, 1893, 1900a, 1900b, 1902b; Howard and Marlatt 1899). His extensive concentration on mosquitoes over this long period of time and his ready familiarity with most of the related work being done throughout the world gave him background to write a valuable book in 1902 entitled simply "Mosquitoes". In this book he developed an outline of the "remedies" known to be effective in reducing mosquito populations. Running through this treatment can be seen a thread of the philosophy that led to the development of modern day pest management concepts.

Howard began by dividing mosquito control methods into two categories: (1) remedial work against the early stages and (2) remedial work against the adults. Control of the early stages was again divided into three subcategories: (a) treatment of breeding places with insecticides, (b) abolition of breeding-places by drainage, diking, or filling, and (c) introduction of natural enemies into breeding-pools which for any reason it may be undesirable to drain or treat with an insecticide. Remedies against adult mosquitoes consisted of: (a) screening, (b) use of adulticides, and (c) repellents. Further, Howard stated that an essential early step in mosquito control was to make a thorough breeding site survey. Also, he advocated resorting to legal measures to insure that individuals comply with ordinances necessary to mosquito control. All of this demonstrates a surprisingly modern comprehension of the fundamentals of abating mosquitoes. Moving on from Howard's early classification, the development of each type of control technique of pre-DDT era will be traced separately.

LARVICIDING.—Time has disclosed that oil is the only one of the many larvicides reported in the literature prior to 1920 which has remained acceptable. Although the first substantiated report of its successful use as a larvicide dates from work done by Howard in 1892, the earliest recommendation known for the use of oil in controlling mosquitoes was published in the American Daily Advertiser in 1793 (Stage 1952).

Following the demonstration by Howard in 1892 that coal oil (kerosene) was an effective larvicide, countless trials and experiments have been made with vegetable and petroleum oils down to the present time. Throughout, the search has been for a goal never quite attained, the perfect larvicide. Ross (1902:33-34), very shortly after this search began, described the need in the following words: "I have long wished to find an ideal poison for mosquito larvae. It should be some solid substance or powder which is cheap, which dissolves very slowly, and which when in weak solution destroys larvae without being capable of injuring higher animals. What a boon it would be if we could keep the surface of a whole pond free from larvae simply by scattering a cheap powder over it, once in six months or so. It is very possible that such a substance exists, but unfortunately we have not yet discovered it." He couldn't realize of course that he was fairly accurately describing DDT, an insecticide not to be recognized for another 40 years.

Delaney (1921) expressed both the satisfaction and frustration with the use of oil for mosquito control that was common to the times, when he summed up a presentation on mosquito larvicides by saying: "In conclusion, petroleum as a larvicide is not entirely satisfactory, but in the absence of a more acceptable larvicide, it is the most practicable medium for larval destruction . . . My brief consideration of larvicides clearly visualizes one fact –– that the ideal larvicide does not exist. Nearly all the larvicides on the market at present have a limited use or obvious disadvantages which make their use impracticable from the standpoint of efficiency."

It is a remarkable coincidence that, in the same year in which Delaney gave voice to his frustrations, a new and entirely different type of larvicide was announced. Barber and Hayne (1921), reading of a discovery by Roubaud (1920) that paraformaldehyde powder dusted onto the surface of the water would be fatally ingested by anopheline larvae, tested a considerable number of toxic dusts and found compounds containing arsenic to be the best. Of these, Paris green was the most efficient. Mixed at a rate of about 1 percent with an inert dust, Paris green was quite effective against anopheline larvae while being harmless to beneficial terrestrial and aquatic animals. The advantages of Paris green for control of anopheline larvae were its cheapness, portability (road dust was commonly used as a diluent), ease of distribution, and the possibility of using it over areas difficult of treatment by any other method then known. Its chief disadvantages were that its use was limited to anopheline larvae (eggs, pupae and culicine larvae were unaffected) and that, like the oils, it was difficult to maintain an effective surface film with it.

Interestingly enough, the pioneer use of the airplane in dispersing insecticides for mosquito control was with Paris green. Employing the technique developed for dusting cotton with calcium arsenate from an airplane (Goad, Johnson and McNeill 1924), W. V. King and G. H. Bradley in 1923 and 1924 had Paris green dusted by Army DeHaviland planes over rice fields and large areas of wooded swamps and lakes filled with aquatic vegetation, obtaining better than 90% control of anopheline larvae present (Howard 1925:13; King and Bradley 1926).

The versatility of Paris green was later increased by mixing it with wet sand and throwing it broadcast over a pool. The sand carried the Paris green below the surface where it was eventually fed upon by culicine larvae. At the same time sufficient Paris green remained on the water surface to kill anopheline larvae also. It proved particularly effective against the salt marsh *Aedes* (Griffitts 1927). In 1935-36, it was found possible to suspend Paris green in water and dispense it with a watering can for culicine larval control (King and McNeel 1938).

The use of Paris green caught on with amazing rapidity and in 1928 L. O. Howard was able to say that the Paris green treatment had gone around the world and that to that date it was the cheapest and most efficient control method known for *Anopheles*, its only fault being that it had no action against eggs and pupae.

Meanwhile, a great need existed to improve the utility of oil as a larvicide and in 1927 Joseph M. Ginsburg, biochemist in entomology at the New Jersey Agricultural Experiment Station, began a study of this subject which was to continue for more than two decades. From the beginning of the use of oil it was commonly believed that it caused the death of mosquito larvae and pupae by suffocation. In 1918, Freeborn and Atsatt conclusively showed that oil kills mosquitoes because of penetration of the tracheae by volatile components of the oil. Several more years were to elapse before this demonstration was fully accepted. Ginsburg's preliminary studies (1927, 1928, 1929) were concerned with a determination of the characteristics which made an oil a good larvicide and were very valuable in bringing some hard data to a subject previously only speculated on.

Because of a continuing concern for the effects, actual or fancied, of oil on plant life, fish, and water fowl, Ginsburg (1930) undertook experimentation in 1929 with the use of pyrethrum as a mosquito larvicide (first reported by Balfour 1913) and succeeded in developing an emulsifiable kerosene extract of pyrethrum which very effectively killed mosquito larvae and pupae without injury to vegetation, water fowl, and fish at a cost not greater than that of ordinary light fuel oil and with a lasting quality not less than it (Vannote and Ginsburg 1931). This, along with the Paris green-sand mixture, marks the beginning of the availability of safe and effective larvicides useful below the water surface. Although emulsifiable products were used as far back as the campaign by Gorgas (1904) to free the Panama Canal Zone from yellow fever and malaria, none of them was adequately safe to use. Subsequently, Ginsburg improved his oil-pyrethrum larvicide in several ways, including devising a formulation that permitted its use in both fresh and salt water (1934, 1935).

ADULTICIDING.–In the pre-DDT era, remedial work against the adult mosquito was much slower to develop than it was against the larva and pupa. The burning of brimstone, punk, and just plain green wood for repelling blood-seeking mosquitoes undoubtedly dates back to very early times. The use of materials which might actually destroy adults is more recent, with pyrethrum probably being one of the first (Howard and Marlatt 1899:17). The burning of sulfur and of tobacco was also practiced (Ross 1902:36), as was fumigation with hydrocyanic acid gas (Bailhache 1903:29). However, little progress was made in the development of adulticides for many years. In 1930, work was reported by the USDA on the use of pyrethrum sprays against adult mosquitoes (Howard 1930:17).

The first real breakthrough in this area was by L. L. Williams (1932). In a search for a method to alleviate the conditions produced by a sudden crop of *Aedes sollicitans*, he used the Ginsburg pyrethrum-oil larvicide without dilution, liberated from a knapsack sprayer into the air on the windward side of a marsh at the usual application rate. This treatment appeared to kill practically all adult mosquitoes in the immediate downwind area. In subsequent work, Ginsburg (1935, 1936, 1937) perfected the use of this method to a point where it was widely adopted by abatement districts to give protection from mosquito annoyance at large outdoor meetings. Power sprayers with high pressure pumps were used. The formula finally used was a 1-10 or 1-15 dilution with water of a pyrethrum-kerosene emulsion. The entire field of adulticiding apparently stems from this work.

Adulticiding received a major forward thrust from the discovery reported in 1942 by USDA scientists Sullivan, Goodhue and Fales of the breakdown and delivery of insecticides by gas propulsion. This was not only the beginning of fogging for adult mosquito control but also was the point of origin for the entire multibillion dollar aerosol industry of the present time. This discovery came coincidentally with that of the insecticidal value of DDT, with the result that method and material were soon wedded into a very potent adulticidal tool.

ABOLITION OF BREEDING PLACES.–Mosquito control workers have always had at least one tremendous advantage over individuals responsible for controlling agricultural insect pests, i.e. they can destroy the breeding grounds of their pest. Those involved with the control of agricultural pests must seek to destroy the pest species without harm to its breeding site.

From the inception of serious mosquito control in the late nineties onward, this advantage was fully realized and exploited. As a result, mosquito control literature in the pre-DDT era is predominantly concerned with habitat elimination and alteration. Throughout nearly all of this period, ditching, diking, and filling, along with elimination of container-type habitats, were the principal techniques used. At the beginning, all such work had to be done with hand labor. Consequently, unremitting efforts were made throughout this era to mechanize as fully as possible all phases of breeding-site elimination.

Although elimination of natural breeding areas through ditching received serious attention wherever mosquito control was attempted, it was particularly utilized with salt marsh mosquitoes because this was the only means of control that could be afforded by communities living on the edges of miles of potential mosquito-producing salt marshes. Consequently, the early technology of mosquito ditching in this country was principally developed on salt marshes and in an area where population pressures caused an early rapid rise in real estate values, i.e. New Jersey. For inland and urban mosquito control, breeding-site management received its principal impetus from the early efforts of Ross (1902) and Gorgas (1904). The work accomplished and the methods employed by these two and many others in initiating efforts throughout the world to eliminate yellow fever and malaria is more fully described in later publications (Ross 1910; Le Prince and Orenstein 1916).

Guidelines for drainage on Atlantic Coast salt marshes were primarily established by J. B. Smith, who could accurately be referred to as the father of salt marsh mosquito control. Beginning just prior to the turn of the century and continuing until his death in 1912, he not only was instrumental in getting the legislation needed to provide state and community financial support for mosquito control, but also was responsible for determining the mosquito fauna of New Jersey, most of the details of the biology of these species, and the measures needed to begin bringing them under control.

All ditches were initially dug by hand, first with ordinary spades and subsequently with specialized two-man and three-man spades. However, for all of the pre-DDT era there was a steady development of ditching mechanization by New Jersey workers. Time does not permit an elaboration of this development but several writers have left illustrated accounts of it (Eaton 1916, 1918; Reiley 1923, 1928; Merhof 1924; Brooks 1929; Reiley and Vannote 1938). By at least 1937, a caterpillar crane with an orange-peel bucket had been adapted to mosquito ditching (Brooks 1937), a forerunner of the ubiquitous dragline. Actually, steam shovels, as well as dynamite, were used for ditching as far back as World War I (Le Prince 1919).

Diking, as a means of reclaiming land, was used in the Metropolitan New York area shortly after the Civil War (Delaney 1916), and once a serious interest developed in pest mosquito control at the turn of the century, was quickly put to use as a salt marsh mosquito control device. Some of the very early work on habitat alteration for mosquito control using dikes was done in California by Quayle in 1906. Dikes were combined with tide gates or pumps to free a marsh of water. As a rule, dikes are used only when the fall of the tide is not sufficient to draw all of the water out of ditches, when ditches have to pass through strata of such composition that it is impracticable or unduly expensive to keep them open, or when the area in question is too low to be drained (Hardenburg 1922:144). On higher marshes, diking was found to dry the marsh out too greatly and to cause a serious shrinkage in its level. An extensive invasion of the area by undesirable plants also usually occurs. These problems can be reduced by putting water back on the marsh during the winter (Brooks 1915).

Impounding as a mosquito control technique was apparently first reported by Van Dine (1922) based on work done on the Louisiana delta. The topography of that area did not permit drainage and it was necessary to devise some other method of control. During 1914, personnel of the Division of Entomology of the U. S. Department of Agriculture observed that under natural conditions there was practically no breeding of *Anopheles* in sections of bayous where open water and clean margins existed. In 1915, they cleared the bed and edges of a bayou and built a dam to maintain water within it. No anopheline breeding was subsequently found in the impoundment. Even with this demonstration, it was not until the TVA malaria control program was established more than 20 years later that serious attention was given to this method of managing both mosquito and wildlife populations (Hinman 1938). It was not until after the end of the pre-DDT era that impoundment received serious consideration as a salt marsh *Aedes* control device.

USE OF NATURAL ENEMIES.–From the very beginning of organized mosquito control efforts in this country, much energy has been expended on utilizing fully the natural enemies already present, introducing non-native natural enemies, and on the mass-production and release of natural enemies. Special impetus was given to these efforts all during the pre-DDT era since the tools for mosquito control already at hand were so limited in their effectiveness.

J. B. Smith in his excellent pioneering publication upon the mosquitoes of New Jersey (1904) devoted 34 pages to the enemies of mosquito larvae found in that area. For all of the natural enemies of the larvae and pupae, except fish, he stated that "so far as our present knowledge goes, we can make no practical use of [them] . . . All that we can do is to make natural conditions as favorable for them as possible." He concluded that fish were the most important from the practical standpoint because they could be transported to places where they were needed, because they would stay where put, and because they lived during the entire season.

This situation prevailed throughout the entire pre-DDT era. There were, during this time, some interesting efforts made to introduce natural enemies into areas where they were not native. L. O. Howard (1910) tells that Albert Koebele imported from California to Hawaii a large number of western salamanders (*Diemyctylus tortosus* Esch.) which had previously been observed to eat mosquito larvae. Howard also describes the introduction of mosquito fish from Texas to Hawaii by way of San Francisco in 1904 by D. L. Van Dine. This introduction was both successful and eventually useful. Howard (1930) reported that C. E. Pemberton brought living larvae of the predaceous mosquito *Toxorhynchites inornatus* from New Guinea to Hawaii. These apparently did not take. Slightly later, R. W. Paine introduced *Toxorhynchites splendens* from Java into Fiji where it did become established, at least for a while (Bishopp 1933).

Equally interesting, but much more futile, were the energetic and long-continuing efforts during the twenties of Dr. A. R. Campbell of San Antonio, Texas to sell the idea of building roost structures for bats in areas with mosquito problems (Howard 1924). He stoutly insisted for a number of years, squarely in the face of contrary evidence from reputable scientists, that this was a practical control for adult mosquitoes. Although support from newspapers kept this idea alive beyond its time, it eventually lapsed into the obscurity which it merited.

ENVIRONMENTAL CONCERN.–Generally speaking the primary concern during the pre-DDT era was to accomplish mosquito control by whatever means could best be used. Wetlands were commonly held to be wastelands. If mosquito control could be combined with the transformation of wetlands into areas useful for agriculture, industry or recreation, so much the better. Realization that wetlands are an indispensable part of the ecosystem on which man's ultimate survival depends did not come until much later.

Although insensitive to total ecosystem considerations, workers of this period were generally aware that mosquito control practices could have undesirable side effects and that practices should be modified to avoid these. For example, in the early days, salt marsh hay production was an important industry along parts of the north Atlantic seaboard. Elimination of mosquito breeding sites on these marshes had to be done without harming this production. The 10-inch ditch developed by J. B. Smith not only permitted this but actually resulted in an increased yield.

The early volumes of the New Jersey Mosquito Extermination Association often voiced concern over the toxic effect of larvicidal oil on fish, and since top-feeding minnows were an important part of the integrated system of mosquito control then in use, this concern eventually led to the development of the less harmful Ginsburg pyrethrum-oil larvicide. The practice of diking salt marshes for mosquito control was later modified or used less often because of the shrinkage and harmful plant succession which occurred on marshes that were too extensively freed of water (Smith 1907).

Serious concern over the effects of marsh alteration work done for mosquito control on marshland wildlife did not develop until the thirties, but even so, this is much earlier than many environmentalist have given mosquito control workers credit for. At the 22nd Annual Meeting of the New Jersey group in 1935, a symposium was held on the relation of mosquito control to wildlife on the salt marshes (Headlee 1935). From this point on, the literature increasingly reported communication between wildlife people and mosquito control workers.

POPULATION MONITORING.–Ability to effectively monitor mosquito populations is another necessary part of pest management. Techniques basic to our present ability in this area were developed by the workers of the pre-DDT era. In the earlier part of this period, larval dipping and adult biting techniques were developed. Both of these methods remain essential today but both have limitations. An automatic method of sampling was needed, one which would operate repetitively over longer periods of time.

Headlee early recognized this need (1922) and began the studies which led to his development of the first mosquito light trap for survey purposes (1928). This was subsequently improved and modified by Mulhern (1934) into the New Jersey light trap as we know it today. The object of many modifications since that day, the mosquito light trap still serves as an essential population-monitoring device.

ECONOMIC THRESHOLDS.–Another essential ingredient of pest management, as we know it today, is the development of economic thresholds, those levels of damage, disease transmission, or annoyance above which it becomes an economic necessity to control the involved pests. The first reference noted in the literature of the pre-DDT era to what might be regarded as an economic threshold is a statement by Spencer Miller of New Jersey in 1914: "I tried once to define a mosquito pest. Dr. Smith told us that every square foot of a salt-marsh breeding area may produce 5,000 mosquitoes in a single brood. An acre contains almost 5,000 square yards. One mosquito to a square yard would be 5,000 mosquitoes to an acre. Surely 5,000 mosquitoes to the acre is a 'pest'.

Somewhat more to the point, Headlee (1932:126) developed an economic threshold for the light trap. From an analysis of light trap data, he concluded: "For average New Jersey conditions it may be considered that when this trap accumulates more than 24 mosquitoes in a single night the density is sufficient to develop trouble for the householder and when it accumulates less than 24 mosquitoes in a single night the density is low enough to be practically negligible from the householder's standpoint." Although not mentioned in this article, it is obvious that Headlee meant these to be female mosquitoes only, because the figure was derived by comparisons with numbers of mosquitoes taken in human biting collections.

SOCIO-ECONOMIC-POLITICAL FACTORS.–Extremely important to the pest management milieu of today are what can be termed the socio-economic-political factors. They were no less important to, nor any less recognized in, the pre-DDT era. From the beginning of organized or community-wide mosquito control in this country at the start of the twentieth century, we find the literature to be replete with accounts of efforts made to educate the citizenry to the values of mosquito control (Miller 1906; Hardenburg 1922). These educational efforts were carried on primarily to arouse the community to push for the passage of enabling legislation and the appropriation of funds that would provide area-wide mosquito abatement districts. Once passed, all too often a fight had to be carried on year after year in the political arena to put down the regularly recurring efforts on the part of some individuals to repeal existing mosquito control legislation.

Economics were of as much importance to the legislatures and citizenry then as now, and for years most mosquito control work was grossly underfunded. Numerous accounts of the financial side of mosquito control appeared during this period as individuals sought to justify the expenditures that had been made and the even greater sums that were being requested each year (Headlee 1938).

Legal restraints imposed to insure that no citizen undoes community effort at mosquito abatement have been with us from the beginning. Possibly the earliest in the United States was an ordinance published by a mayor of Winchester, Virgina in 1900, requiring householders to oil all water on their property at least once each month (Howard 1902a:174). In New Jersey, the Duffield amendment to the State Health Law, passed in 1904, placed water in which mosquito larvae bred among the nuisances that could be abated by local boards of health at citizen's expense (Smith 1904:142).

CONCLUSIONS.–From the preceding review, it is apparent that all of the elements considered now to be essential to a viable mosquito control program were known and utilized in the pre-DDT era. It is true the DDT era and the post-DDT era have brought us a greatly increased knowledge of mosquito biology and behavior, an extensive refinement of control technology (including one entirely new control method—autocidal control), and the exciting philosophy of pest management. However, the DDT era and the post-DDT era have not brought the freedom from mosquito attack which our constituents have come to believe is possible. It is difficult for some to understand why this anomalous situation exists, particularly when one considers that almost the first organized mosquito control efforts in the world, the cleanup of *Aedes aegypti* and the resulting elimination of yellow fever in Havana, Cuba in 1902, along with a similar accomplishment in the Panama Canal Zone in 1906, both by Colonel William Gorgas, were not only completely successful but occurred in the earliest days of the pre-DDT era.

Essentially devoid of significant biological inputs, as these two programs were, it is little wonder that the term "mosquito engineering" was used in 1901 and that the statement could confidently be made that "Mosquito extermination is essentially an engineering problem" (Weeks 1903). Yet, if analyzed, it will be found that the yellow fever and malaria control programs of Ronald Ross and William Gorgas were pest management systems in the elemental sense of the word, even if they had not filled in many of the biological values in the systems model governing the population sizes of the vector mosquito species. The species having the critical economic impact was selected out, its economic threshold empirically established, its involved biological and behavioral characteristics determined, the limits of the area needing control marked off, the appropriate population management techniques adopted, and a total operational program put into effect. To check the progress of the work, population monitoring procedures adequate to the purpose were established.

To my way of thinking, the reason these programs had such complete success is that, unlike most mosquito control programs since, unlimited manpower was available and there was no concern over socio-economic factors or over a political-legal basis for the work because it was all done within areas under military control. In other words, it comes back to a fact all too well known to us, i.e. that man left to a free decision on all matters can frequently be his own worst enemy. On the other hand, give us a lever to man's actions and a fulcrum on which to rest it and we can literally move the world. Outside of dictatorial force, which is contrary to the American philosophy, our principal lever to mens' minds today is persuasion through education and public relations, and here for us probably lies the ultimate key to economic and environmentally sound mosquito control. A versatile and innovative approach to the control of public opinion is the element most wanting from our mosquito pest management systems today, not fundamental biological data or suitably advanced technology.

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EPA's STRATEGY FOR IMPLEMENTATION OF THE FIFRA

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During the last few decades, pesticides have become increasingly important to the processes of food and fiber production, insuring public health and sanitation, protecting natural resources, and improving human comfort and well being. However, since pesticides are biological poisons and are not always specific in time, place or target of their chemical activity, their proliferation in the environment has caused known serious human health and environmental problems and may be causing others not now well understood. The need for federal government involvement in the control of pesticides was perceived early and was principally addressed by the FIFRA Act of 1947. Since December, 1970, the responsibility of administering this Act has rested with the Environmental Protection Agency (EPA). In October, 1972, the Act was amended to give broad and new pesticide control authority to this agency. Fully implementing the Act as amended between now and October, 1976, is an essential responsibility of EPA and of the states and their agencies.

Pesticides, unlike most air and water pollutants, are intentionally released into the environment in order to produce their intended benefits. Any action regulating pesticide use must be based on a careful assessment of its effect on those activities to which pesticides represent an important beneficial input. In brief, the major elements of the 1974-76 EPA strategy to implement FIFRA are: (1) to control product availability or supply control; (2) to control the use of pesticides; (3) to improve and integrate hazard evaluation systems; and (4) to improve our understanding of pesticide problems, of corrective mechanisms, and of testing and measuring methods.

The backbone of the pesticide regulatory program is the registration process. It serves to keep highly hazardous chemicals off the market and through classification and other restrictions to control the use of others. The new Act requires re-registration and classification of all currently registered pesticides by October, 1976. A similar requirement applies to those pesticides which are currently registered by states for use in intra-state commerce. It is estimated that these statutory mandates will add over 40,000 registration actions to the normally submitted registration petitions which require processing. Emphasis will be placed on those pesticides which are of a persistent and bioaccumulative nature and those which potentially present long term health risks or special toxicities to household users. Pesticides currently involved in litigation proceedings will not be re-registered before hearings are completed. Initial supply control as underwritten by product registration must be enforced to be effective. A concerted program over the last few years of labeling, efficacy, and quality control enforcement has substantially reduced the number of gross violations. The new act gives enforcement authority in these areas through establishing registration, books and record requirements, and surveillance at the point of manufacturing rather than in the market place. The first step in this process is the registration of all manufacturing and formulating establishments.

USE CONTROL.– Labeling plays a crucial role in assuring the proper use of pesticides. Labeling is approved at the time of registration as providing adequate instructions and precautions to prevent adverse effects on health and the environment. However, three factors bear recognition: (1) the label is limited in the amount of information it can contain; (2) it has been shown that users often do not read printed instructions, and (3) questions arise as to how well the users understand what they have read. The major thrust will be to restrict the use of compounds known to be hazardous to the applicator or his immediate environment when used in accordance with common practices. Such compounds will be available only to private applicators (that is, farmers) and to commercial applicators who have demonstrated competence in their use. This restriction should significantly reduce accidents by keeping hazardous substances out of the home and out of the hands of untrained individuals.

Certified applicators must know how to follow label instructions, to augment them when necessary consistent with label intent, be knowledgable of safety principles, and be required to demonstrate competence in using application equipment. Additional use control efforts will include public information programs such as media advertising directed toward motivating the user to read and adhere to labeling instructions. In addition to restricting certain pesticides to qualified users, the Act gives the administrator authority to impose other restrictions. These may include, for example, seasonal or geographic poundage limitations on total amount, or use of a pesticide only in conjunction with an integrated pest management system. Certain other clearly needed programs will be instituted to deal with adverse health and environmental effects. These include specific requirements for child-proof packaging for home use products, disposal and storage regulations in enforcement, development of dissolvable packaging and modified formulations of hazardous compounds. Control of spillage and food and clothing contamination during transportation of active ingredients and formulation products will be implemented. Protective clothing, lack or non-use of, which has been implicated in large numbers of accidents, to applicators and farm workers, will be promoted. Effort will also be directed toward medicating adverse health effects in farm workers due to pesticide exposure.

HAZARD EVALUATION.–The need to protect the general population from possible irreversable health effects, especially from those pesticides used on food and feed crops, leads to the third major thrust of the pesticide strategy, that of developing an effective hazard evaluation system. EPA has a responsibility for formulating a national plan for monitoring pesticides in cooperation with other federal, state and local agencies and for specifying studies to be conducted during experimental testing of new pesticides and coordinating with other agencies in hazard evaluation activities. As an early warning system it should feed information back to the registration process by identifying possible harmful effects of pesticides and their by-products not previously recognized. The hazard evaluation system will have direct input to cancellations, suspensions, and other actions, reclassification by providing a method to identify chemicals requiring in-depth review, and by supplying data to support subsequent review procedures.

The final major thrust of the insecticide strategy is a vigorous program of investigation both to support current techniques and program options to meet future regulatory needs. The research program will have two major components to meet these objectives. The first relates to scientific research and model development to support regulatory actions, the second consists of studies to evaluate specific questions of policy and strategic planning.

APPLICATOR CERTIFICATION.–The applicator certification program is an attempt to insure that applicators have sufficient knowledge to prevent acute injury to human health and environmental effects in or near areas being treated with pesticides. To be certified, an applicator will have to be aware, for example, of the potential toxicity of pesticides and know what steps can and should be taken to prevent himself and other individuals from being needlessly exposed. Similarly, he must have sufficient knowledge to prevent the most obvious types of localized environmental damage, including acute injury to nontarget species and runoff into streams and lakes. Towards these ends, the proposed standards of competence stress "practical knowledge", that is, the possession of pertinent facts and abilities to use them in dealing with problems and situations likely to be encountered in an applicator's work.

By October, 1976, all registered pesticides must be classified for either general use or restricted use. A general use pesticide is defined in the Act as one which will not generally cause unreasonable adverse effects on the environment when used in accordance with its labeling or widespread and commonly recognized practices. Such pesticides normally will be available to the public. A restricted use pesticide is defined as one which may generally cause unreasonably adverse effects on the environment, including injury to the applicator, unless it is subject to additional regulatory restrictions beyond labeling. FIFRA provides first that if a pesticide is classified for restricted use because of potential hazard to applicators or other individuals, it can be used only by or under the direct supervision of a certified applicator. If a pesticide is classified for restricted use because of potential environmental hazard, it can be used only by or under the direct supervision of a certified applicator or in accordance with other regulatory restrictions imposed by the administrator.

Section 4, which provides for applicator certification, reflects the intent of congress that states assume the primary responsibility for this activity. States are expected to develop and to administer applicators' certification programs based on standards of competence which meet or exceed those prescribed by the administrator. State certification plans must be reviewed and approved by the administrator and for this purpose must be submitted to him by October, 1975. EPA believes strongly that applicator certification can be most effectively handled at the state level, for proper consideration can be given to the particular characteristics of agricultural and other uses of pesticides within each state and to existing mechanisms for licensing applicators.

The proposed certification standards are designed to insure that applicators would have a practical knowledge of the basic elements of pesticide usage. Certification will require the applicator to be competent and aware of the need for proper use and the consequences of misuse. It is designed to insure that the applicator knows the importance of correct placement of pesticides to avoid or minimize entry into the environment other than to target areas, knows how to prepare the proper and safest formulation for a given situation, and is aware of what constitutes poor handling, mixing and storage practices, the leading causes of pesticide accidents. These standards will not require that the applicator fully comprehend the scientific basis for the labeling directions, but rather that he knows how to follow the directions and has a sufficient knowledge to augment them in each use situation to prevent adverse health and environmental effects and to assure proper use of pesticides.

SPECIFICS OF REGULATIONS.–The regulations establish a system of categorization based on occupation. Ten categories have been developed. Three are of special significance, as they involve demonstration applications, regulatory activities, and public health programs. They have been structured to require especially high levels of proficiency and understanding. The ten categories are – agricultural pesticides control, subdivided into plant and animal; forest pest control; ornamental and turf pest control; seed treatment; aquatic pest control; right-of-way pest control; industrial, instititional, structural and health-related pest control; public health pest control; regulatory pest control;and demonstration pest control.

Section 171.4a requires that commercial applicators' competence be determined through written examination and, as appropriate, demonstration testing. Section 4b sets forth general standards to be met by all commercial applicators. State standards must be equivalent to or may exceed EPA standards. The general standards would require commercial applicators to have practical knowledge in the following areas: problem identification, interpretation of labeling instruction, application techniques, and safety. Two matters on safety: acute human health hazard in the form of injury or death represents a highly visible, easily understood danger. Field application techniques are directly related to the dermal, respiratory and overall exposure that an applicator experiences. Each applicator must know the use of protective clothing and equipment, common intoxication systems and first aid procedure. The certified applicator is required by these standards to have the operational competency necessary to minimize pesticide poisoning.

Section 4c sets forth specific standards for each commercial applicator category. They identify areas of practical knowledge that are particularly important in each category. As an example: "Applicators shall demonstrate practical knowledge of regulated pests, applicable laws relating to quarantine and other regulation of pests, and the potential impact on the environment of restricted use pesticides used in suppression and eradication programs. They shall demonstrate knowledge of factors influencing introductions, spread and population dynamics of relevant pests. Their knowledge shall extend beyond that required by their immediate duties, since their services are frequently required in other areas of the country. . ."

Finally, I wish to point out that Section 4 of FIFRA provides for the development of some additional regulations and these will identify the requirements for an acceptable state certification plan. Consideration is also being given to spelling out provisions which have to be included in the state plan with respect to matters such as examinations and methods to be used to determine applicator competence and to the issuance of credentials to certified applicators. EPA will welcome comments and suggestions regarding these, or any other aspects of FIFRA's requirements. Section 4 appeared in the Federal Register as of February 22 and there will be a 30-day period for comments. This will be a 30-day period in which all comments are considered before issuance of the final regulations.

CALIFORNIA's SAFETY AND HEALTH PROGRAM

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I would like to present a brief overview of California's safety and health program. We need to start with the federal act so that we understand the federal and state relationship that has now been established. The federal act was passed by Congress and signed into law by President Nixon in 1970 and went into effect in 1971. It mandates a national worker safety and health program which applies to every work place where there are one or more employees. Congress recognized the need for states to administer the program within their respective jurisdictions. Therefore, Section 18 allows states to retain administration of the program by submitting a state plan where assurances are made and all federal criteria are met.

Why a state plan? California has had a worker safety and health program for over 40 years. It has been different from today's program, as it was more or less service oriented, although it did have some teeth if there were serious hazards. There were tagging procedures whereby we could shut a place down. We also had criminal sanctions, which we did use in cases of gross negligence and when people were hurt or killed. Our choice was somewhat limited. We knew we must either redirect our program to meet the federal criteria or we faced federal preemption. More specifically, California could provide broader coverage than the federal program by including public agencies, which were not covered in the federal program. In California there are about one million workers in state, county, city, school, and other local jurisdictions. California's program provides public workers the same rights and protection as private employees. It also treats public employers the same as private employers with one exception, that is, civil fines do not apply to public agencies. California can devote more resources by obtaining 50% federal funding, can retain its professionals and therefore can provide more input into the program. Employers and employees can deal with a program which is administered closer to home. This is important in standard setting enforcement and appeals. California also provides consulting services in the program.

California's plan was submitted to the regional office in September, 1972, and after regional review it was submitted to Washington on December 15, 1972. The summary of California's plan was published in the federal register on January 5, 1973, and there was a period of 30 days for comment. It was finally approved on April 24, 1973.

Three points should be made about California's state plan. First, it was developed with the assistance of an advisory council, composed of prominent persons from management, labor and the public. Although no votes were taken, the council's views represented a concensus between management and labor. The second point is that this is a developmental plan. The federal criteria recognize that it takes time to complete and implement a program as comprehensive as OSHA. We have three years from the time the plan was approved to completely implement this development. Thirdly, California's plan encompassed three blends, existing state law, or those items which worked well in the past, the federal mandate, including the requirements that we had to meet in order to have state jurisdiction, and the work of our Assembly select committee on industrial safety. We had a series of investigations by this committee as a result of some unfortunate incidents, and their considerations were taken into account.

When we were in reasonable accord on our state plan, we applied for grant funds to administer and enforce the program, and on June 5, 1973 we received a five million dollar grant from the U. S. Department of Labor. We also worked with our California Legislature on enabling legislation. AB150, after much debate, was signed into law on October 2, 1973.

The Agricultural and Services Agency is one of four cabinet level agencies which exercises policy direction over several departments, one of which is the Department of Industrial Relations. It is the Agency responsible for developing and implementing the state plan. Within it is a small administrative unit which I supervise as program manager. We serve as the liaison or focal point with the federal government and we also coordinate the many activities at the state level.

We have an Occupational Safety and Health Standards Board whose responsibility is to set standards for worker safety and health and also to consider applications for permanent variances from standards. This Board also acts as an appeal body from the granting or denial of temporary variances by the Division of Industrial Safety. The Board is composed of seven members, two representing management, two labor, one from the field of safety, one from health, and one public member.

The Division of Industrial Safety is the responsible enforcement agency. Since January 1, 1974, compliance inspections have been conducted as prescribed by the state plan and our state statute, AB150. The Division also provides consultation services, provided by 18 persons, 9 in the north and 9 in the south. Their responsibility is to assist employers and employees to conform to the statutes. The Division is also conducting seminars throughout the state to inform the public of the requirements of the state plan.

The State Department of Health has an interagency agreement with the Division for technical support and training. It also conducts health studies, either at the request of the Division of Industrial Safety or on its own. It also has responsibility for evaluating and developing recommendations for standards and variance requests. The State Fire Marshall's office also has an interagency agreement. It's role is similar to that of the State Department of Health in its consideration of fire standards.

The final organizational entity is the Occupational Safety and Health Appeals Board. It hears appeals from enforcement actions, such as penalties, citations and abatement periods. It is a three-man board, appointed by the Governor. It now has a contract with the state Office of Administrative Hearings to conduct the first level of hearings. Eventually it will be hiring its own hearing officers.

California's Department of Food and Agriculture has responsibility for a comprehensive pesticide program. This is a program under AB246 (1972). Because federal OSHA has not been involved in the field of pesticides, we have kept the pesticide program as administered by the Department of Food and Agriculture somewhat separate from the Occupational Safety and Health Program. The pesticide program of the Department of Food and Agriculture is much broader in scope than just worker safety. In other words, it takes into consideration the environment, the consumer and several other issues not related to workers.

The Administration's goals relative to occupational safety and health programs are:

First.–To remove the federal field enforcement people from this program. During the last two years we have lived under concurrent jurisdiction – the federal people had responsibilities in the occupational safety and health field and so did we under state law. Two groups of inspectors were covering the field, inspecting under two sets of standards, a confusing situation for both employers and employees in California. As of January 1, the federal OSHA people in Region IX are no longer out in the field inspecting. They have converted to a monitoring role.

Next.–We want to meet and where appropriate, exceed the federal criteria.

We wish to preserve California's expertise in the field of occupational safety and health.

Finally.–We wish to administer this occupational safety and health program fairly.

These goals and their accomplishments are specifically directed toward the overall program objective to assure so far as possible California's working men and women safe and healthful working conditions.

RESPONSIBILITIES OF THE CALIFORNIA DEPARTMENT OF FOOD AND AGRICULTURE

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The California Department of Food and Agriculture has a responsibility for regulating the sale and use of pesticides in this state. This includes several features, such as the registration of all pesticides that are offered for sale in this state, the licensing of professional applicators of pesticides and pilots who are in the business of applying pesticides, licensing of dealers who sell pesticides for agricultural use, and licensing of persons who make recommendations for agricultural pest control. Then we regulate certain pesticides as restricted materials requiring permits for use. We enforce pesticide residue requirements on raw agricultural commodities, and recently adopted regulations on worker safety, for protection of workers who are exposed to pesticides by handling, mixing, and applying them.

The Department has a staff working throughout California, however many of the requirements are enforced at the local level by county agricultural commissioners who work under the supervision of the Director of the Department. One of the important features of this program as it relates to vector control work is a provision in the Health and Safety Code, Section 2426, which authorizes the Department of Health to enter into cooperative agreements with local agencies. All vector control agencies have entered into this agreement, according to Richard F. Peters of the Department of Health. This has important ramifications. The Food and Agriculture Code itself recognizes this relationship in that it defines agricultural use and in the definition it excludes local vector control agencies that have formalized such agreements. It is a tribute to the leadership and guidance of the Department of Health in working with local agencies.

What is the impact on the state as a result of developments at the federal level? For some time the California Department of Food and Agricultural has registered about 10,000 pesticide products annually. Of these, about half are not shipped out of the State and therefore do not require federal registration. The Department generally has used federal guidelines in registering these products. However, with the new amendments to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) that became effective in 1972, all products must be Federally registered after October, 1974. In addition, the states cannot require any different labeling or packaging. This will have a considerable impact in California, since up to now about 5,000 products have been registered only by the State. In addition, under the law the Department has granted authorization for use of pesticides in special instances in conflict with the label. The Department will no longer have this authority after October, 1974. The new law does authorize states to register pesticides for special local needs if they have been granted authority by the Environmental Protection Agency (EPA) and if the uses involved have not been cancelled by EPA. California will seek authority to grant registrations under this provision of law at the appropriate time.

Another feature of the new Federal legislation is the provision for certification of applicators, the law requiring certification of commercial and private applicators. The full impact of this new requirement will not be known until we know how EPA plans to classify pesticides. There have been consultations with the states in discussing plans for classification of pesticides. We have seen drafts of plans but we do not know yet how they will be finalized. It is expected that they will be published in the federal register within the next few months. However, even after we see the plan we will not know the full impact because it will depend on how the scheme is actually applied to a given product.

The new law authorizes states, if approved by EPA, to enter into the certification of applicators and California plans to enter into this program. We are very much interested in the standards for certification of applicators which were published on February 22, 1973. Probably there will be many pesticides classified for restricted use, and there will be many people in California seeking certification under these new requirements. The law also refers to the training of people for certification. For some time we have been working with the Agricultural Extension Service of the University of California in developing training programs for persons who are intending to be qualified as pest control advisers under state law. The University of California has taken the leadership to develop these training programs – there are courses being presented at different levels, by state colleges, community colleges, and by the Agricultural Extension Service itself. Federal support for these training programs has been sought, and we are looking for federal financial support to assist us. Training is a very expensive activity and requires extensive resources. We work closely with EPA – the Region 9 office has been very responsive to the needs of California and has helped obtain information from the Washington headquarters.

Workers' safety is an important issue in California. There has been a history of concern for protection of workers for many years. About 1962 the State Department of Industrial Relations in the Division of Industrial Safety adopted general safety orders requiring medical supervision of workers exposed to highly toxic organic phosphate pesticides. These regulations were also considered in the Department of Agriculture regulations governing restricted materials and governing commercial applicators. However, during the past few years, with greater concern for the highly toxic pesticides and also with the greater militancy of agricultural labor, there has been renewed attention in the state legislature for the protection of workers. This resulted in new legislation in 1972, Assembly Bill 246. These requirements are incorporated in the Department of Food and Agriculture Code. The law declares that it is necessary to provide for the safe use of pesticides and for safe working conditions for farm workers, pest control applicators and other persons handling, storing or applying pesticides or working in or about pesticide-treated areas. The law requires that the regulations that are to be developed are the mutual responsibilities of the Departments of Health and of Food and Agriculture. They are to be promulgated by the Department of Food and Agriculture. The requirement was made that these regulations were to be established by the first day of the legislative session of 1974, January 7.

The Department of Health made its recommendations to the Department of Food and Agriculture. A public hearing was held, and regulations were adopted on January 7. These regulations caused quite a reaction, and there were many persons who asked for further time to comment on them. As a result, the hearings will be reopened in March. In their present form they establish many requirements for employee safety. They specify the maximum number of hours a person may work in handling these materials; they require medical supervision; they provide for reentry intervals for entering certain crops which have been treated with certain pesticides. Many persons are very concerned because they believe there is too big a burden placed on the employer. We have had many comments from vector control agencies, pointing out that certain features are difficult to comply with, and some have suggested that vector control agencies be exempted entirely since they are in the cooperative agreement with the Department of Health. All statements filed with the Department will be given careful consideration.

WHAT PRICE REGULATION?

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This session was conceived in order to put into better perspective what has become a very bewildering emergence of regulation as a way of life in a field of public service that has heretofore enjoyed only limited regulation. There are different levels of association between federal and state governments and local programs throughout this country and elsewhere in the western hemisphere. In California vector control, we have existed in a relatively regulation-free state-local relationship since organized mosquito control began in 1915. Oldtimers may recall that the way of life of mosquito control in the distant past was essentially drainage, water management, filling, the discrete employment of mosquito eating fish, and supplemental reliance on diesel oil or other petroleum materials. Somehow a combination of these approaches managed to bring about a reasonable degree of mosquito prevention and control. Then, in 1946, we got into this modern miracle insecticide era, and a new control technology began in which insecticides could be superimposed upon the environment, both on water and on surfaces where mosquitoes were expected to alight, and for many years obtained, on this basis, very effective control.

But concurrent with this development in mosquito control, agriculture began to express itself with an even more affirmative use of pesticides. It is probable that many of the more profound management and preventive principles of agriculture also yielded to the abundant presence of the modern insecticides. Then we find ourselves suddenly confronted with evidence of environmental pollution, of occupational hazards, and to some extent actual related deaths and injuries. Then the ecology movement appeared in which many of the proponents did not even know how to spell the word; and the politicians heard clamors for – "we must do something, we must take drastic action, we must prevent this pollution from continuing." Now, we have become no longer part of a miracle insecticide era – we are about to start on a regulation era – regulation to the extent that if we do not express some degree of concern and objection before it gets too big it could dwarf all of the technological successes of the past and leave us wondering about the future.

I speak as a member of the California Department of Health, wishing to put into perspective the fact that there are bonafide needs for pesticides, used discretely, in conjunction with the regulation of pesticide abuses. In California vector control we have enjoyed what I would call a good understanding with local agencies, in which enforcement as such has never been necessary to the extent that we have ever had to call to the attention of any governing board "you are not doing your job properly." This is a power of the California Department of Health if indeed the public is at risk from abuse of local responsibility. Instead, over the years we have developed a working relationship with local control agencies involving an affirmative program of training by our Vector Control Section which has been willingly responded to by the members of the California Mosquito Control Association (CMCA). For many years, even before EPA, even before certification mandates were in prospect, an understanding had been reached between our Vector Control Section and the CMCA that certification was inevitable, necessary, and desirable. To prove it, we have already announced that on April 5, 1974, a statewide certification examination will be held for every practitioner of mosquito control in California . . . well over a year – almost a year and a half – before the EPA mandate requires such certification to be in effect. I do believe this connotes no apprehension about operator safety and no apprehension about mosquito control technology, because this program as planned contemplates not only an examination on how to use pesticides properly with respect to the environment, to man, to livestock, to wildlife, and to operator safety, it also contemplates using pesticides in their proper relationship to physical and biological methods of control. We believe that in our statewide program we cannot use insecticides disregarding other relationships, possible adverse impacts and side effects.

We have come to this point, not because of regulations, but because of a program conviction. This has also been characteristic of the American Mosquito Control Association (AMCA) as well as of the CMCA. Many years ago Robert L. Vannote and others in the AMCA developed a Fish and Wildlife Management-Mosquito Prevention Control Coordinating Committee which was dedicated to the position that fish and wildlife management and mosquito prevention and control were not in conflict, but were part of the same package as they relate to human endeavor.

It hardly needs mention that when I represent the California Department of Health and advocate pesticide usage, since my Department has already been identified as the consultive agency on how to regulate effective avoidance of personal injury and mortality, both to operator and to those associated with side effects of pesticide usage, that vector control could never survive within our own Department if indeed our cooperative agreement failed to heed the health parameters in this matter.

At this point, mosquito and other vector control finds itself in the awkward position of being caught up in a comprehensive regulatory program where we feel that some acknowledgment needs to be made, not total exception, but recognition of the affirmative aspects of a program that has been contemplating the conditions and situations requiring regulation for a decade or more. Some of the mosquito abatement people are weighing at this point, with resistance to insecticides having now limited their effectiveness, whether or not they are indeed subject to the regulatory provisions of the new prospective safety regulations. To begin with, one of the powers of the mosquito abatement act, and a health statute at that, is that a district "may take all steps necessary" to obtain the control of mosquitoes. The implication is: to protect the public against mosquito borne diseases and the deterrent impact of mosquito pests to normal living. Is this statute in itself, i.e., this charge, transcendent to some of the other considerations that are now coming into existence as primary considerations to public well being? Also, in terms of the level of pesticide use, in modern mosquito control technology, the amount of time which a control operator now spends in applying insecticides is often little or none throughout the day, inasmuch as surveillance – the finding of larvae to control – is the primary task. The amount of time engaged in spraying after finding them is an extremely small part of the total work time.

In response to the current regulations under development within the Department of Food and Agriculture, a survey was made within the CMCA as to what would be the financial impact of meeting the regulations as originally proposed. The returns, representing districts ranging from several operators to 60-man operators, show an expected increased cost range from several hundred dollars up to several hundred thousand dollars to implement the facility, equipment and materials changes required. At the January 4, 1974, hearing called by the California Department of Food and Agriculture, I recommended that local California vector control agencies be exempted from full compliance with the operator safety regulations as proposed. These facts would seem a profound basis for favorably considering this recommendation: All vector control agencies in California use less than half of 1% of the total statewide pesticides applied annually; mosquito control pesticide dosage rates amount to 5-10% of those used in agriculture; the operator safety record in vector control over the past quarter century is excellent; pesticide usage in California vector control is drastically declining annually in large part due to resistance to pesticides; and, the existence of a comprehensive cooperative agreement between the California Department of Health and all local vector control agencies pertaining to all aspects of vector control. This recommendation recognizes the need to add only those appropriate and applicable regulations contained in the California Department of Food and Agriculture proposal to the cooperative agreement, with enforcement to be carried out by the Department of Health, Vector Control Section. Otherwise, a direct relationship on all aspects of vector control between the California Department of Health and all the local vector control agencies in California would no longer exist. Such a relationship has proven very effective in the past; its continuation is requested by all the local agencies concerned.

PANEL: DEVELOPMENTS IN WORLD WIDE VECTOR CONTROL – AN OVERVIEW

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The bylaws of the American Mosquito Control Association (AMCA) reveal the idealistic spirit and scientific educational purposes of the Association to promote research on and control of mosquitoes and related subjects and to disseminate knowledge thereon throughout the world. Specific definitions of the purposes and typical activities appear in the original "Articles" by which AMCA became incorporated in New Jersey in 1948 [Mosq. News 8(4):91] and in the revised Articles for reincorporation in 1974.

Initially the impact of AMCA upon the world-wide programs was small, but as this Association grew its publication Mosquito News came to be recognized and read wherever in the world scientists are combatting the mosquitoes. Progress in the altruistic purposes of AMCA has not been rapid, nor has it been easy! However, through the extraordinary efforts of its members, it has been possible to offer to the world the knowledge generously provided by its members, which is the greatest asset of AMCA!

There have been obstacles – for example, the language barrier. Some day Mosquito News may be translated into several languages. The mail has been distant, and sometimes slow and unreliable. Differences in economic standards and rates of exchange have sometimes been very great. Many years ago Harry H. Stage tried to overcome the economic barrier by founding the "Good Neighbor Club" which helped greatly in introducing Mosquito News into some countries. In 1969 the immensely useful "World-Wide Committee" was formed and is becoming increasingly active. As developing resistance by mosquitoes to chemical control is severely limiting vector control in widely separated parts of the world, AMCA and its World-Wide Committee are able and ready to help – and could well be called upon to serve as the vehicle to coordinate the needs for technical knowledge with the immense resource of highly competent personnel who make up this Association.

AN OVERALL REVIEW OF THE WHO PROGRAM OF MOSQUITO CONTROL (ANTIMALARIA PROJECTS)

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The malaria eradication program, which began 17 years ago, is the most extensive mosquito control program or public health disease control program ever undertaken by mankind. It is unparalleled in its geographic coverage, the means employed, and the number of people protected.

It is still operational in 92 countries on the five continents, but at the peak of its operation covered 120 countries. Ten years ago it was estimated that malaria eradication would cost over $2 billion. Then it was protecting over one billion persons directly and more than 60,000 tons of DDT powders were used annually.

DDT was then as now the lifeline of the program, but many developments have since taken place. Total coverage residual spraying of the inside of houses with DDT caused a drastic drop in malaria transmission wherever it was applied, but gradually showed signs of inadequacy and weakness, first in areas where elusive vectors played an important role in the transmission of malaria and later where vectors began to develop tolerance and resistance. The problem of resistance could be overcome by using other residual insecticides (temporarily in some cases) but the exophily and exophagy of vector Anophelines could not, and a radical change in the methodology of control of mosquitoes was needed.

As early as 1956 some malaria eradication programs (as in Jordan), had to resort to source reduction and oiling in addition to DDT spraying to control *Anopheles sergenti* and *A. superpictus*. Behavioristic changes in some vectors and their avoidance of sprayed surfaces required special strategy and new techniques in mosquito control operations.

In 1964 the World Health Organization began a vast program of investigations and staff training in antimalaria measures other than residual spraying. First priority was given to antilarval operations, and the latest techniques, materials, and equipment used in the field were reviewed. Those feasible for application in malaria programs were selected and gradually introduced, first in trials and then in field operations. Senior staff were sent on observation tours of the United States of America's mosquito abatement organizations, and the teaching of antilarval operations was extended at the Malaria Eradication Training Centers (METC). Seminars and conferences were held to train the national and international staff in the new technology, requirements of these operations, and in their planning and organization.

THE CHANGE OF STRATEGY.–By 1967, it was established that some countries had financial, administrative, and operational difficulties in continuing successful malaria eradication programs. Others could not begin a program in the foreseeable future. Thus the world-wide program faced several alternatives: to continue and expand eradication measures despite serious technical, financial, and administrative problems; or to relax the time-limited criteria and allow flexibility in the duration of programs and phases. The latter option was chosen and the Fifteenth Session of the Expert Committee on Malaria (1971) recommended the implementation of malaria control programs where eradication is not feasible.

The revised strategy widened the scope of mosquito control methods employed and allowed diversification of measures based on feasibility studies and cost/benefit relationships, rather than on purely technical justifications and complete interruption of malaria transmission as practiced previously in malaria eradication.

THE PRESENT.–There are now more than 20 major antimalaria programs carrying out antilarval operations in addition to residual spraying. These are programs having technical problems, e. g., vector resistance to chlorinated hydrocarbons, exophily or exophagy, population movement, etc. They are planning or using the combinations of mosquito control measures more suitable to various operational areas.

Larviciding with chemicals is at present the principal method used against larvae, but other antilarval operations are rapidly gaining importance. The use of larvivorous fish, as an inexpensive and effective biological antimosquito measure, is expanding. Other biological enemies are being studied.

The malaria programs in Iran, Iraq, Afghanistan, India, and the Sudan have developed the needed operational organization and logistics and are making large-scale use of *Gambusia* fish. Other programs also undertake similar activities on limited scales. The results have been extremely encouraging. Where an adequate fish population could be maintained, there was a drastic drop in the number of larvae (sometimes complete disappearance). In Afghanistan where fish were introduced in the rice paddies and ponds at 6-10 fish per square meter, within days the numbers of *A. pulcherrimus* larvae were reduced by 95% and were maintained at that level for the rest of the transmission season. The few larvae found were mostly of the first or second stages. The cost of operations in an area of 7500 km^2 with 400,000 population was reported at $0.02 per capita.

Use is being made of other species of larvivorous fish, especially those native in some countries. Somalia is investigating dissemination of *Notobranchius Guntheri*, native in the country. The guppy (*Poecilia*) is under investigation in a number of programs.

Because there are possible hazards involved in the introduction of fish to new areas, WHO in cooperation with FAO is currently investigating such implications and the precautions that should be taken.

For chemical larviciding, newer chemicals have replaced the older ones. Temephos (Abate) is the larvicide most commonly used because of its effectiveness and greater safety. These factors are important in a larvicide to be used where the material is applied in remote areas by semi-skilled laborers. Malaria programs in Jordan, Sudan and Cyprus are making large-scale application of Temephos.

Equipment requirements are under investigation. A number of ULV machines are being tested for use in rural and urban areas. ULV application of pesticides by airplanes has also been tried and has been shown to have a place in combating malaria epidemics and in problem areas.

The source reduction and engineering approach to malaria control has made less progress in the programs. There is an acute need for training of staff in these activities, especially in areas with water development schemes and agricultural extension projects.

THE COMPREHENSIVE APPROACH TO MOSQUITO AND VECTOR CONTROL.–It is generally agreed that measures used for the control of malaria vectors are effective against mosquito vectors of other diseases, yet this has seldom been explored and pursued, or given the importance it deserves in programs for the control of malaria and other parasitic diseases. Some parasitic diseases, e. g., filariasis, may be transmitted by malaria vectors and where this happens, antimalaria operations directed against mosquito vectors may also control filariasis. Also, leishmaniasis has been virtually eliminated from areas where residual spraying was systematically carried out to control malaria.

In many cases, a minor modification or extension of methods used against one mosquito species would be adequate to make them effective against other vectors. Therefore, it may be feasible to include such changes, without the need for much additional resources. The consequences of such an approach, apart from its obvious economical, operational, and public health benefits would serve the cause of malaria control and be an added justification for the initiation or continuation of antimalaria programs.

A broadened concept of comprehensive mosquito control can be applied to the control of vectors and intermediate hosts of parasitic diseases. The causative agents of major parasitic diseases are transmitted by a number of arthropods or they may pass part of their development in other animals. Several species of flies, mosquitoes, fleas, bugs, crustacea, and mollusks are involved as vectors or intermediate hosts. The complexity of selecting control measures that would yield common benefits and effectiveness therefore can be visualized. A closer review, however, shows that some control measures used against mosquitoes are in fact directly effective against other vectors or intermediate hosts. Residual spraying used against mosquito vectors proved effective against *Triatoma* bugs, the vector of Chagas disease (American trypanosomiasis). Similarly, other operations used against mosquitoes are equally effective against the snails which are the intermediate hosts of schistosomiasis. There are occasions where two control methods may be combined and applied in one operation, such as larvicides and molluscacides used in combined application. These more sophisticated approaches require a careful study of the life cycles of the intermediate hosts and vectors concerned, and of the local operational and ecological conditions. Where possible, the combined approach will be considered to facilitate control operations and simplify organizational and manpower needs. The resulting savings may be important.

THE WORLD HEALTH ORGANIZATION'S PROGRAM AGAINST MOSQUITO VECTORS OF DISEASE OTHER THAN *ANOPHELES*

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INTRODUCTION.—Aside from malaria, mosquitoes are vectors of a number of other diseases of public health importance. The WHO has therefore undertaken extensive programs of research on the ecology and control of these mosquito vectors and, in addition, has operational programs for the control of some of them.

FILARIASIS.—It has been estimated that there are 200,000,000 persons in the world infected with Bancroftian filariasis; in most of Africa the vectors of this disease are *Anopheles gambiae* and *A. funestus.* On the East African coast and in urban areas throughout the tropics the main vector is *Culex pipiens fatigans.* Extensive municipal vector control programs have been developed by many countries against this mosquito due to its being a vector of filariasis and because of its importance as a pest. Many of the earlier programs failed due to a lack of adequate information on the biology, insecticide susceptibility, and most effective means of control. The WHO established a Filariasis Research Unit in Rangoon, Burma in 1962 and for eight years carried out a program of research on the bionomics of *C. p. fatigans,* and field tested numerous insecticides against this species. A method of control was developed which has had a high degree of efficiency and is more economical than most municipal vector control programs previously in existence. The Organization is now seeking to introduce this methodology into other urban centers and demonstrations have already been performed in Dar es Salaam, Rangoon and Bangkok, while others are planned for other large cities in the tropics. An active program of field testing of newer insecticides against this species is now being carried out at the Organization's Vector and Rodent Control Research Unit in Jakarta, Indonesia.

Active *C. p. fatigans* control programs assisted by the Organization have been carried out in Sri Lanka, Burma, Thailand and elsewhere.

AEDES VECTORS OF ARBOVIRUSES.—*Aedes aegypti* is the vector of at least two extremely important arboviruses in various parts of the world, i. e. yellow fever, dengue and the dengue related haemorrhagic fever. This species has been responsible for severe urban epidemics of yellow fever in the western hemisphere and since the late 1940's has been the subject of a hemisphere wide eradication campaign that has succeeded in eliminating the mosquitoes from numerous countries and territories in the western hemishere. This program has been impeded by the development of insecticide resistance and the re-introduction of resistant *aegypti* into areas where it has previously been eradicated. Further research is being undertaken on control methods to overcome such recurrences.

Extensive research has been carried on at the WHO *Aedes aegypti* Research Unit in Bangkok, Thailand, on Haemorrhagic Fever in Southeast Asia. Based on the ecological and insecticide studies at this Unit, methods were developed for a long-term effective and economic method of controlling the breeding of *aegypti*, and for a number of procedures for controlling epidemic outbreaks of Dengue Haemorrhagic Fever by ULV applications against the adult mosquitoes. Additional tests are being carried out on new types of equipment and new insecticides, at the Vector and Rodent Control Research Unit in Jakarta, Indonesia and in collaboration with national governments.

In Africa, where *Aedes simpsoni* is one of the most important vectors of yellow fever, WHO sponsored collaborative ULV aerial application trials have shown that it is possible to obtain rapid control of this species for brief periods when necessary during epidemic outbreaks of disease.

The Organization has an extensive computer mapping program of the *Stegomyia* sub-genus to assist national governments in determining where *Aedes*-borne disease is likely to occur.

AID POLICY ON MALARIA

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BRIEF, HISTORICAL BACKGROUND.—The U. S. Agency for International Development and its predecessor agencies have been assisting national malaria programs in developing countries since the early 1950's. Assistance has been in the form of commodities (insecticides, vehicles, spray equipment, laboratory equipment, etc.), technical advisory services (including full time technical advisors and short term consultants), local currency support, and training fellowships. Since 1958, AID has supported WHO in its worldwide malaria eradication efforts. At the peak of AID participation in the worldwide malaria eradication program, the Agency was providing approximately 70 technical advisors on a full time basis in 26 countries and was providing commodity assistance to an additional 10 countries. In 1966, AID reached an agreement with HEW/CDC whereby CDC carried out the responsibilities of the malaria eradication program on behalf of AID. In 1969, the Director-General of WHO presented a report "Re-examination of Global Strategy of Malaria Eradication". This report recognized the serious problems of achieving eradication of malaria in certain countries and recommended strategy reviews of the programs to determine the need for revising the malaria eradication strategy on a case-by-case basis. In 1970, AID announced a policy of multilateralization of technical advisory services. This policy was based on the premise that for approximately 20 years both AID and WHO had been providing training fellowships for national malaria personnel in the fields of malariology, epidemiology, entomology, health education administrative management, and logistics as well as the techniques of malaria eradication. It was considered that since the individual countries had an adequate cadre of professionally trained personnel, and that WHO would continue to provide technical advisory services, it was not necessary for AID to do so. In the period since the announcement of this policy, AID has reduced its technical advisory personnel overseas from 50 technicians to the present number of five advisors in three countries.

This policy of multilateralization was widely misinterpreted to mean that AID was withdrawing its support from the worldwide malaria eradication effort. This was not the intent and a clarification and restatement of policy was issued in July of 1973. The pertinent points are as follows:

The U. S. Government supports the WHO global strategy of malaria eradication. In the implementation of U. S. support under the foreign assistance program, current AID policy places emphasis on the following:

1. Countries which demonstrate a willingness to help themselves by providing whatever resources they have available to carry out the program.
2. Realistic assessment of assisted projects to obtain a sharper definition of those targets which can be reasonably expected to be reached within a time-limited effort and those which are likely to be delayed due to administrative, technical or political problems.
3. Retention of malaria eradication as the ultimate objective for projects which meet and maintain the minimum WHO and AID conditions (as expressed by the Fourteenth WHO Expert Committee on Malaria, 1968, and in conformance with the Twenty-second World Health Assembly resolution on malaria, 1969.)
4. Maintenance of the option to support malaria control activities where projects do not currently meet eradication criteria, if the economic, social, or political value of the project merits support.
5. Promotion of multilateralization of technical services through encouraging assisted governments to request advisory services from WHO while effecting an orderly withdrawal of U. S. scientific advisory technicians.
6. Continuation of support in the context of foreign assistance policy to research, commodities, local costs, and evaluation; cooperation with other U. S. agencies and WHO in assisting multilateralization of technical services; and consideration of interim provision of staff assistance in managerial areas where WHO may not be able to provide such staff.
7. The A.I.D. Office of Health, Technical Assistance Bureau (TA/H) has an overall central responsibility for development of AID policy for malaria eradication and assuring its implementation. TA/H has a direct responsibility for AID central support to malaria research projects. Assistance to the country projects is implemented through the AID Regional Bureaus with technical backstopping from the Office of Health.

(Under a 1970 Memorandum of Understanding between AID and HEW, the Center for Disease Control of the Public Health Service worked directly with AID in carrying out the responsibility for the malaria eradication program. This responsibility included provision of staff, expediting commodity assistance, planning and participating in evaluations and providing headquarters backstopping for these activities.

This Memorandum of Understanding which established AID/HEW relationships was terminated June 30, 1973 and on July 1, 1973 AID resumed the responsibilities formerly delegated to HEW/CDC required to carry out current AID policy on malaria.)

CRITERIA FOR CONSIDERING ASSISTANCE TO COUNTRY MALARIA PROGRAMS.—AID assistance to country malaria programs will be considered when:

1. The country demonstrates its own interest and concern for malaria through the provision of an adequate budget and staff to carry out the program.
2. There is a critical need to protect a substantial U. S. investment in terms of gains already made or a need to prevent malaria from becoming a deterrent to other country development programs.

3. The country provides a malaria plan which is technically, administratively, and financially sound and is based on an AID review of the recommendations of a joint WHO/LDC evaluation team.
4. Available resources within the country have been mobilized and available external sources of assistance have been explored.

CURRENT INVOLVEMENT OF AID IN MALARIA.–

A. Operational Programs.

AID grant funds are still being used in Nepal and Haiti. However, in both countries these are in effect terminal grants, as the programs are being converted to general health/communicable disease programs. AID loan funds are still being utilized to support country malaria programs in Ethiopia, Brazil, Paraguay, Ecuador and the Central American countries. With the exception of Brazil, all of these loans either have terminated or are near termination, but AID has a continuing responsibility for evaluation of these programs. Loans to provide support to the malaria programs in Indonesia and Pakistan are currently under consideration in Washington. A joint AID-WHO strategy review of the Thailand malaria program is planned for the near future.

B. Research.

AID's primary thrust in malaria research lies in support of the Malaria Immunity and Vaccination project under contract to the University of New Mexico. AID is also supporting through the National Academy of Sciences a workshop on the Development and Production of a Human Malaria Vaccine. AID is supporting a research project with Dr. Robert Metcalf of the University of Illinois on Development of Biodegradable DDT. In the field of pesticides, AID is also supporting through WHO a research project for the Development of Fenitrothion as an alternative to DDT. In the field of mosquito control, AID is supporting a research project on the Development of Genetic Control of *Aedes aegypti* under contract with the University of Notre Dame.

WORLD WIDE SURVEILLANCE OF *AEDES AEGYPTI*

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Nearly three-quarters of a century after the discovery that yellow fever was transmitted by *Aedes aegypti*, epidemics of the virus diseases that this synanthropic species of mosquito transmits are still occurring. The 1969 outbreak of yellow fever in interior West Africa involving at least 60,000 cases in five countries, and the 1971 outbreak of dengue in northern Colombia involving 416,000 cases, are instances of the continuing recrudescence of this vector problem. Now that epidemics of dengue haemorrhagic fever are running at about 1200 cases with 300 deaths per year in Thailand, and are spreading to Malaya and Indonesia, and during a period when Central American countries are battling with reinfestations of *Aedes aegypti*, it is clear that a continuing and world-wide surveillance of the distribution and density of this vector is really needed.

Records of the occurrence and distribution of this species commenced in 1901, when Howard had identified it as *Stegomyia fasciata* Fabricius. In 1920 it was re-identified by Dyar as *Aedes aegypti* Linnaeus, and the records of its occurrence to date were collected under this name by Kumm (1931). The first determinations of density were made by Connor and Monroe (1923) in South America; they introduced the House Index (the percentage of houses that are positive for the species) and the Container Index (the percentage of water-filled containers found positive for the larvae). Breteau (1954), working in French West Africa, introduced what is now known as the Breteau Index (the number of positive containers per 100 houses).

As we all know, the larvae of *A. aegypti* typically develop in small bodies of clean water, similar to those which filled the tree-holes which are their native habitat in Africa. Stores of drinking water are excellent habitats, e. g., the large ceramic jars of southeast Asia, the earthenware pots of Africa, and the water casks of sailing vessels. The discarded metal containers of the so-called developed societies, such as drums, tin cans and best of all old tires, are fertile outdoor breeding grounds. In making an *aegypti* survey, up to 50 dwellings are taken in any locality, and every potential breeding site on the premises, outdoor and indoor, is examined.

In 1966 the French Overseas Scientific and Technical Research Organization commenced, with the support of WHO, surveys of the density of *aegypti* and related *Stegomyia* species in the new countries of francophone West Africa. Subsequently, WHO promoted surveys in various parts of the eastern hemisphere by means of Contractual Technical Agreements with Ministries of Health, by sending consultants to strategic areas, and by staff members of the several Vector Research Units that it was establishing in certain centers. Thus from all sources some 12,000 records have been accumulated, with new accessions running between 1 and 2 thousand per year.

The objective being the collection and subsequent dissemination of this needed information in readily digestible form, advantage was taken of the computer at WHO head-

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quarters for accession, storage, computation and print-out. The print-out decided upon is in the form of maps, on which the presence or absence, or the density, is indicated at each place surveyed by a single symbol. Thus with respect to distribution there are records of the disappearance, the reappearance, and even the first appearance. With respect to density, the indices determined, which may be one or more of the three types of index already mentioned, are reduced and averaged to a density figure on the scale from 1 to 9.

Table 1.—Average House, Container and Breteau Indices for each of nine Size-Classes of *Aedes aegypti* infestation.

Breteau-Index Size Class	Number Samples in Class	Average House Index	Average Container Index	Average Breteau Index
1- 5	17	3	2	4
6- 10	17	7	4	8
11- 24	18	16	8	18
25- 49	23	30	15	37
50- 74	21	42	24	62
75- 99	18	57	34	88
100-149	21	64	34	122
150-199	14	73	34	170
200+	23	82	46	324

The manner in which conversion factors were obtained from one index to another, so that a density figure could be obtained based essentially on the Breteau Index, is as follows. The 172 assessments recorded from various places by 1971 where all three indices had been simultaneously determined were grouped into nine classes according to the magnitude of their Breteau Index (Table 1). Then the average House Indices were plotted against the average Breteau Indices (Figure 1), and the average Container Indices likewise. From the curves so obtained, the House Indices and Container Indices equivalent to Breteau Indices of 5, 10, 20, 35, 50, 75, 100 and 200 can be read off. Thus a table was constructed for nine degrees of infestation (nine density figures) against the corresponding ranges of the three indices (Table 2).

The indices found at any locality are entered on a computer form, along with its coordinates of latitude and longitude to the nearest minute. The computer prints out the

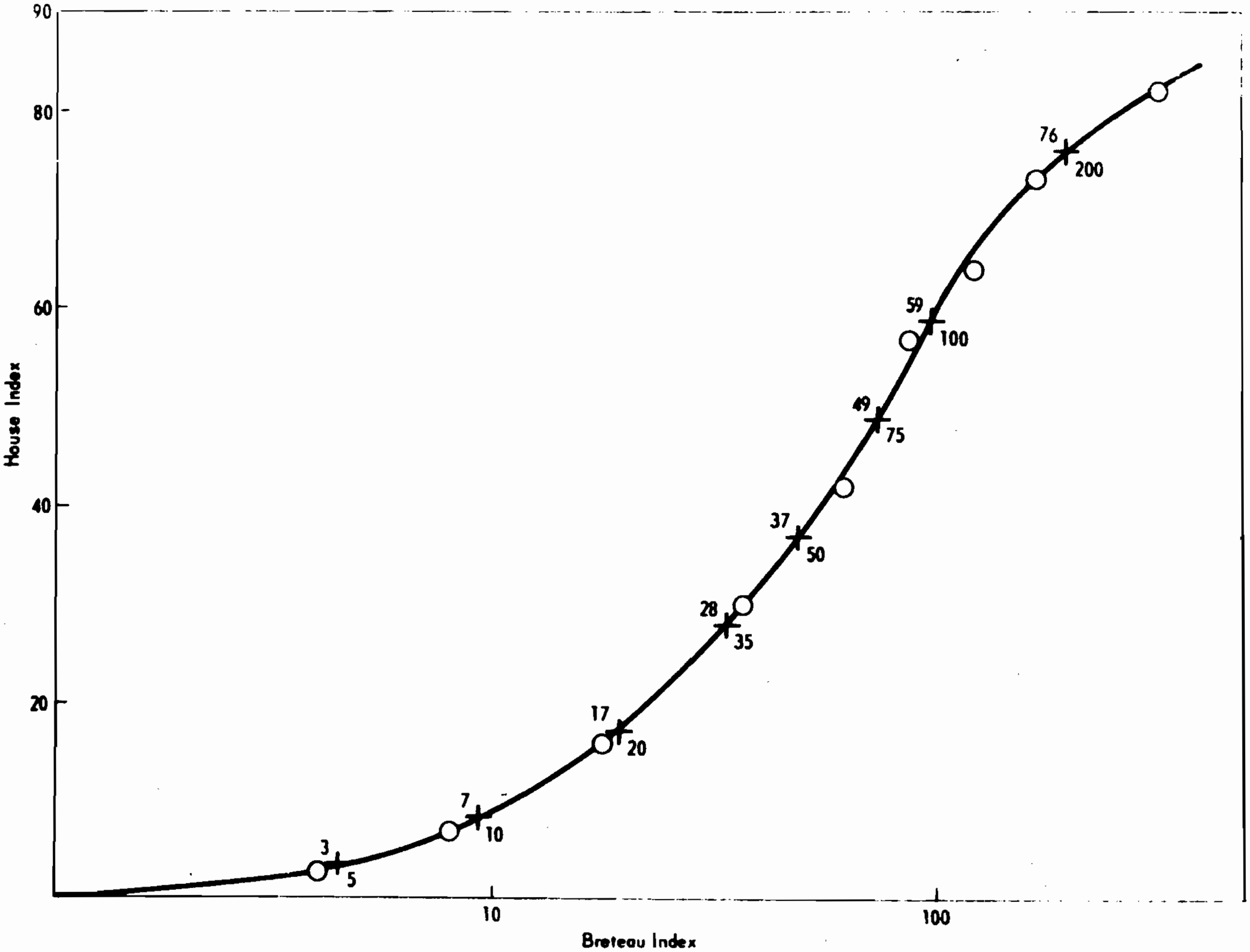

Figure 1.—House Index plotted against Breteau Index : average for nine size classes.

Table 2.—The density figures corresponding to the larval Indices found.

Density Figure	House Index	Container Index	Breteau Index
1	1- 3	1- 2	1- 4
2	4- 7	3- 5	5- 9
3	8-17	6- 9	10- 19
4	18-28	10-14	20- 34
5	29-37	15-20	35- 49
6	38-49	21-27	50- 74
7	50-59	28-31	75- 99
8	60-76	32-40	100-199
9	77⟨	41⟨	200⟨

density figures in map form, on a rectangular rather than an equidistant projection. Where more than one kind of index had been determined in any one assessment, the computer prints out the average of the two or three density figures indicated. Where many assessments have been made at any one place, the computer is instructed at present to print only the highest figure. Records of mere presence of *aegypti* are marked with a P, early records with an E, and records of absence with an X. In order to avoid dense aggregations of figures in certain areas of the print-out, in the interests of legibility those records located in the same square of 3 minutes' side are taken as referring to the same place. The computer program was drawn up by Mr. B. Sletta of the Data Processing unit at WHO headquarters.

The figures for *Aedes aegypti* are printed out on a scale of 1:5 million on a roll of computer paper; the sections are then cut out and mounted on white bristol-boards, and a transparency with the country boundaries is placed over them. A total of 34 maps are thus made for the Eastern Hemisphere, and are reproduced on a reduced scale of 1:8 million to form a brochure.

If one examines the map including most of West Africa (Figure 2) it may be seen that *aegypti* density figures of five and above are found in southeastern Mali, western Upper Volta, northern Ghana, southwestern Niger, northern Nigeria, and southern Togo and Dahomey. This density, corresponding to a Container Index of 30 or more, and a Breteau Index of 50 or higher, constitutes a focus of infestation (Pichon et al. 1969) which on the basis of experience in the 1965 epidemic in Senegal (Chambon et al. 1967) can be expected to propagate an urban yellow fever outbreak should the infection enter from monkey reservoirs which might be present in the countryside. Similar high indices are to be found in northern Cameroon, coastal Gabon, in Luanda, Angola, and in western Senegal. Of the 11 foci of infestation mentioned above, six have been the site of YF epidemics either in 1969 or 1971. In East Africa, on the other hand, the *aegypti* indices are almost universally generally low, with notable exceptions in a very few villages.

Over in Southeast Asia, extremely high indices are to be found in the Mekong delta and around the Gulf of Thailand, a density figure of nine being usually found in the towns and villages there. In the Philippines these figures are usually six to nine, while figures of four to eight are found in northern Thailand, Cambodia and west Malaysia. These areas have been the site, during the past 20 years, of epidemics of dengue haemorrhagic fever (DHF) transmitted by *A. aegypti*; densities of grade four (House Index ⟩15, Breteau Index ⟩22) have proved sufficient in Singapore to engender concentrations of DHF cases (Chan et al. 1971). While this vector is fortunately now scarce in northern Borneo, Taiwan and Australia, it is becoming more abundant in the islands of the Southwest Pacific, with density figures approximating six. It is also fortunate that at present the yellow fever virus is not found east of the African continent.

Proceeding westwards in Asia, density values around three are found in Bangladesh and eastern India, while infestations are sporadic in western India and scarce in southern India, Sri Lanka and Pakistan. Across the Atlantic, in the Caribbean area, infestations of density figure 4-5 (House Indices 21-47) were present in Jamaica in 1970, while in Alabama in 1964 it was found that 23 out of 64 towns had a density over three (House Index more than 10). This is to be contrasted with the situation in Colombia in 1922, where most House Indices were in excess of 50 (density figure 7-9).

In Africa, the populations of *A. aegypti* breeding in the forests, towns and villages produce adults which are jet black with white stripes, and these have been described as subsp. *formosus*. In certain of the ports and coastal sailboats, adults are found which are orange in color; this form when encountered near Brisbane was described as *queenslandensis*. Both of the above forms coexist in certain villages of southeastern Tanzania and coastal Kenya, *queenslandensis* breeding in the houses and *formosus* in the surrounding vegetation. Of the two forms, the domestic *queenslandensis* has been found by olfactometer test to be the much more anthropophilic of the two. It is commonly encountered in Mauritius and the Seychelle islands, along the Arabian coast, and from Sudan sporadically across the interior of West Africa to Gambia. In Asia and America, the prevalent form is mahogany in color; specimens of this form were taken from Kuala Lumpur, Malaysia by Mattingly (1962) as being the type form (*typicus*) to constitute a neotype of *Aedes aegypti* L., this being necessary because the original description published by Hasselquist in 1672 was obviously of the common palaearctic species *Aedes caspius*.

The world-wide surveillance of WHO extends to other close relatives of *aegypti* in the subgenus *Stegomyia*. *Aedes africanus*, *luteocephalus* and *metallicus* keep the yellow fever infection going among monkeys, the first species in the rain forests, the second extending farther north into the savanna, and the third with a more eastern distribution in Africa. *Aedes simpsoni*, which breeds in the water-filled axils of tropical plants such as bananas and cocoa-yams, was an important vector of yellow fever in the great YF epidemic of 1960-62 in Ethiopia. *Aedes vittatus*, with a distribution extending from the Atlantic coast of Africa to Southeast Asia, is a vector breeding in rock-holes; it is not uncommon in domestic containers in India. *Aedes albopictus*, which extends from Madagascar to Japan, is an important vector of dengue and DHF in the Oriental zoogeographical region, breeding in feral rural sites. It is now

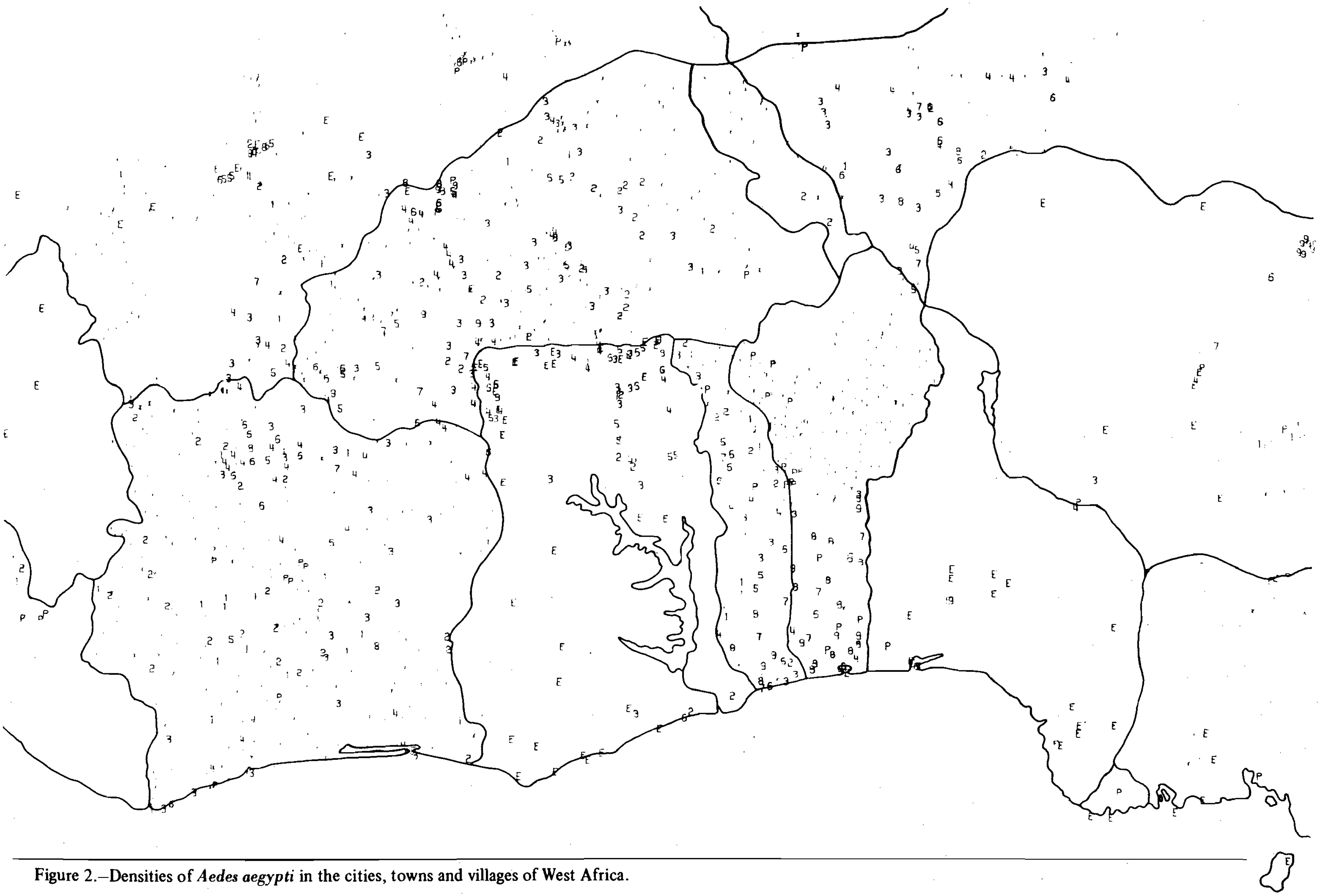

Figure 2.–Densities of *Aedes aegypti* in the cities, towns and villages of West Africa.

spreading into the Australian zoogeographical region, normally occupied by species of the *Aedes scutellaris* complex, which are vectors of dengue on the Pacific islands.

The principal vector, *Aedes aegypti,* reached its high-water-mark of distribution in the 1930's (Figure 3), when it extended up to its bioclimatic limits of the 10°C. January isotherm (or just north of it) in the Northern Hemisphere and the 10°C. July isotherm in the Southern Hemisphere. Thus its continuous and year-round distribution had extended north to Norfolk, Va. and Cairo, Ill., with local or occasional infestations in localities extending to St. Louis and Boston, while occurring in San Francisco and even coastal British Columbia. Infestations were present around the Mediterranean, on the Black Sea coast and across to Baku on the Caspian, and in certain ports on the Bay of Biscay. In South America, *aegypti* had penetrated almost to the headwaters of the Amazon and Parana river systems, and its coastal distribution extended south to Buenos Aires and Valparaiso. In the Far East, it had extended north to southern Japan, south to Australia, and further east to the main Pacific islands.

This spread could be detected once its importance as a disease vector was realized and a watch was kept for its arrival, – Hong Kong 1902, Bucaramanga (Colombia) 1909, Haiphong (N. Vietnam) 1915, Santa Cruz de la Sierra (Bolivia) 1916. In 1903, the only infested localities in west Malaysia were Singapore and Port Swettenham, while by 1955 all the towns but not all the villages of the Malayan peninsula were infested. All the evidence points to a spread of this species of African origin which started with sailing ships dating from the Portuguese explorations of the 15th century, and later with the slave trade on the east-west Middle Passage from West Africa to the Caribbean and the Atlantic states, and yet later with the East African slave trade of the 19th century out of Zanzibar. Once arrived at the new ports, the species would be spread inland along the lines of communication.

Following on the abatement of *Aedes aegypti* in the coastal cities of the Americas during the first 20 years of the 20th century, which was sufficient to eliminate the urban foci of yellow fever, the control extended to the hinterland during the 1930's; the arrival of DDT in 1945 made it possible to aim at total eradication of this vector. Thus eradication was achieved from Bolivia by 1948, from all Brazil by 1950, from Ecuador, Peru, Chile, Uruguay and Paraguay by 1955, and from Argentina by 1959. In Central America, eradication was achieved from Panama, Costa Rica and Nicaragua by 1955, and from El Salvador, Guatemala and Honduras by 1957. It was almost achieved in Mexico. A United States eradication program started in Puerto Rico, the Virgin Islands and Florida in 1964 but was discontinued in 1968.

During this period, practically all the Caribbean islands remained infested, along with northern Colombia, Venezuela and the Guianas, and the United States north to Tennessee and North Carolina. Between 1965 and 1971 re-infestations appeared in Mexico, El Salvador, Guatemala, Panama, and Para state of Brazil; spot re-infestations in Buenos Aires and Escuintla, Costa Rica were promptly eliminated. The major source of these re-infestations was the importation, from infested areas, of used tires in which the eggs of *aegypti* can survive for at least a year.

If we compare the distribution map for the 1970's (Figure 4) with that for the 40 years previously, it can be seen that in addition to its elimination from most of South America, *A. aegypti* has also disappeared from the Mediterranean area, the only recent records being Algiers in the 1950's and Desenzano, northern Italy, in 1971. The species has apparently disappeared from Egypt clear down to Djibouti beyond the southern end of the Red Sea. It has been eliminated from Hong Kong, the Ryukyu islands and southern Japan, from the Mascarene islands, and from Oahu among the Hawaiian islands; among the Atlantic islands, the Azores, the Canaries and Bermuda are now free of *aegypti.*

On the other hand, in addition to increasing in density on the Pacific islands of the New Hebrides, New Caledonia and the Society Islands, *Aedes aegypti* has extended its range among the Solomon Islands and Ellice Islands during World War II, and appeared on Niue for the first time in 1972. When one also considers the hazards of re-infestation of liberated territories, it is clear that a continuing surveillance of the distribution of this vector is essential. In view of the increasing prevalence of dengue in the Caribbean, and of DHF in southeast Asia, it is also essential that this surveillance be made in terms of vector density. If by some mischance the virus of yellow fever should gain a foothold in Asia or Oceania, this surveillance along with control operations could become of crucial importance.

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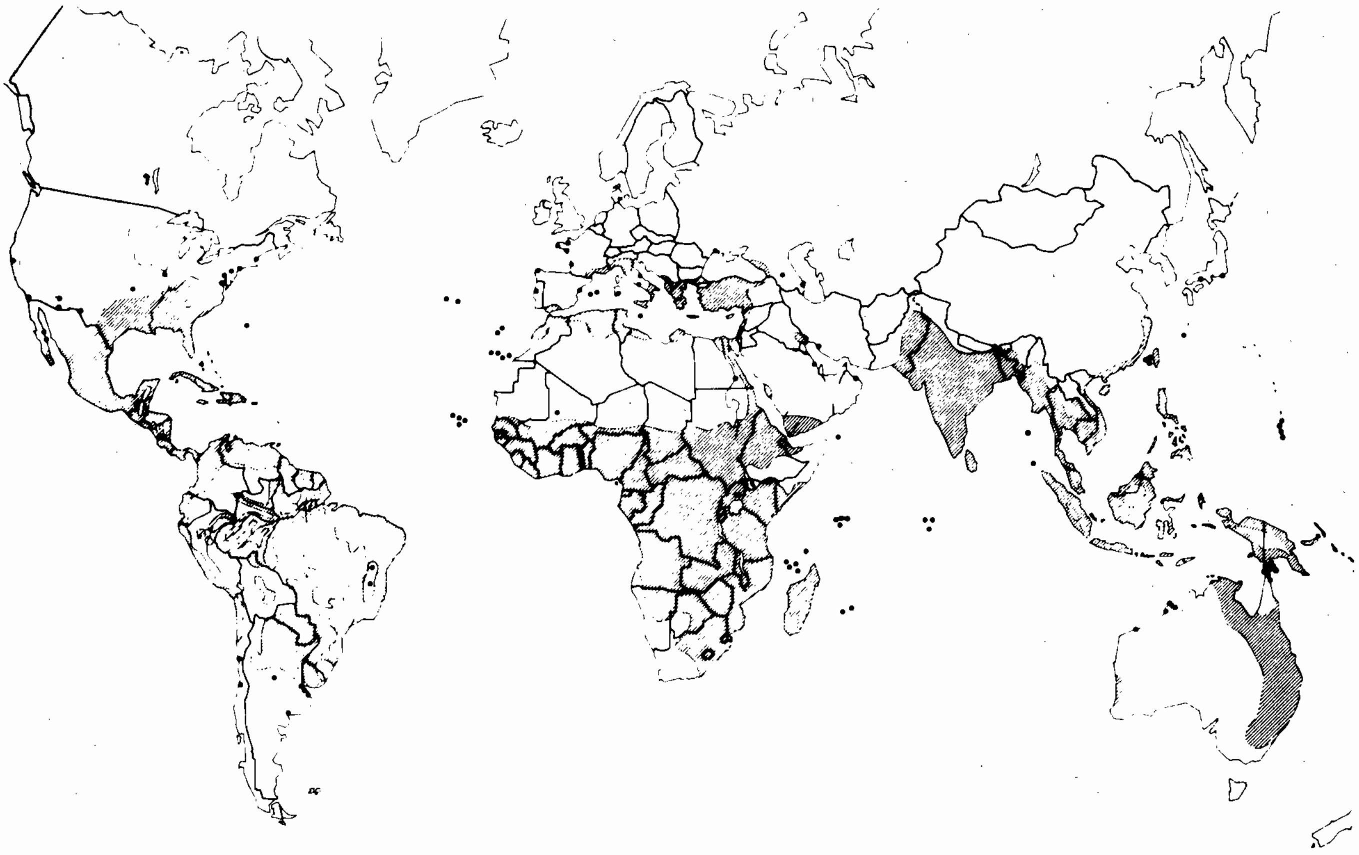

Figure 3.–Geographical distribution of *Aedes aegypti*, ca. 1930.

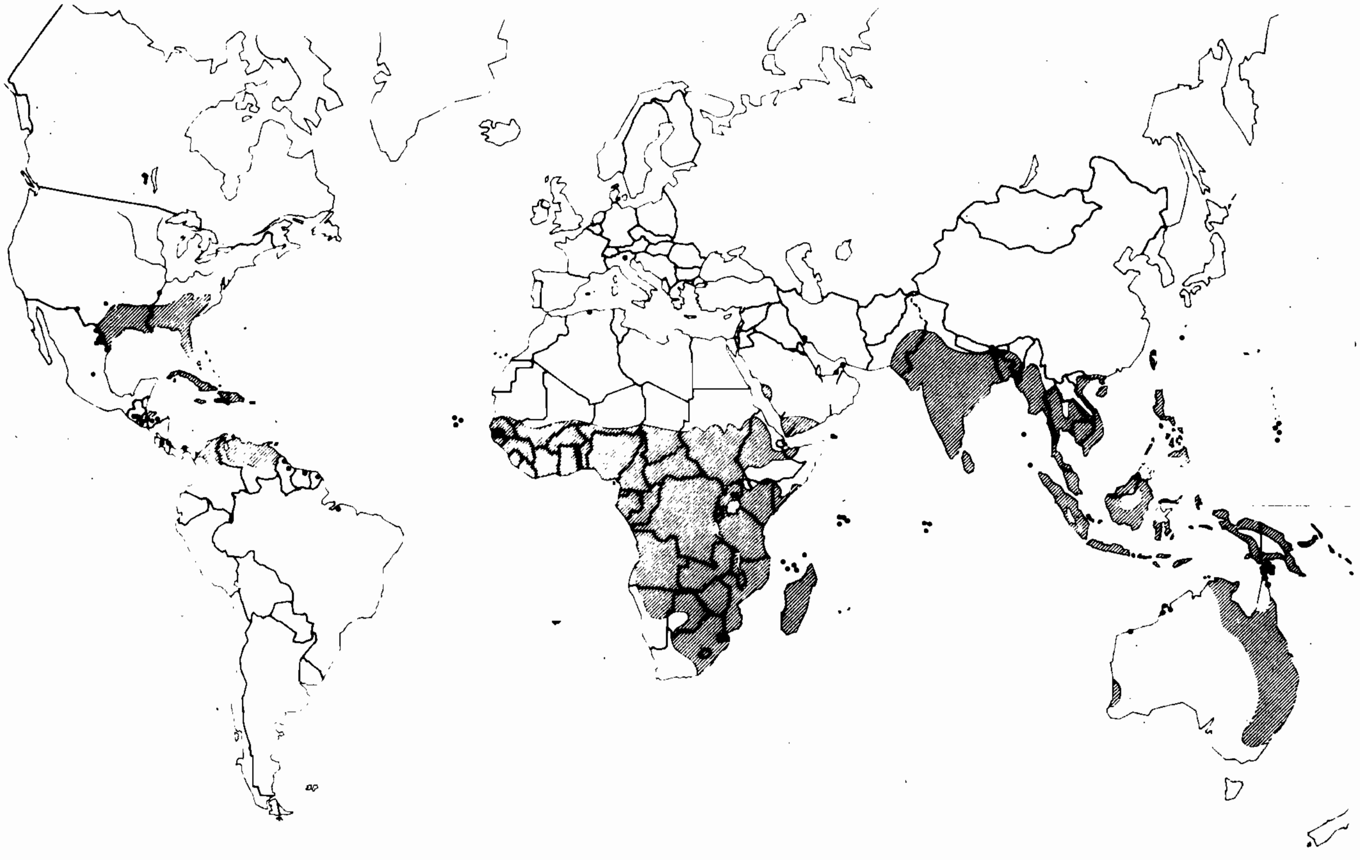

Figure 4.–Geographical distribution of *Aedes aegypti*, ca. 1970.

TAXES – LOCAL, STATE AND FEDERAL

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Federal spending and federal taxes – the link is as inescapable as death and taxes – or to quote the words of the song about "love and marriage" they go together like a horse and carriage . . . you can't have one without the other". And you can't cut one without the other in spite of the "pie in the sky" suggestion that all we have to do to slash taxes is to plug the tax loopholes.

All governments combined spent $410 billion last year. According to Tax Foundation, Inc. of New York, federal, state and local tax collections for fiscal 1973 will be the equivalent of $5,070 per American household. In 1960 the total tax figure was $2,400 per household; this represents an increase of $2,670 (or over 100% in 13 years).

Estimates of the total tax bite from the income of the average American vary from 36 to 40%, with the Federal share variously estimated as low as 20 or as high as 25%. The breakdown in estimated federal taxes for the year ending June 30, 1974 is as follows:

Personal Income Tax	$118.0	billion
Corporation Tax	43.0	billion
Social Security Taxes	66.3	billion
Excise Taxes	17.1	billion
All other revenue	25.6	billion
Total Federal Taxes	$270.0	billion (1970)

Total budget receipts have increased from $112.7 billion for fiscal year 1964 to an estimated $265 billion for fiscal 1974. The new budget which has just been submitted is $304.4 billion.

It is no accident that during this same period budget spending priorities of the Federal government have shifted. For example, in 1965, $41.86 of each $100 the Federal government spent was used for national defense, $6.25 was used for commerce and transportation; $21.70 was used for the income security function and $8.75 was used to pay interest on Federal securities.

Today the national defense budget is estimated at 30%, a dramatic reduction, while income security has increased to an estimated high of 30.51%, a jump of almost 9%, primarily in trust fund activities – old age and survivors insurance, federal employees retirement, unemployment and railroad retirement, etc. Public assistance and grants for social services are the major Federal funds programs accounting for about 19% of total outlays for the income security function. Trust funds account for about 81% of these outlays.

The education and manpower functions have been increasing steadily from 1.93% in 1965 to more than 4% in total outlays for the last three years. The health function has increased from 1.4% of total outlays in 1965 to more than 8% estimated for fiscal year 1974. Budget functions of community development and housing have increased in relation to total outlays for the period with continued annual deficit financing; also interest on the national debt has accelerated. Interest is now the second largest item of the budget.

One factor which makes the problem of controlling both the size and composition of Federal spending particularly acute is the fact that future budget increases are likely to be concentrated in the categories termed relatively uncontrollable.

In March of 1967 President Johnson appointed a Commission to study the Federal budget concepts and presentations. In October of that year the Commission rendered its report recommending that the budget be presented on a single unified basis, thereby eliminating the three budget concepts employed previously (the Administrative budget, the consolidated cash budget and the National income account budget). This method was adopted and has been in use since the Johnson administration.

The uncontrollable categories include expenditures from social insurance trust funds, veterans benefits, public assistance including medicaid, agricultural price support, military retirement payments, postal operations, revenue sharing, operation of the legislative and judicial branches of government and last, but not least, the cost of servicing the public debt. These categories account for an estimated 75% of total spending. The interest burden this fiscal year is expected to exceed $30 billion, a sizable obligation for the American taxpayer.

A study of changes in the pattern of budget outlays reveals what may be a progressive shift in economic theory. But upon closer examination it may prove not so much a matter of economic theory as of ideology, an income leveling force in favor of greater personal income equality. We might ask ourselves whether we choose to view government as a huge machine for the redistribution of wealth, taking from those who have more and giving to those who have less; or do we believe instead that free market forces are the most effective means to stimulate expansion of the economy with presumed benefits reaching all or most segments of society. To pursue the "pie in the sky" analogy, is the question: "How to slice the pie?" or "How to bake a bigger pie?"

Assuredly there are many shortcomings in the tax law that need study and improvement. The year 1972 was a year of prolonged Congressional hearings and a plethora of legislative proposals; but major decisions were deferred until 1974. Following hearings on windfall profits, the House Ways and Means Committee is expected to bring forward some significant changes but predictions on the nature of the reforms vary.

The answers are far more complicated than most spokesmen for mere loophole closing claim. If we accept the obvious link between federal spending and federal taxes and if we rule out alternatives such as the "value added" tax imposed by other industrial nations, we are left with several other possible alternatives, some with self-evident drawbacks. Income tax could be boosted, or a surtax imposed to produce needed revenue. Congress could subject some of the major now-protected forms of personal income to the full impact of the tax rate schedule in a move toward developing a comprehensive tax base. Congress might allow the large annual deficits that have prevailed to continue into the future permitting them to become a fixture of the federal fiscal system through the 1970 decade, with inevitable inflationary impact.

Or attention can be given to altering the relationship of the federal government to state and local spending programs. The proliferation of grants-in-aid programs pleases no one. Federal revenue sharing returns some money to state and local governments. But in general state and local governments must move in the direction of assuming more of the tax load and the difficult task of redistribution if this is the goal. In my opinion the public interest is best served when the traditional and more responsible role of government requires the officials to raise the revenue that they will eventually spend. Responsibility is too easily shifted when this function is divided.

Leadership pronouncements in both Houses of Congress during 1973 make it clear that income tax reform will be a major goal of legislative efforts. However, final action may fall short of announced aims and could be much less significant and extensive than in the Tax Reform Act of 1969. Yet the importance of the way in which the tax burden is distributed cannot be overrated at a time when federal, state and local governmental revenues equal about 40% of total personal income. The load should not only be equitable among economic groups and individuals but also have the least harmful effect on economic growth and stability.

First imposed in 1913 at rates from 1 to 7%, income taxes rested principally on only a small number of wealthy persons. The income tax was turned from a class tax into a mass tax during World War I when the levy was extended downward to reach lower incomes. Its rate scale elevated to between 6 and 77%. The 1920's saw a series of reductions in rate scale until the trend reversed in the 30's when the scale climbed from 23 to 94% and the number of taxpayers multiplied ten-fold. Tax cuts were small and it was not until 1964 that the 14 to 70% scale was adopted which is still in effect.

Congress can more effectively control taxes by controlling expenditure growth. Practicing fiscal restraint and enacting comprehensive budget control legislation which would place the fiscal affairs of the Federal government on a sound basis would provide the vehicle for systematic policing of taxing and spending programs. Such a bill has passed the House of Representatives and Senate action is expected on companion Senate legislation.

After the tough decisions have been made, what remains is to sort out the specific reforms that matter the most in terms of both fairness and simplicity and to work out the knotty problems of intergovernmental finance. The most immediate problems are the finances of the states and local governments. Taxes are here to stay! We can hope but can't promise that they will be reduced. But it is in our power and therefore it is our duty to have a fair system of taxation and one that raises enough money to escape the crisis aura that hangs over too much Federal, state and local finance today.

THE NEW VECTOR-BORNE DISEASES DIVISION BUREAU OF LABORATORIES, CDC

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The Fort Collins, Colorado, Laboratories of the Ecological Investigations Program were organizationally transferred to the Bureau of Laboratories of CDC on July 3, 1973. Staffs from Fort Collins and Atlanta were combined to form a new Vector-Borne Diseases Division, headquartered at Fort Collins. Dr. Thomas P. Monath will report for duty as Chief of the new Division in July 1974. Until then, Dr. Archie Hess will serve as Acting Chief. Dr. Hess will retire from the Public Health Service at the end of 1974, having reached the mandatory age for retirement. Mr. David T. Pegg will serve as Assistant Chief for Administration, and Dr. Jack D. Poland will be Chief Medical Epidemiologist for the Division.

The new Vector-Borne Diseases Division will conduct field and laboratory research on the ecology, epidemiology, and control of arthropod-borne diseases; will provide consultation, laboratory services, and epidemic aid to State, Federal, and international agencies; and will train technical and professional personnel. It will also serve as the World Health Organization's Arthropod-Borne Virus Regional Reference Laboratory for the Americas.

The Vector-Borne Diseases Division will be comprised of seven branches as follows: (1) Plague Branch, Dr. Allan M. Barnes, Chief; (2) Immunochemistry Branch, Dr. Dennis W. Trent, Chief; (3) Water Resources Branch, Dr. A. D. Hess, Chief; (4) Arbovirus Ecology Branch, Dr. W. Daniel Sudia, Chief; (5) Vector Ecology Branch, Dr. D. Bruce Francy, Chief; (6) Vertebrate Ecology Branch, Dr. Thomas P. Monath, Acting Chief; and (7) Arbovirus Reference Branch, Dr. Charles Calisher, Chief.

It is hoped that this new organization will enable CDC to provide better services in the area of vector-borne diseases, including those associated with water resources development. The staff of the Division will continue to maintain close liaison and collaboration with State health department personnel.

MONTHLY COMPARISONS OF 1973 LIGHT-TRAP COLLECTIONS OF *CULEX TARSALIS* MOSQUITOES IN A FOCUS OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS VIRUS TRANSMISSION

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Since 1967, investigators from the School of Public Health at UCLA have conducted research into arbovirus activity in the Imperial Valley of California. From 1970-72, a focus of western equine encephalitis and St. Louis encephalitis transmission was defined at Finney Lake. The area, about 30 miles north of Mexico, is southeast of the Salton Sea. There is a normal annual rainfall of only 2.3 inches and the elevation is approximately 170 feet below sea level.

Using data from earlier collections, two sites located in Figure 1 in the Finney-Ramer Wildlife Area were selected for study. One was on an east-west levee between Sheldon Pond and the Alamo River. Here, one CDC light-trap, with dry ice as an attractant, was suspended from the trees or bushes (4 to 8 feet above the ground) every 100 feet. This 15-trap transect was 1,400 feet long.

The second site consisted of an ecotone on the north shore of Finney Lake. In this locale, a grid of nine traps, positioned 50 feet apart in rows of three, was established. They ranged from the densely vegetated shoreline to a bluff rising abruptly 15 feet to the prevailing plateau level of the Valley floor. Except for one open space leading to the center of the grid, the site consisted of a dense stand of tamarisk trees. Numbered metal plates were permanently affixed at each trap site to insure precise placement of the light-trap during each monthly collection period. The entire Finney-Ramer area is surrounded by vast irrigated fields in which such crops as lettuce, tomatoes, melons, sugar beets and cotton are rotated.

Beginning in February, 1973, and continuing through January, 1974, monthly trips were made to ascertain trap night yield, trap site yield, species proportions, seasonal variation and total number within the focus.

This paper reports the initial analysis of these data, with emphasis on *Culex tarsalis* since this species comprised 110,000 (92 percent) of the 120,000 mosquitoes collected.

A summary of the transect collections is presented by Table 1, showing the total number of mosquitoes collected. In May, the *C. tarsalis* population increased to its highest levels, but dropped by 75 percent from June to July, a very hot period in the Valley. Collection numbers began to rise again through September and October but only to the April level. *Culiseta inornata* was prominent during the cooler months of the year, even exceeding *C. tarsalis* in March.

Average trap night values on the transect are shown in Figure 2. A marked drop occurred from June to July, after a rise in May. The top line of the figure presents the average of the three most productive trap sites, numbers 1, 5 and 6. The bottom line presents data from the three least productive sites, numbers 7, 8 and 11. The curves follow a similar pattern.

Table 1.–Mosquitoes collected from transect at Finney-Ramer Wildlife Area, Imperial Valley, California (1973-74).

Month	*Culex erythrothorax*	*Culex tarsalis*	*Culiseta inornata*	Other Species[1]
February	1	2,466	222	0
March	1	1,016	1,201	9
April	2	1,922	681	2
May	75	14,340	246	292
June	1,300	29,177	148	50
July	4	5,247	0	3
August	0	930	0	2
September	1	1,727	0	0
October	0	2,086	4	3
November	0	591	3	2
December	40	836	35	0
January	7	960	98	0
TOTALS	1,431	61,298	2,638	363

[1] *Aedes dorsalis, A. vexans, Aedes* spp., *Culex pipiens quinquefasciatus.*

Table 2.–Mosquitoes collected from grid at Finney-Ramer Wildlife Area, Imperial Valley, California (1973-74).

Month	*Culex erythrothorax*	*Culex tarsalis*	*Culiseta inornata*	Other Species[1]
February	0	476	97	0
March	15	256	287	1
April	6	195	262	1
May	1,456	9,462	436	72
June	2,770	9,840	91	11
July	486	12,121	0	26
August	86	7,755	0	3
September	40	5,382	4	0
October	1	2,602	8	9
November	0	307	9	0
December	4	248	40	0
January	0	208	111	0
TOTALS	4,864	48,852	1,345	123

[1] *Aedes dorsalis, A. vexans, Aedes* spp., *Culex pipiens quinquefasciatus.*

Table 2 gives the results of the grid collections. These also show the marked increase in *C. tarsalis* numbers in May. This trend continued upward to a peak in July and slowly diminished through October, as the season progressed. In the grid, *C. inornata* numbers exceeded *C. tarsalis* in March and April.

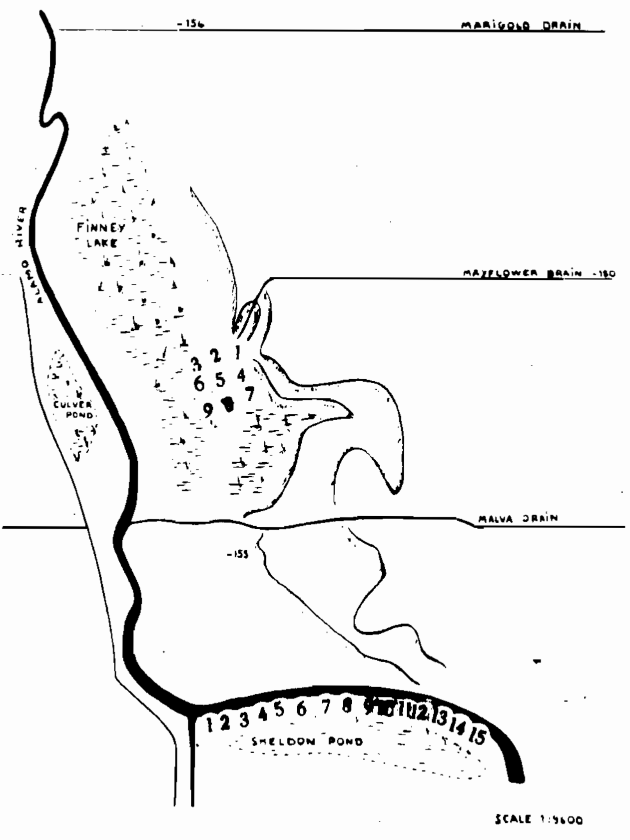

Figure 1.–Finney-Ramer Wildlife Area.

The average trap night yield shown in Figure 3 illustrates the dramatic increase in numbers from April to the July peak on the grid. Through October an average of 160 mosquitoes was collected in each of the nine traps. The bottom line is the average of traps numbered 1, 4 and 7, those located near the bluff. The top line shows catches in traps 3, 6 and 8, located near the lake.

The two trap night curves are compared in Figure 4. There was a marked rise and fall on the transect; in the grid, the numbers increased in May and continued above the transect values through October.

The average maximum and minimum temperatures, and the ranges of temperatures recorded during each collection period at the Soil Conservation Center just southwest of Brawley rose to a peak in July and then gradually decreased. The average relative humidity recorded at 9:00 a.m. was below 30 percent during collection periods in July, August and September.

The authors attempted to determine why the two sites, within 0.8 mile of each other, yielded catches that were so different. Perhaps proximity to water and favorable breeding habitat adjacent to the grid, and the protection from the 100-plus degree temperatures among the dense tamarisk trees offered conditions conducive to survival of the mosquitoes. By contrast, the transect was in a relatively exposed, unprotected site with a river on one side. Possibly the numbers of mosquitoes in the May-June transect collections reflected an excess of mosquito production elsewhere, with decreased survival as the temperature increased.

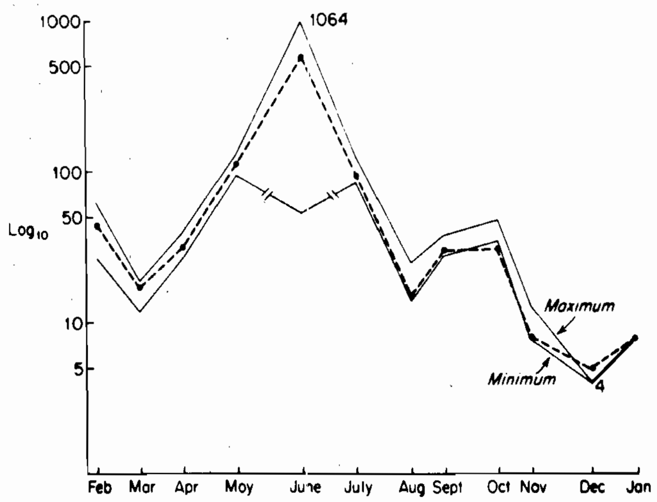

Figure 2.–Average number of mosquitoes collected per trap night from Sheldon Pond transect.

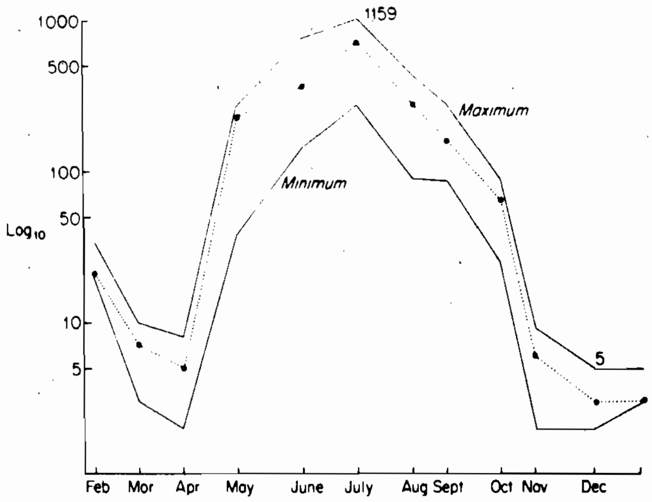

Figure 3.–Average number of mosquitoes collected per trap night from Finney Lake grid.

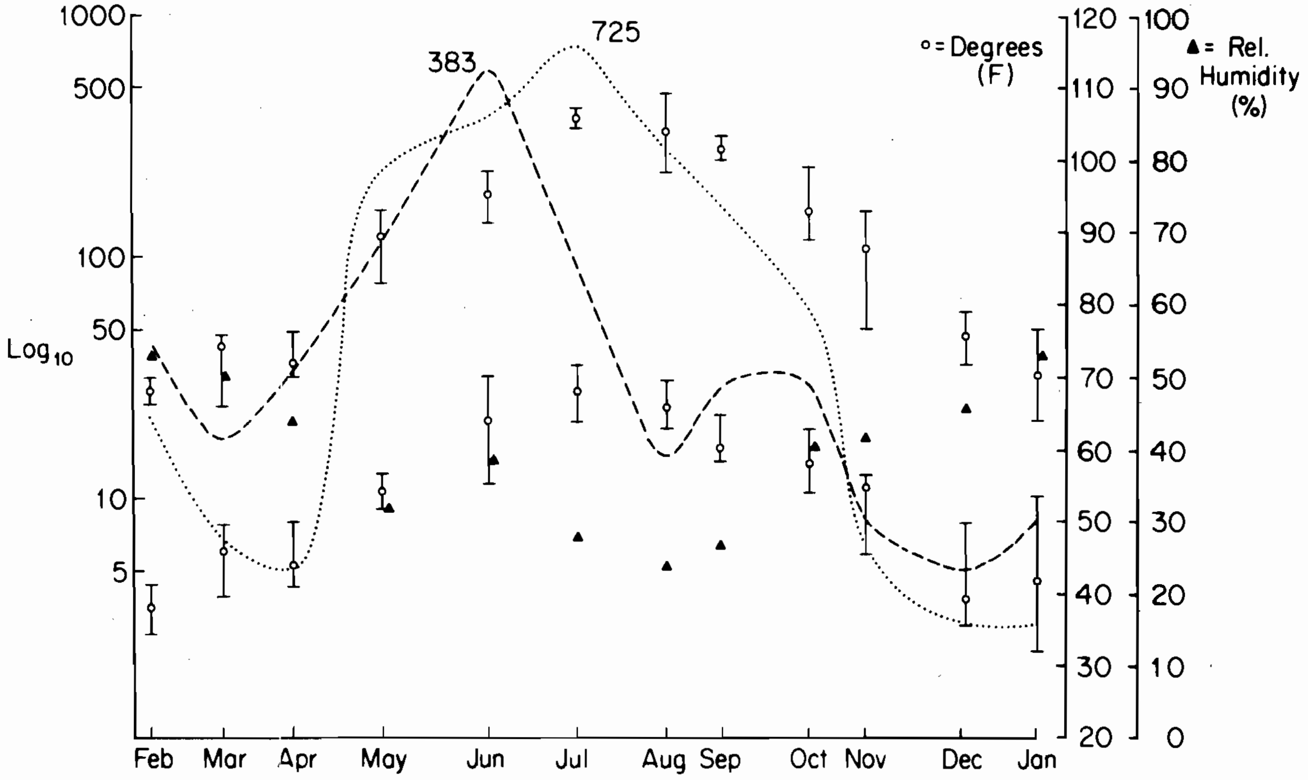

Figure 4.–Average number of mosquitoes collected per trap night from grid and transect.

DIFFERENTIAL PATTERNS OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS VIRUS ISOLATIONS FROM *CULEX TARSALIS* MOSQUITOES COLLECTED AT TWO SITES IN IMPERIAL VALLEY

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Culex tarsalis mosquitoes have long been recognized as the rural vector of western equine (WEE) and St. Louis encephalitis (SLE) viruses in western USA. While the relationships of the mosquito to epidemics of human disease have been well analyzed (Reeves and Hammon 1962) much remains to be learned about the dynamics of *C. tarsalis* as a virus vector so as to monitor these mosquitoes as indicators of potential epidemics and to more clearly determine where and when to apply vector control.

Investigations initiated in August 1967 at the State of California Wister Wildlife Refuge at the southeastern end of the Salton Sea were extended toward the interior of Imperial Valley in 1970 after it had been established: 1) that *C. tarsalis* was active throughout the year and 2) that it was infected and potentially transmitted WEE and/or SLE viruses from as early as April until as late as November each year.

By 1971 it had been determined that the erratically fluctuating results of virus vector studies at the Wister Refuge reflected the great changes in habitat management, and that this locale encompassed an edge phenomenon indicative of what was occurring more centrally in Imperial Valley. Mosquito collections in 1971-1972 along a fourteen mile transect, paralleling the Alamo River from the Finney-Ramer Wildlife Refuge to the Salton Sea, confirmed this hypothesis and showed that consistent results in regard both to mosquito catches and virus isolations were obtained in the Finney-Ramer Area six miles northeast of Brawley. Therefore, it was decided to intensify mosquito studies in this more stable ecological area.

Results of the first year's study of mosquitoes collected in a transect and grid, which were designed to quantitate habitat, site and trap yield using CDC light traps, have been reported in a companion paper (Clark et al. 1974). The data show that in spite of apparent similarity of the two collection areas, the trap yields are quite different. These contrasts expose probable differences in the microclimate characteristics. Analysis of the virological data bears out these differences but in a different way. Clues as to sources and maintenance of virus infection in *C. tarsalis* are also beginning to emerge.

MATERIALS AND METHODS.–Permanently installed wire hooks with associated number plates enabled repetitious suspension of the light traps in the same exact places along the transect and in the grid as shown in Figure 1. Six volt storage batteries contained enough energy to operate the light traps consistently for five to seven nights. Approximately two pounds of dry ice in a brown paper bag were placed near the light trap as an additional attractant.

After sunrise, the catching bags were removed from the light traps and immediately placed in styrofoam boxes with dry ice as refrigerant. On arrival at the field laboratory, the bags were sequentially emptied into an aluminium container on a slab of dry ice. Mosquitoes were separated from other debris and transferred to plastic vials which were transported on dry ice to UCLA where they were stored at – 70°C in a Revco freezer until examination for species and pooling. This was done in enamel pans on a slab of dry ice. Pools were accumulated in glass tubes prior to trituration in 1.8 ml of 20% buffered normal rabbit serum in a mortar. The suspension was then centrifuged at 2,000 rpm in the cold and the supernatant inoculated for virus isolation.

A substantial portion of each month's pools was inoculated intracerebrally into suckling mice. Because of the large number, additional pools were inoculated into tissue culture for detection of virus by cyto-pathic effect. Positive tissue culture harvests were passed IC into suckling mice. Brains from mice showing pathogenic effects were harvested and

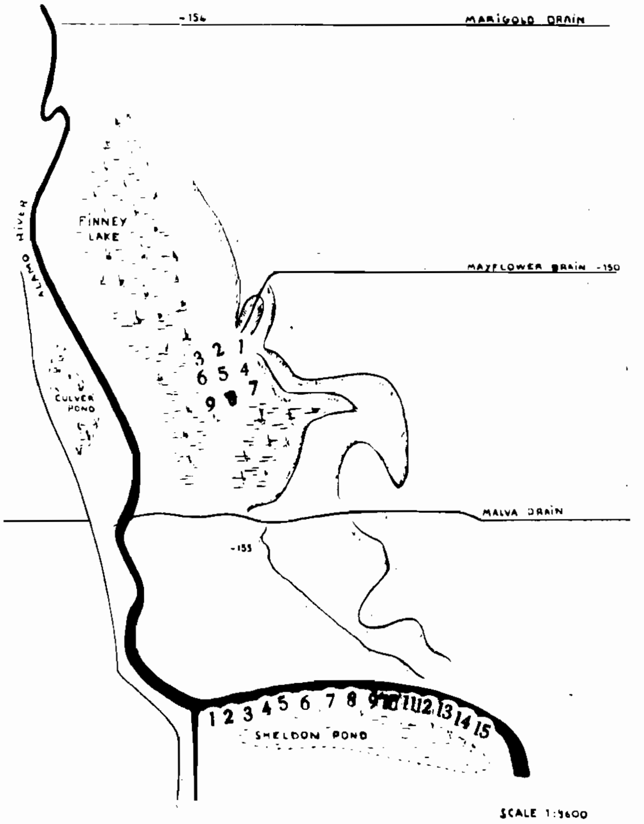

Figure 1.–Finney-Ramer Wildlife Area.

suspended into bovine albumin phosphate saline at pH9 for preparation of a crude antigen. These antigens were then tested for hemagglutination of goose red cells. Those that hemagglutinated were subsequently tested at optimum pH with hyperimmune ascitic fluid for WEE and SLE viruses. Fourfold or greater inhibition of agglutination established the identity of the virus. Those strains which did not hemagglutinate initially were passed IC in suckling mice for further attempt to produce an hemagglutinin. Those that did not hemagglutinate were then tested in complement fixation reactions using additional hyperimmune ascitic fluids for Turlock and California viruses which have also been isolated in the study area.

The results reported here encompass almost all of the viruses isolated from February 1973 through January 1974. The few remaining for definitive identification will not appreciably change the patterns derived nor the statistical conclusions because they are from sites and months that already provide most of the isolations reported here. There is also one other technical question regarding SLE virus isolations. Analysis of all the data questions whether tissue cultures are as sensitive for isolation of SLE virus from mosquito pools as suckling mice. Therefore, the number of SLE isolations reported may reflect less SLE virus transmission than if all mosquito pools were inoculated into suckling mice.

Table 1 summarizes by month total *C. tarsalis* caught, numbers of pools constituted and numbers of WEE and SLE virus isolations obtained. So far the total year's catch of 110,038 *C. tarsalis*, divided into 2,423 pools has yielded 192 isolates; 136 WEE and 56 SLE. A few strains remain under study and will be added to the totals when identity is certain.

The bottom lines of Table 1 give the percentage of positive pools for WEE and SLE isolations. Although in earlier years WEE virus has been isolated as early as April and SLE as late as November, isolations from the transect and grid in 1973 appear to be restricted to June, July, August and September. SLE virus was isolated from a site on the New River in Imperial Valley on 4 April 1973 (Magy, Personal communication).

Because of interest in specific origin of virus isolations, mosquitoes were pooled by single trap sites. There were practical reasons why every pool could not consist of exactly fifty mosquitoes. Occasionally there were too few in the last lot to total fifty, or the residue of one trap site collection exceeded fifty by several more. Therefore, in June, 690 or 82% of the pools consisted of exactly 50 mosquitoes while 58 contained more and 94 fewer than fifty. In July, 257 or 72% had fifty; 44 more, 55 fewer. In August, 116 pools (61%) were of fifty; 30 more and 47 fewer. In

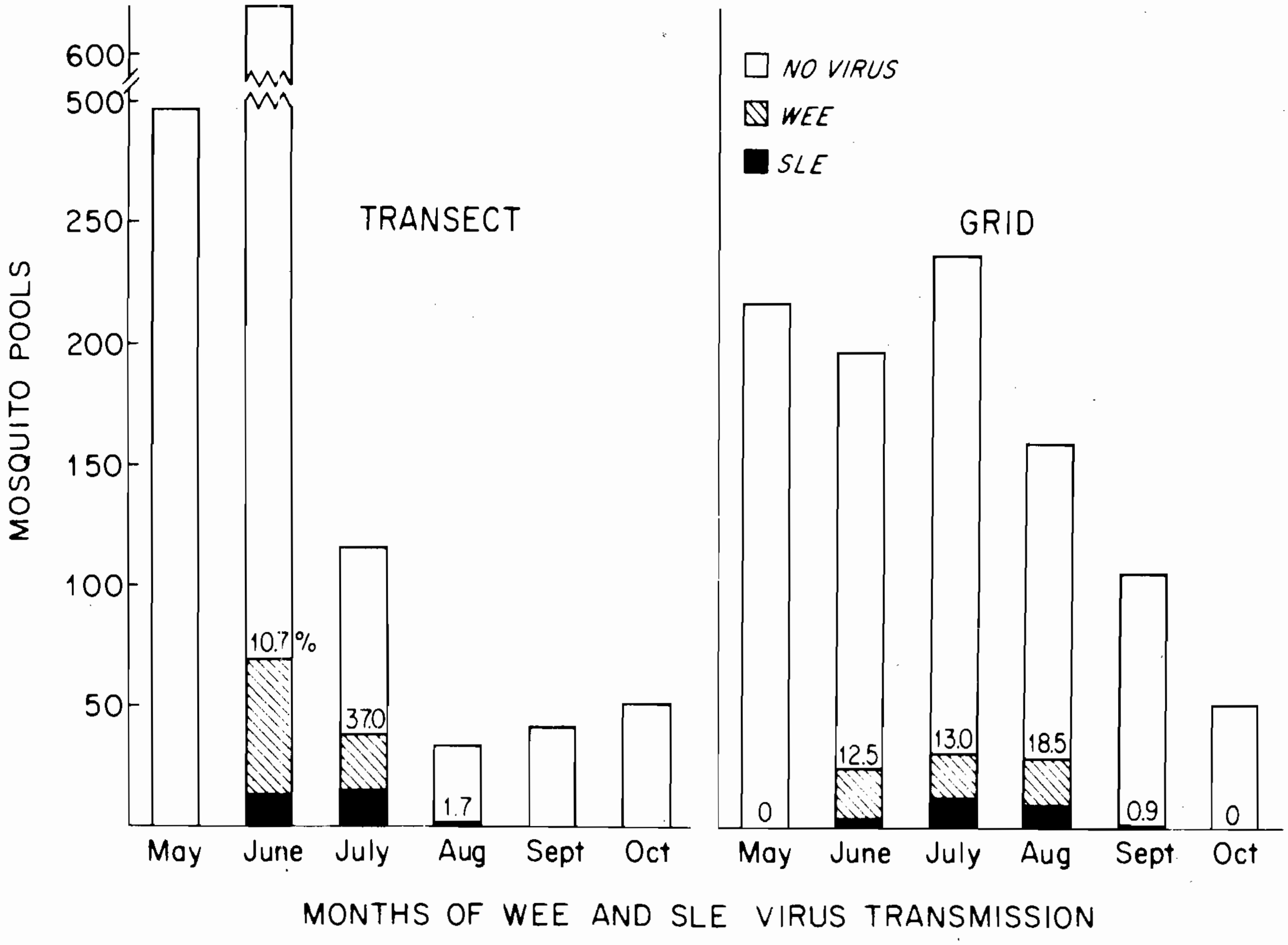

Figure 2.–Comparison of *Culex tarsalis* mosquito transmission of WEE and SLE at two sites at Finney Lake, Imperial Valley.

Table 1.–1973 virus isolations from *Culex tarsalis* mosquitoes collected at Finney Lake, Imperial Valley, California.

	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	Total
TRANSECT													
MOSQUITOES	2,466	1,016	1,922	14,319	29,089	5,247	930	1,727	2,086	591	836	960	61,189
POOLS	60	26	46	286	640	117	33	41	51	19	23	29	1,371
WEE	0	0	0	0	55	22	1	0	0	0	?	0	78
SLE	0	0	0	0	13	15	1	1	0	0	0	0	30
ISOLATIONS	0	0	0	0	68	37	2	1	0	0		0	108
GRID													
MOSQUITOES	472	256	196	9,462	9,840	12,121	7,755	5,382	2,602	307	248	208	48,849
POOLS	16	11	9	228	202	239	160	108	52	12	11	9	1,057
WEE	0	0	0	0	19	19	20	0	0	0	0	0	58
SLE	0	0	0	0	3	12	10	1	0	0	0	0	26
ISOLATIONS	0	0	0	0	22	31	30	1	0	0	0	0	84
TOTALS													
MOSQUITOES	2,938	1,272	2,118	23,781	38,929	17,368	8,685	7,109	4,688	898	1,084	1,168	110,038
POOLS	76	37	55	514	842	356	193	149	103	31	34	38	2,428
WEE	0	0	0	0	74	41	21	0	0	0	0	0	136
SLE	0	0	0	0	16	27	11	2	0	0	0	0	56
ISOLATIONS	0	0	0	0	90	68	32	2	0	0	0	0	192
WEE/POOLS	0	0	0	0	8.9	11.5	10.9	.07	0	0	0	0	
MIN INF RATE	0	0	0	0	2/1000	3/1000	2/1000	1/8000	0	0	0	0	
SLE/POOLS	0	0	0	0	1.9	7.6	5.7	.13	0	0	0	0	
MIN INF RATE	0	0	0	0	1/2000	3/2000	1/1000	1/4000	0	0	0	0	

September it was 82 (55%) fifty, 21 more and 46 fewer. The overall rate for June, July and August is 76% of the pools that contained exactly fifty mosquitoes.

The lower lines in Table 1 give the percentage of WEE and SLE virus positive pools along with the calculated minimum infection rates. If the formulae of Chiang and Reeves (1962) are applied then the infection rates per thousand *C. tarsalis* mosquitoes are substantially higher, ranging from 8-12 per thousand for WEE and 2-4 for SLE.

The trap yield on the transect reflects the bimodal curve observed previously in Imperial Valley (Knudsen, Work and Vanis, to be published), while the grid shows a slow decline in mosquito population through the summer months. Figure 2 compares the percentage of infected pools from the transect by month with 10% positive in June, rising to 37% in July, and dropping to almost none in August. It appears that while there is a precipitous drop in *C. tarsalis* population within reach of the transect, there is a substantial increase in infection rates with a proportionate increase in SLE compared to WEE.

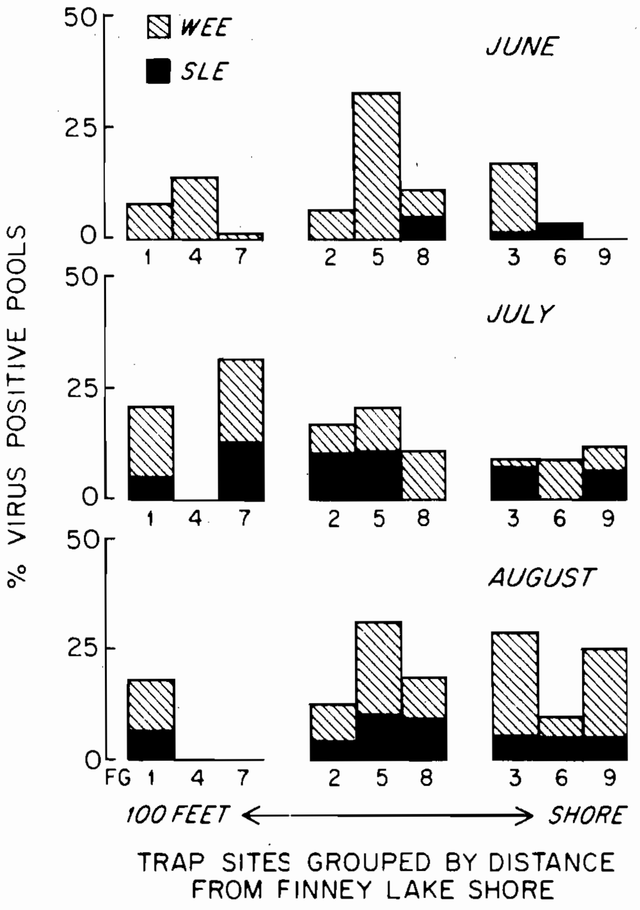

Figure 3.–Monthly shift of WEE and SLE virus yield from *Culex tarsalis* according to trap distance from Finney Lake.

Early seasonal appearance of WEE with a sudden peak in July followed by increase in SLE virus has been observed previously elsewhere. These data indicate that there is a source of infection with WEE and SLE viruses in June and July at Sheldon Pond with a virtual crash of the infected mosquito population by August.

On the other hand, the percentage of positive pools in the grid shows a steady increase with a proportionate rise in SLE positive pools. In the face of a decrease in total catch in July, August and September this could indicate more favorable conditions for survival of infected mosquitoes and/or continued prevalence of a virus source for infection of mosquitoes, possibly with more SLE viremic vertebrates than for WEE viruses. Disappearance of infected mosquitoes by October could reflect departure or immunization of vertebrate providers of virus within the sampled habitat.

An additional clue to such an hypothesis is given in Figure 3 which shows a shift in percentage positive pools from throughout the grid in June toward the shore of the lake in August. It should also be noted that there is substantial evidence of WEE in the grid in August compared to its virtual absence on the transect, as shown in Figure 2. Again, this may indicate that the site of mosquito infection is in the aqueous habitat of Finney Lake.

The trap success in total numbers of mosquitoes caught varied substantially according to site along the transect. When the percentage of virus positive pools is plotted in Figure 4, the yield of the transect light traps is relatively comparable with one remarkable exception.

Transect Trap 2 hangs over the Alamo River with a northern exposure toward Finney Lake. It is the only site which is fully screened by thick vegetation from a southern exposure toward Sheldon Pond. It is the only trap site on the transect which failed to collect a single pool of virus infected mosquitoes in June, July and August even though 23 pools consisting of 931 *C. tarsalis* mosquitoes from it were examined during that period.

On a dark night most light traps along the transect can be spotted from the bluff above the south shore of Sheldon Pond. This is the direction of scrub bushes and agricultural fields, a habitat in which cottontails, *Sylvalagus auduboni* and *Perignathus* rodents are in sufficient abundance to be routinely trapped. Sheldon Pond is a favorable habitat for coots, ducks, grebes, herons and shorebirds, as well as carp, bullfrogs and beaver.

This directs further inquiry to the south and east as a possible source for virus infected or virus infection of *C. tarsalis.*

CONCLUSIONS.–Quantitative data from monthly collections of *C. tarsalis* mosquitoes along a transect between Sheldon Pond and the Alamo River, and a grid between the shore of Finney Lake and a bluff demarcating an arid expanse from a tamarisk habitat, confirms that *C. tarsalis* mosquitoes are active throughout the year in the Finney-Ramer Wildlife Refuge.

In spite of increased numbers of mosquitoes collected in the winter months no isolation of WEE and SLE viruses were obtained other than in the hot months of June, July, August and September.

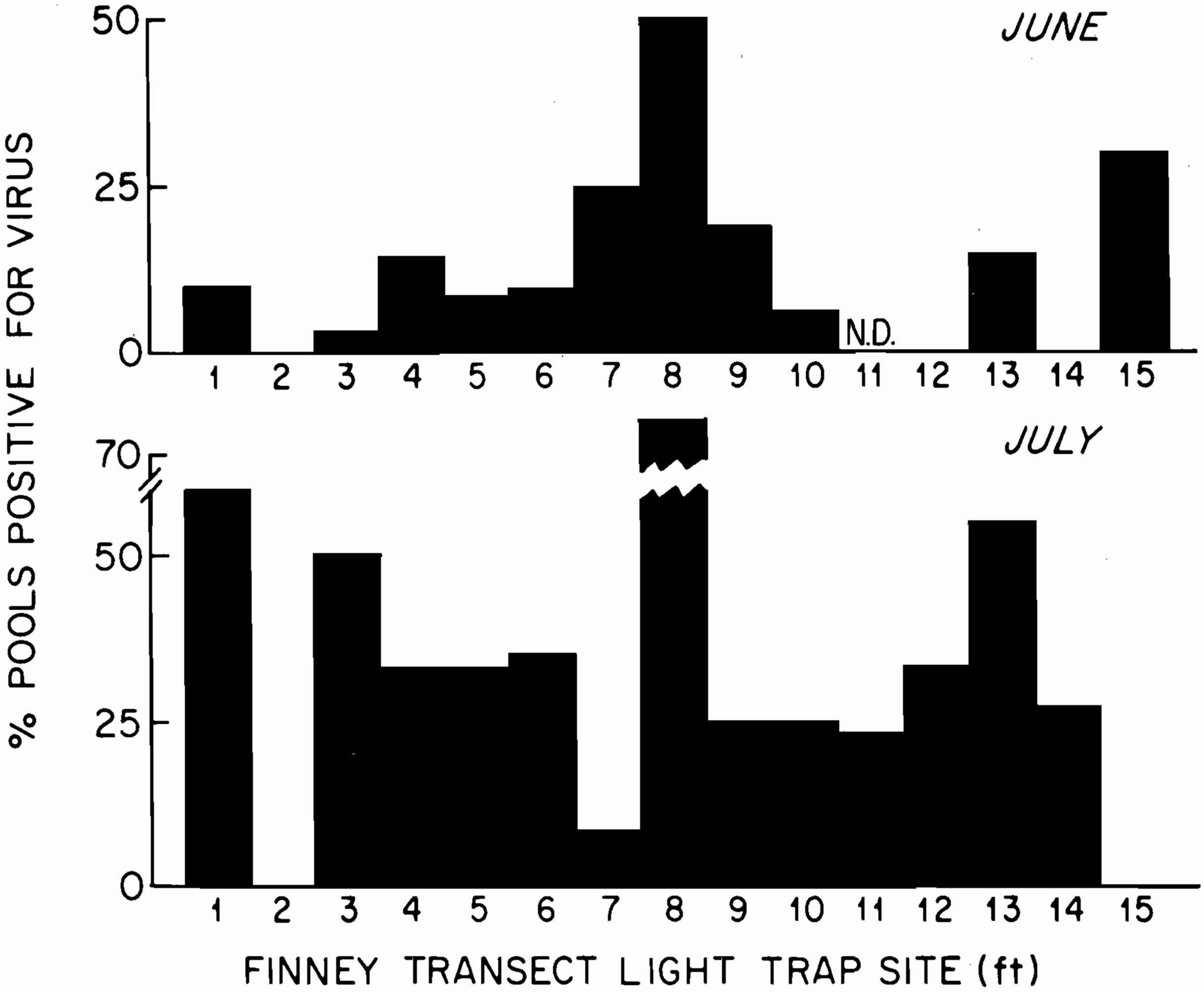

Figure 4.– June-July 1973 transmission period light trap site yield of WEE/SLE viruses from pools of *Culex tarsalis* collected on Finney transect.

Distinct differences between the two light trap patterns were observed not only in regard to trap night numbers but in percentage yield of *C. tarsalis* pools positive for virus. The more exposed transect collections yielded high infection rates for WEE in June and July with virtual disappearance of virus in August whereas infection rates in the grid continued to rise through August reflecting either longer survival of infected mosquitoes or continued presence of viremic vertebrate sources of virus for mosquitoes in the adjacent aqueous habitat of Finney Lake, or both. Detailed study of such vertebrates at Sheldon Pond early in the summer and in Finney Lake through September are indicated.

Also of importance are microclimate studies of temperatures and humidity in the two different collecting localities.

Not only have the data collected so far clearly established differences between trap success and virus positive mosquito pool yield between these two sites which are only half a mile apart; clues have appeared as to where to look for the sources of virus that are ingested by *C. tarsalis* mosquitoes.

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VECTOR COMPETENCE OF *CULEX TARSALIS* IN RELATION TO FUTURE ENCEPHALITIS CONTROL

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ABSTRACT

Vector competence studies conducted in California during 1972 and 1973 have demonstrated conclusively that a single mosquito species, *Culex tarsalis*, can vary greatly in its susceptibility to infection with western equine encephalomyelitis (WEE) virus. These variations in vector competence were observed when colonized and field strains of *C. tarsalis* were fed either on viremic hosts or on pledgets soaked with a mixture of WEE virus, defibrinated blood and sucrose. A seasonal variation in vector competence was found for *C. tarsalis* collected at monthly intervals during the summer of 1973 along Poso Creek in Kern County. Preliminary studies have suggested that the ability of infected mosquitoes to transmit WEE virus also might vary.

These results have made us revise our thinking on vector competence and to consider genetic as well as non-genetic variables that might affect transmission cycles of encephalitis virus. If susceptibility of *C. tarsalis* to infection with arboviruses is genetically controlled, then potentially the gene for viral susceptibility could be introduced into susceptible field populations of *C. tarsalis* along with chromosomal translocations. Such a dual introduction could provide a genetic basis for control of vector populations and encephalitis. These alternate mechanisms for control of insect vectors and the diseases they transmit need to be evaluated in view of the increased genetic resistence of the vectors to chemical insecticides.

AEDES MELANIMON AS A VECTOR OF WEE VIRUS IN CALIFORNIA

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ABSTRACT

Ecological studies on western equine encephalomyelitis (WEE) virus in the Sacramento Valley revealed that WEE virus was maintained during the summer by two separate but intrarelated transmission cycles: one involved *Culex tarsalis*, wild birds and jackrabbits and the other involved *Aedes melanimon* and jackrabbits. The existence of these transmission cycles was documented by WEE viral infection rates in field populations of *C. tarsalis* and *A. melanimon*, host feeding preferences of the 2 mosquito species and WEE viral antibody prevalences in wild birds and mammals. Experimental infection studies demonstrated that *A. melanimon* was a competent vector of WEE virus. This is the most conclusive evidence to date to indicate that *A. melanimon* might be an important vector of WEE virus in California.

VECTOR-HOST ASSOCIATIONS OF *AEDES TRIVITTATUS* AND THEIR IMPORTANCE TO THE NATURAL HISTORY OF TRIVITTATUS VIRUS IN IOWA

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ABSTRACT

The feeding patterns of *Aedes trivittatus* were studied to determine the possible vertebrate host(s) of Trivittatus virus in central Iowa. Antisera for precipitin tests were prepared in rabbits except for an anti-cottontail serum which was prepared in chickens. Of the 590 blood meals tested, 409 (69.3%) were of rabbit origin

Of the 77 mammal sera collected from the same area, 21 had neutralizing antibodies against Trivittatus virus, including 10 of 22 sera from eastern cottontail rabbits. Results from serological and host preference studies support a hypothesis that the eastern cottontail rabbit (*Sylvilagus floridanus*) is important as a vertebrate host of *A. trivittatus* and is probably important in the maintenance of Trivittatus virus in central Iowa.

Sentinel rabbits placed in the study area in March 1973, converted to seropositive for Trivittatus virus by June 27th. Trivittatus virus was isolated from a blood clot collected from one of the rabbits on June 20th, a date nearly coinciding with the first adult population peak of *A. trivittatus*. The association between early seasonal virus activity and the first brood of *A. trivittatus* suggests the possibility of virus in *A. trivittatus*.

ECOLOGICAL AND CONTROL STUDIES ON JAPANESE ENCEPHALITIS VECTORS IN TAIWAN[1]

Carl J. Mitchell[2]

ABSTRACT

Intensive studies were made by the World Health Organization, Japanese Encephalitis Vector Research Unit, during 1970-1972 on the ecology and control of demonstrated and potential mosquito vectors of Japanese encephalitis in Taiwan. *Culex tritaeniorhynchus*, *C. annulus*, *C. fuscocephalus*, and *C. pipiens fatigans* were the principal species studied. Data relating to one or more of these species were collected and analyzed with reference to seasonal abundance (Mitchell & Chen, 1972a), host feeding patterns (Mtichell, Chen & Boreham, 1972), insecticide susceptibility (Mitchell & Chen, 1972b), control by chemicals (Mitchell, Chen & Okuno, 1972), susceptibility to infection by a parasitic nematode (Mitchell, Chen & Chapman, 1972), seasonal infection rates with JE virus (Okuno, Mitchell, Chen, Wang & Liu, 1973), and vector potential as determined by experimental transmission studies (Okuno, Mitchell, Chen, Hsu & Ryu, 1973).

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[1] These studies were carried out under the auspices of Vector Biology and Control, World Health Organization, Geneva.

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PROGRESS REPORT ON THE HEMOGLOBIN CRYSTALLIZATION TECHNIQUE TO STUDY MOSQUITO BLOOD MEAL IDENTIFICATION

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ABSTRACT

The hemoglobin crystallization technique for identifying host blood meals successfully yielded crystals from some species of rodents, rabbits, carnivores and ruminants.

Modifications from the previously described method were reported. Although phylogenetically distinct groups generally depict a different crystal morphology, a common form is often found within each group.

The catalogue of crystal types is continuously being enlarged. A concentrated effort is being made on primate and bird bloods because of their involvement in the epidemiology of arboviruses and malaria. Crystals from these two groups are difficult to obtain, but generally form if the preparations are kept at 4°C and are left up to four days.

Crystals obtained from the known host blood meals (usually of *Aedes aegypti*) were found to be identical to the whole blood sample. A series of slides taken after various periods of digestion from *A. aegypti* fed on guinea pig indicated that the crystals retain their characteristic form, but become smaller with digestion time. They were identifiable as guinea pig crystals up to 48 hours after ingestion.

Culex pipiens, *Anopheles freeborni* and *Aedes sierrensis* have all given positive results using guinea pig as host. In a preliminary test, however, positive results were obtained with a pheasant host for *C. pipiens* but not for *A. aegypti.*

Our technique, though still in an early phase of development, presents an inexpensive and useful supportive method for blood meal identification.

EAST-WEST DISTRIBUTION OF TREE-HOLE MOSQUITOES IN NEBRASKA

Steele R. Lunt[1] and George E. Peters[2]

ABSTRACT

Extensive field collecting was done in an east-west direction from eastern Iowa across Nebraska into southeastern Wyoming and northeastern Colorado. 434 separate samples were taken from 199 different tree holes resulting in a total of 2,976 larvae, pupae, and adults. Five species were collected. *Aedes hendersoni* Cockerell was found along the entire collecting route. *Aedes triseriatus* (Say) was found only in Iowa and the eastern half of Nebraska. *Orthopodomyia signifera* (Coquillett) was found at 10 widely separated sites in Iowa and the eastern two-thirds of Nebraska. *Orthopodomyia alba* Baker was collected from one site in Iowa (new state record) and three sites in eastern Nebraska (new state record). *Anopheles barberi* Coquillett was collected from seven sites in Iowa and the eastern two-thirds of Nebraska.

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SOME FACTORS INFLUENCING PRODUCTION OF PEST MOSQUITOES IN TUCSON, ARIZONA[1]

John L. McDonald[2] and Gary S. Olton[3]

The mosquito problems of Arizona are scattered, sporadic, and discontinuous in almost every sense. Some people have lived in Arizona for 35 or 40 years without having a single mosquito bite. Many of the mosquito problems in Tucson are essentially confined to: (1) areas near washes and ditches which hold water after rains; (2) cattle feeding areas or horse stables where watering troughs are rarely emptied and mosquito breeding is nearly continuous; (3) irrigation areas where poorly maintained ditches or side pools form mosquito breeding sites; and (4) urban and rural areas where overwatering causes standing water and subsequent mosquito breeding sites.

This investigation of the mosquito fauna of the Tucson area was made to provide data concerning the ecological conditions which promote or prevent mosquito production in this rapidly expanding urban area. The primary considerations were ambient temperature, rainfall, and subsequent mosquito complaint calls received by the vector control office of the Pima County Health Department in Tucson.

Figures 1-3 show that mosquito complaints received by the Pima County Health Department were most abundant in the months of June through October. In 1971, the number of complaints closely coincided with the incidence of summer rains after maximum temperatures recorded for that year were reached (Figure 1). In 1972, the numbers of mosquito complaints were followed with the occurrence of unusually long and heavy summer rains, but not the fall rains. Probably this was in part due to high temperatures during the July rains and cooler temperatures in October when the later heavy rains occurred (Figure 2). In 1973, the number of complaints was greatest during the summer rains. The unusually heavy rains of February and March coincided with cooler temperatures, failing to produce significant mosquito complaints, (Figure 3).

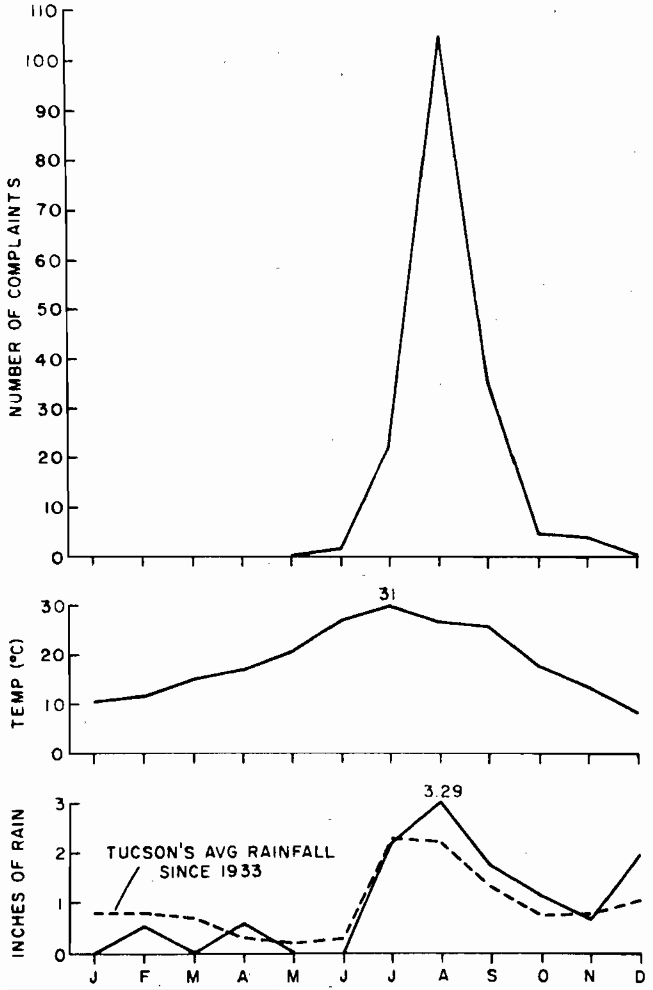

Figure 1.—Pima County Health Department mosquito complaints for 1971 versus temperature and rainfall of that year.

These data indicate that mosquito complaints and production of mosquitoes in Tucson occur mainly when periods of high summer temperatures and heavy rainfall coincide.

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[1] This research was supported in part by the Office of Naval Research, Microbiology Program, Naval Biology Project, under contract No. N00014-74-C-0017, NR 306-097.

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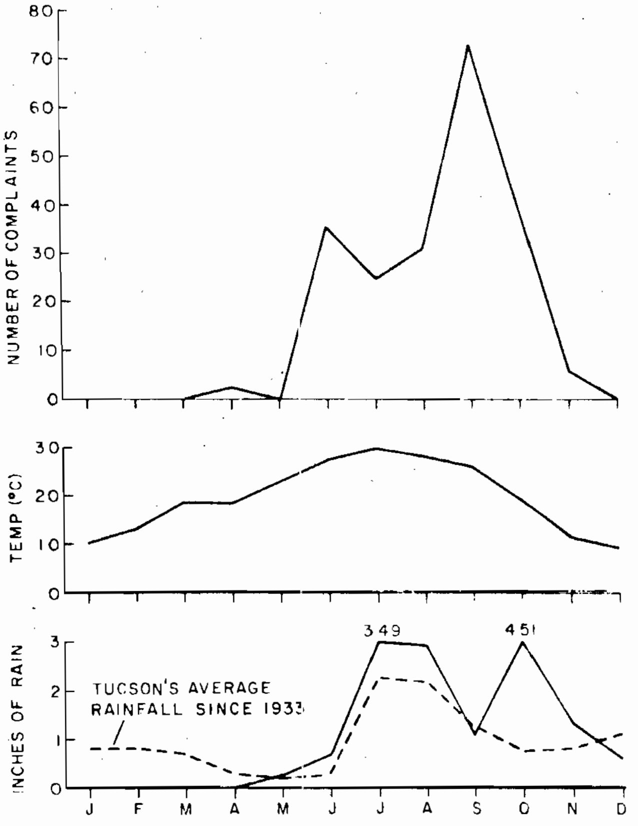

Figure 2.–Pima County Health Department mosquito complaints for 1972 versus temperature and rainfall of that year.

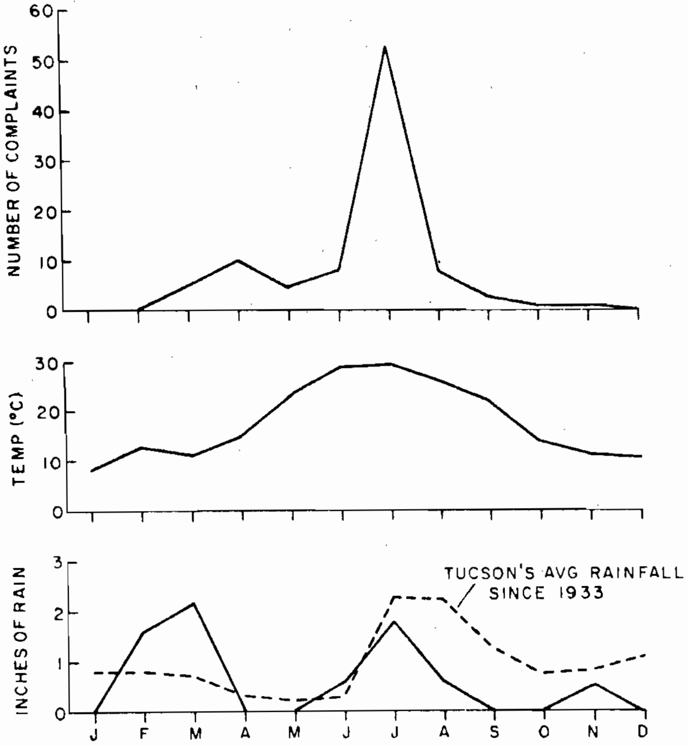

Figure 3.–Pima County Health Department mosquito complaints for 1973 versus temperature and rainfall of that year.

ADDITIONAL RECORDS OF CULICINE AND CHAOBORINE MOSQUITOES FROM THE MOUNTAINS OF ARIZONA AND NEW MEXICO

Theodore A. Wolff[1], Lewis T. Nielsen[1] and Jay H. Linam[2]

Nielsen, Wolff and Linam (1973) reported new distributional records of snowpool *Aedes* mosquitoes from Arizona and New Mexico and cited pertinant literature. Continued investigations during 1973 have revealed three additional new state records of culicine mosquitoes, *Aedes increpitus* Dyar in Arizona, and *A. cinereus* Meigen and *Culiseta impatiens* (Walker) in New Mexico, as well as additional distributional data on other species. In addition the chaoborines, *Chaoborus americanus* (Johannsen), *Eucorethra underwoodi* Underwood and *Mochlonyx sp.* Loew are reported for the first time in New Mexico.

Aedes increpitus was collected in Apache County, Arizona, VI-6-73, 8,500 ft., 1 mile north of Greer along a tributary of the Little Colorado River in a partially shaded overflow pool surrounded by willows. The larvae were associated with *A. implicatus* and *A. pullatus.*

Previously we reported *A. cinereus* from Conejos County, Colorado, just north of the New Mexico border and stated that *A. cinereus* undoubtedly occurred in New Mexico (Nielsen et al. 1973). The present report of this species from Rio Arriba County, Chama, New Mexico, VI-11-1973, 7,860 ft., confirms the presence of *A. cinereus* in New Mexico. Mature larvae and pupae were collected in a partially shaded pool containing heavy growths of sulphur bacteria. Associated with *A. cinereus* were larvae of *Culiseta incidens, C. inornata, A. implicatus,* and *A. pullatus.*

Culiseta impatiens females were collected in Rio Arriba County, New Mexico, VI-9-1973, 9,250 ft., biting in the forest in the vicinity of Trout Lakes. Associated biting adults were *C. incidens, C. inornata,* and *A. cataphylla.*

Aedes fitchii which had been previously known in Arizona only from the North Rim of the Grand Canyon (Richards, Nielsen and Rees 1956) was found to have a much wider distribution in that state. Additional larval collections of *A. fitchii* were found in several pools in southeastern Arizona in Apache County, 10-15 miles East of McNary on Highway 260, VI-6-1973, 8,800-9,050 ft. All were semi-permanent pools in mountain meadows.

An extensive survey of the White Mountain area in Apache County revealed numerous sites containing larvae of snowpool *Aedes*. In Apache County, at Highway 473 and the North Fork of the White River, V-7-1973, 7,850 ft., in pools fed by melting snow and stream overflow we found *A. cataphylla, A. implicatus* and *A. pullatus.* Along Highway 273 which runs southeast through the White Mountains to Alpine, we found numerous snowpools containing *A. cataphylla* and *A. pullatus,* V-7-1973, 8,500 - 9,500 ft. On the same date we also found several containing *A. implicatus* and *A. pullatus* in the Hannagan Recreational area in Greenlee County, 9,000-9,500 ft.

In May of 1973, we investigated the following mountainous areas at elevations above 7,500 ft.: southwest of Flagstaff through Coconino and Navajo Counties; the Pinalino Mountains in Graham County; the Santa Catalina Mountains near Tucson in Pima County; the Chiricahua Mountains in Cochise County. Suitable habitat for snowpool *Aedes* in these regions was rare and all areas were negative.

The San Francisco Mountains near Flagstaff contain the highest elevation in Arizona, 12,670 ft. This area is of volcanic origin, the surface is extremely porous and there are no permanent streams. We made a survey of this area in May, 1970, and found no suitable habitat for snowpool *Aedes* production.

During June 3-5, 1973, we collected the Kaibab Plateau from Jacob Lake to the North Rim of the Grand Canyon. The area contains numerous snowpools above 8,000 ft. Large larval populations of *A. cataphylla* and *A. fitchii* were found throughout the area. *C. inornata* larvae also were collected.

We have now surveyed virtually all of the mountainous regions of Arizona with elevations likely to support snowpool *Aedes.* It appears that the distribution of these species is restricted to *A. cataphylla* and *A. fitchii* on the North Rim of the Grand Canyon portions of the Kaibab Plateau, and *A. cataphylla, A. fitchii, A. implicatus, A. increpitus,* and *A. pullatus* from the White Mountains and Mogollon Rim in the southwestern part of the state in Apache and Greenlee Counties.

Nielsen et al. (1973) listed 10 species of snowpool *Aedes* from the mountains of northern New Mexico. During May and June, 1973, we made additional collections in other mountainous areas throughout the state and found larvae which extend the ranges of several of the species we had previously reported. In Santa Fe County in the Pecos Wilderness at Steward Lake, VI-22-1973, 11,000 ft., we found *A. cataphylla, A. hexodontus* and *A. pullatus.* In the Mogollon Mountains of southwestern New Mexico in Catron County in the Gila Wilderness area at Ben Lilly Forest Camp, V-11-1973, 8,100 ft., we found snowpools along Willow Creek containing large numbers of *A. implicatus* associated with smaller numbers of *A. cataphylla.* Both the Pecos and Gila Wilderness areas are extensive regions with large areas containing snow mosquito habitat. It is likely that other *Aedes* species will be found there.

We also surveyed the Sierra Blanca and Sacramento Mountains in southcentral New Mexico in Lincoln and Otero Counties, with negative results. Both areas have elevations above 8,000 ft., but the regions are restricted in size, drainage is rapid and suitable snowpools do not appear to be present.

Larvae of chaoborine mosquitoes also were collected in the mountains of northern New Mexico during the course of our surveys. *Eucorethra underwoodi* Underwood was taken

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in Rio Arriba County, Trout Lakes area, VI-9-1973, 9,250 ft., in shaded forest pools in association with *Aedes*, including *A. cataphylla*, *A. communis*, *A. implicatus* and *A. pullatus*.

Larvae belonging to the genus *Mochlonyx* Loew were found in the same pools with *E. underwoodi*. None were reared to adults. The immature stages of *Mochlonyx* are not separable to species at the present time. However, of the three known Nearctic species of *Mochlonyx*, the wide distribution of *Mochlonyx velutinus* (Ruthe) in North America with Colorado and Utah records (Cook 1956), suggests that this is likely the identity of the species collected in New Mexico.

Larvae and pupae of *Chaoborus americanus* (Johannsen), from which adults were reared, were collected at Trout Lakes VI-9-1973, 9,300 ft., from a semi-permanent pool which contained *A. excrucians*. Larvae of *C. americanus* also were taken in the Trout Lakes area on VIII-28-1973, 8,100 and 9,200 ft. At the lower elevation the larvae were associated with those of *Culex tarsalis* and *Culiseta inornata*, and at the higher elevation with those of *C. inornata*. Cook (1956) in his revision of the Nearctic Chaoborinae reported *C. americanus* as ranging across North America from the Atlantic Coast to the Uinta Mountains of Utah, with New Jersey and Southern Illinois as the southern limits. The New Mexico record represents a considerable southern extension of the known range of this species.

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THE INFLUENCE OF SLOPE AND FREQUENCY OF FLOODING OF A BREEDING DEPRESSION ON MOSQUITO POPULATIONS IN SALT MARSHES

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ABSTRACT

During the years 1970 to 1972, a study was conducted in Carteret County, North Carolina to determine the effects of mosquito control ditching on populations of *Aedes* and *Anopheles* mosquitoes in salt marshes dominated by *Juncus roemerianus.*

While collecting the data necessary for this evaluation, it was observed that in sites with similar flooding frequencies, the proportion of *Aedes* larvae in mosquito populations was higher in the more steeply sloped depressions. These observations initiated a study to determine the specific influence of slope on mosquito populations in sites with similar flooding frequencies.

All data on slope index, flooding frequency and larval-pupal numbers for each selected breeding site were analyzed by multiple regression.

According to the analysis, the combined influence of the slope, the reciprocal of flooding frequency, and the ratio of flooding frequency to slope on the proportion of *Aedes* and *Anopheles* populations in breeding depressions seems to be quite significant (R^2=.73 at $p > .0001$).

It was found that the highest proportions for *Aedes* at any given flooding frequency ranging from 10 to 35 floodings per month occurred in sites with slope index values falling between 0.25 and 0.45. The proportions of *Aedes* collected in samples increased rapidly as the slope increased from 0.05 to 0.15 and decreased gradually beyond 0.45.

The significance of the relationships between these independent and dependent variables as revealed by the statistical analysis may prove useful in locating potential *Aedes* breeding sites in large sections of *Juncus* salt marsh.

INTRODUCTION.—A large percentage of the approximately 300,000 acres of coastal wetlands in North Carolina consists of irregularly flooded salt marsh dominated by the plant species *Juncus roemerianus* Scheele (black needle rush). Six species of mosquitoes are found in these marshes. The most important of these are *Aedes sollicitans* (Walker), *A. taeniorhynchus* (Weidemann), and at times, *Anopheles bradleyi* King.

During the years 1970-72, a study was conducted in Carteret County, North Carolina, to determine the effects of mosquito control ditching on populations of mosquitoes in *Juncus* salt marsh. During the study, specific objectives were carried out. These included the determination of the temporal and spatial distribution of each species breeding in the marsh and correlating to each breeding site such factors as frequency of flooding, plant cover, and the distance from maximum high tide level.

While collecting the above data, it was observed that in sites where the flooding frequency was similar, proportions of *Aedes* larvae were higher in those depressions whose sides were more steeply sloped. It has been known generally that sites with lower flooding frequencies usually have a greater proportion of *Aedes* in their mosquito populations than those sites with higher flooding frequencies (Buttrick 1913, Connell 1939, and Provost 1968). The influence of depression slope, however, on the proportions of *Aedes* and *Anopheles* in sites with similar flooding frequencies has not been extensively studied. Some studies by Travis and Bradley in 1943, showed that the density of *Aedes* mosquito eggs of salt marshes in Florida was somewhat higher on irregularly contoured marshes than on flat ones. Most of these eggs were found on the tops and sides of hummocks or potholes within the marsh.

The purpose of this paper is to present the analysis of data collected on the physical and flooding characteristics of breeding sites in salt marshes in an attempt to determine the importance of their relationships on mosquito populations.

MATERIALS AND METHODS.—Data on slope and frequency of flooding were obtained from breeding sites located in Carteret County, N. C. (See Figure 1.)

Estimation of the slope index was determined by dividing the value for the deepest point at each site by the average distance from this point to the edge of the depression (See Figure 2). The higher the slope index calculated, the steeper the slope.

The flooding frequency on the marsh was determined by a series of Leupold Stevens Type F tide gauge recorders. These were set up, one each, at North River, Davis, Kings Point and Newport River. Data on flooding due to rain was obtained from Climatological Data published by the U.S. Department of Commerce. Weather Stations at Morehead City and Cedar Island were used as representatives for the study areas.

All sites were visited at least three times a week. At most visits larval-pupal numbers were determined by means of the sampling procedure reported by Belkin (1954). Representative samples of larvae and pupae were processed for identification.

All data were analyzed by multiple regression using a standard statistical program developed by Barr and Goodnight at N. C. State University. This regression procedure was used not only to determine how important the independent variables (slope index and flooding frequency) are in relation to salt marsh mosquito populations, but also to determine whether they have any significant value for prediction.

RESULTS.—Table 1 gives the results of the statistical analysis. According to this analysis, the combined influence of the slope index, the reciprocal of flooding frequency, and the ratio of the flooding frequency to slope index on the proportions of *Aedes* and *Anopheles* seems to be quite significant (R^2= .73 at $p > .0001$).

The graph in Figure 3 illustrates how these factors influenced the species composition of breeding sites. It shows

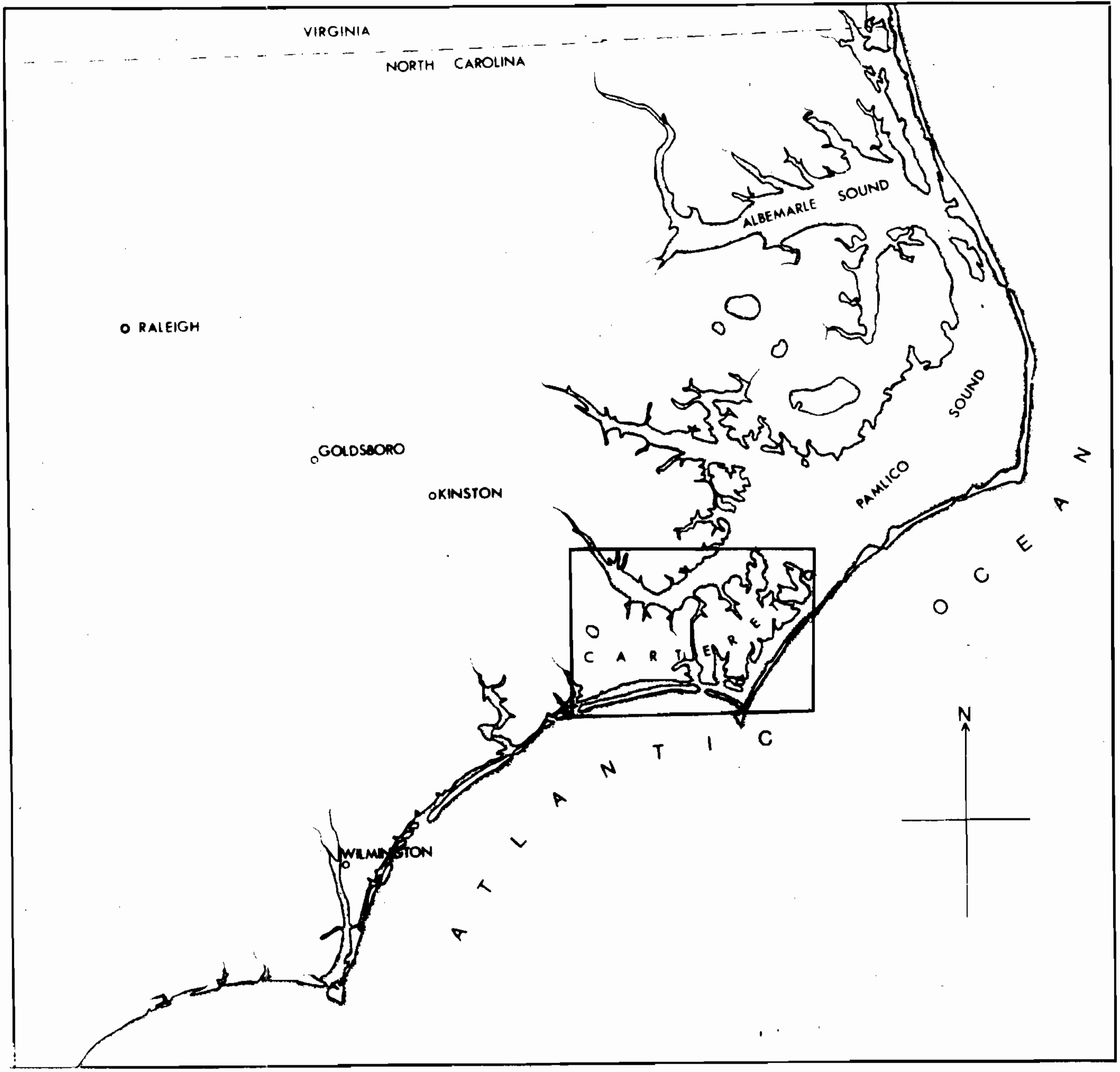

Figure 1. - Area of North Carolina where study was conducted.

the change in the proportion of *Aedes* found in a sample with increase in slope index at selected values of flooding frequency. The highest proportional values for *Aedes* at any given flooding frequency occurred in sites with slope indices ranging between 0.25 and 0.45. The very highest values occurred at a flooding frequency of 5/month, and the lowest were at 35 floodings per month. In sites where the floodings ranged from 10 - 35 per month, the proportion of *Aedes* collected in samples increased rapidly as the slope increased from 0.05 to 0.15 and decreased gradually beyond 0.45. Change in slope had little or no effect on the proportions of *Aedes* in sites flooded five or less times a month.

DISCUSSION.- The results of the statistical analysis revealed certain relationships between the physical characteristics of a depression, the flooding frequency, and the mosquito populations in salt marshes. By determining the slope index and the flooding frequency of a breeding depression, a rough prediction, using the information in Table 2, could be made about the potential proportions of *Aedes* and *Anopheles* larvae that could be collected. For example, a breeding depression with a slope index of 0.65 and a flooding frequency of ten would yield a mosquito population consisting of approximately 81% *Aedes* and 19% *Anopheles*. For values of both independent variables falling between

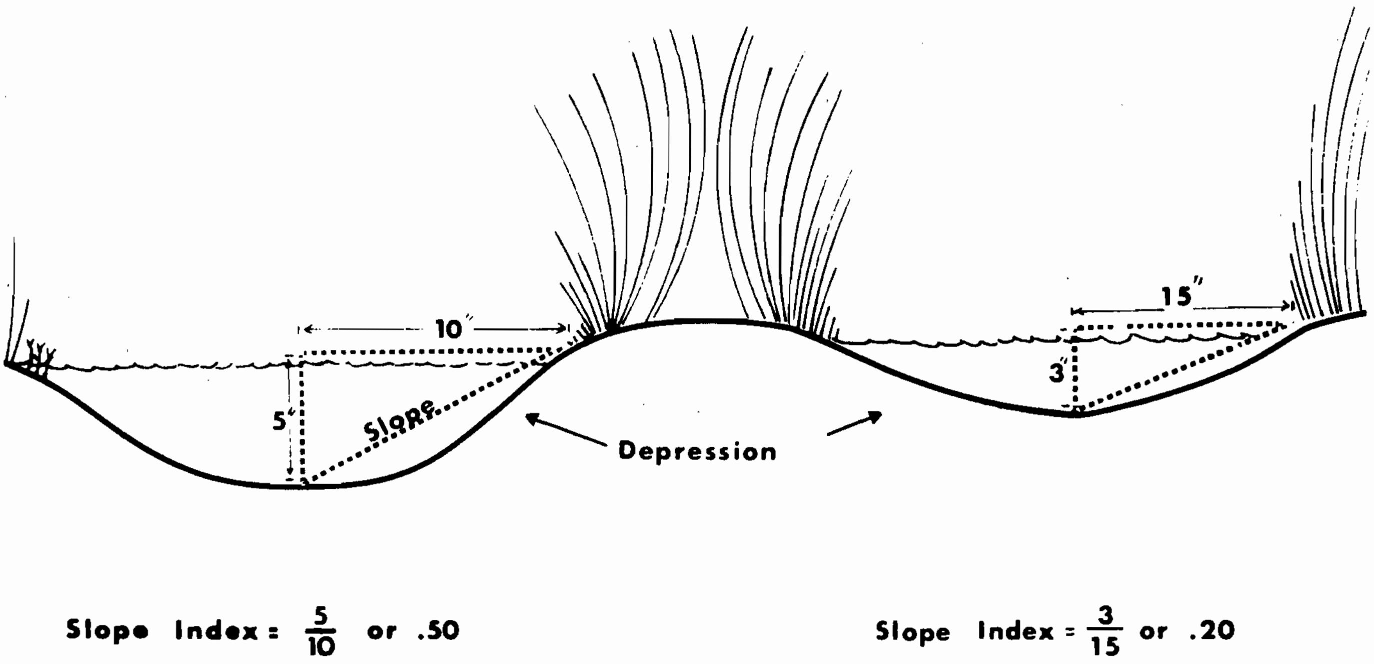

Figure 2.—Determination of slope index.

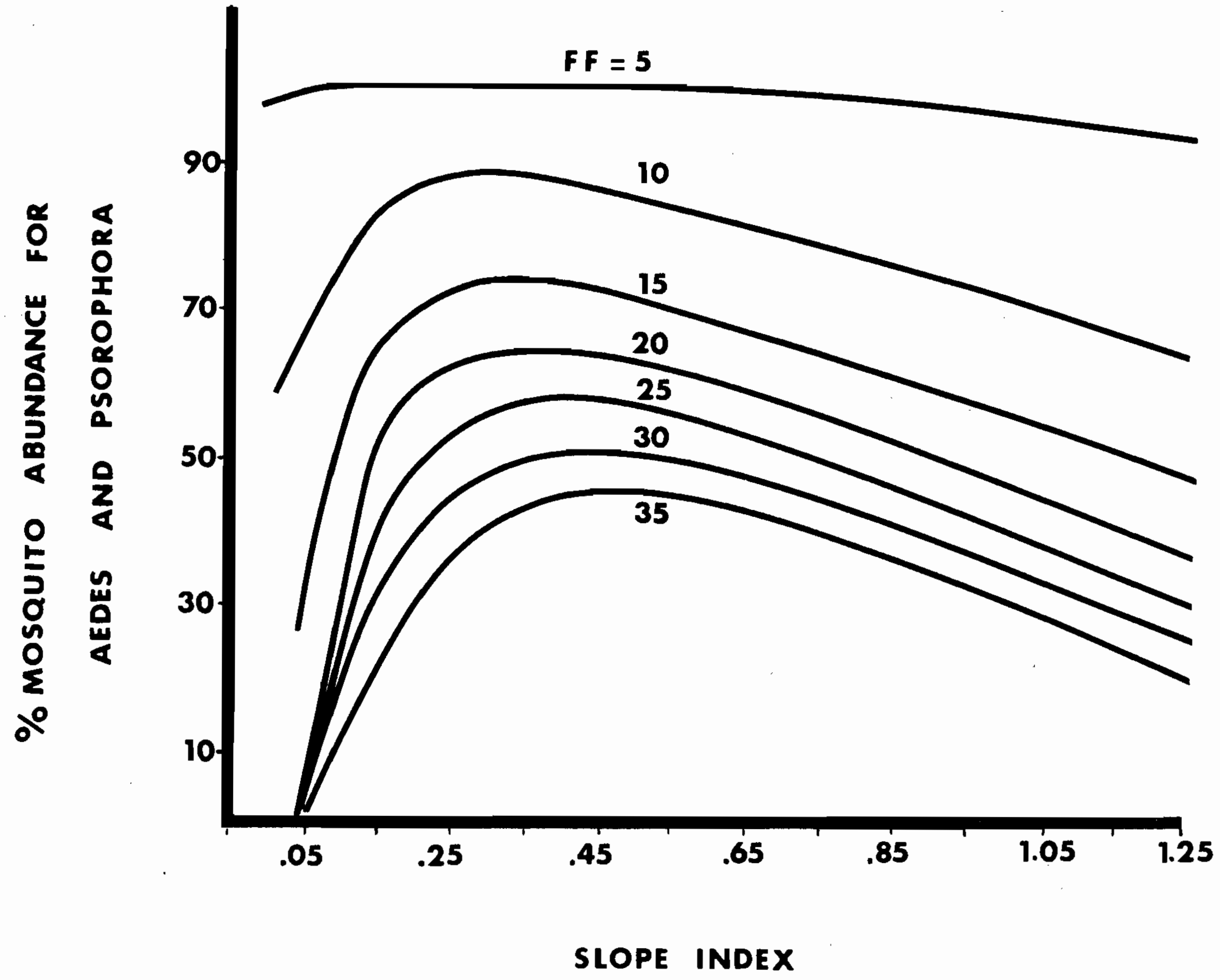

Figure 3.—Response to slope index at 7 frequency of flooding values.

Table 1.–Significant parameters and results of statistical analysis.

Parameter	Regression Coefficient	F – Test
Slope Index (S)	−0.4940	3.66171°
$\frac{1}{\text{Frequency of Flooding (FF1)}}$	5.2061	15.97301^{**}
$\frac{\text{Freq. of Flooding}}{\text{Slope Index}}$	−0.0034	21.16088^{**}
$\left(\frac{FF}{S}\right)^2$	0.0239×10^{-4}	15.79464^{**}
$R^2 = 0.72889^{***}$		

*** $P > 0.0001$
** $P > 0.01$
* $P > 0.05$
o $P > 0.10$

the values listed in Table 2, the formula below the table can be used to obtain predicted proportions.

It should be emphasized that the formula only gives a value which represents that proportion of the total number of larvae collected from a site during the entire breeding season that would be a particular species. It does not give values which represent actual numbers that might be collected.

Since the independent variables used in the regression formula account for about 73% of the variation in the data, further study should continue to determine the other factors which would account for the remaining 27%. This additional information would help to improve the formula and the accuracy of the predictions. It could be improved to the point where, in time, it might become an acceptable procedure for determining what sections of a salt marsh contain the highest proportions of *Aedes* mosquitoes, without having to conduct extensive and long term larval surveys.

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Table 2.—Determination of expected breeding potential for *Aedes* spp. (%).

	Frequency of Flooding (FF)						
Slope Index (S)	5	10	15	20	25	30	35
.05	100	65	30	5	0	0	0
.15	100	85	68	53	40	30	20
.25	100	87	72	61	52	44	37
.35	100	86	72	63	55	49	43
.45	100	85	71	62	55	50	45
.55	100	83	70	60	54	49	44
.65	100	81	67	58	52	47	42
.75	100	78	64	55	49	44	40
.85	100	75	61	52	46	41	37
.95	97	72	57	48	42	37	33
1.05	96	68	53	44	38	34	30
1.15	95	65	50	40	34	30	26
1.25	93	61	45	36	30	26	22

% Expected Breeding Potential

The above potentials determined by following formula:

$$\underline{\text{Aedes}}\ \% = \left[\text{SIN} \left[\left(.78284 + (-.494)S + \left(\frac{5.206}{FF}\right) + (-.0034)\left(\frac{FF}{S}\right) + .024(.0001)\left(\frac{FF}{S}\right)^2 \right) 57.29 \right] \right] 100$$

THE USE OF MINNOW TRAPS TO MONITOR POPULATION TRENDS OF *GAMBUSIA AFFINIS* IN RICE FIELDS

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There is an increasing use of the mosquito fish, *Gambusia affinis affinis* Baird and Girard for the biological control of mosquitoes in California rice fields. California workers have evaluated the stocking rates of *Gambusia* and subsequent mosquito populations in rice fields and have concluded that an effective level of mosquito control could be obtained if fish were planted at a rate of ½ pounds of fish to the acre (330 to 400 fish/acre) (Hoy and Reed 1971, Hoy et al. 1971, Hoy et al. 1972). Little information was obtained in these studies on the relative size of the *Gambusia* population as the season progressed. It was felt that a study of this factor would lead to a better understanding of the biological control effectiveness of the fish population. This paper covers a study made during the 1972 season to test a trapping method of evaluating rice field populations of *Gambusia*.

MATERIAL AND METHODS.—Fish were seined from local drains for use in this study. Due to the limited availability of *Gambusia*, the number of rice fields for this study was limited to 6 small fields. Fish were sized by using a 3/8 inch mesh nylon netting during the seining process. Fish were transported from the ditches directly to the fields in an aerated tank mounted on a pickup. Small aquarium nets were used in counting fish as they were distributed in the field. The aquarium nets were calibrated by averaging the number of fish contained in three full scoops. Over 85% of the fish used were gravid females.

Fish were stocked at a rate of about 100 fish[1] per acre and dumped in alternate rice paddies at the paddy corner opposite the paddy water inlet box and on the downwind side of the field whenever possible. No fish were stocked in the first and last paddies (field inlet and outlet paddies). Figure 1 is a composite illustration of a typical study field.

Eight mesh wire minnow traps were used for capturing and counting fish in the rice fields. Traps were placed in the rice fields 24 hours prior to fish introduction. One trap was placed in each paddy and was located about 10 feet into the paddy opposite the outlet of the paddy water control box (Figure 1). All paddies in the field were sampled (trapped). Traps were checked for fish immediately prior to fish introduction, fish counted and recorded and the trap returned to the site. Traps were then examined for fish at 24-hour intervals for the next 3 to 5 days.

Two fields were stocked May 9, one on May 10, and 3 on May 24. Routine sampling for fish was started June 8 on the first fields stocked when fish had been in the rice field 4 weeks. On June 15 the fields stocked on May 24 were trapped, the fish had been in these rice fields 3 weeks at that time. This weekly schedule was followed through August, thus making 6 2-week trapping periods. Minnow traps were placed in the field for 24 hours and removed during each of the 6 periods. The fish were counted and returned to the field. Water temperatures were taken at the field inlet and outlet boxes. Air temperatures were recorded at the beginning and end of the trap sampling period.

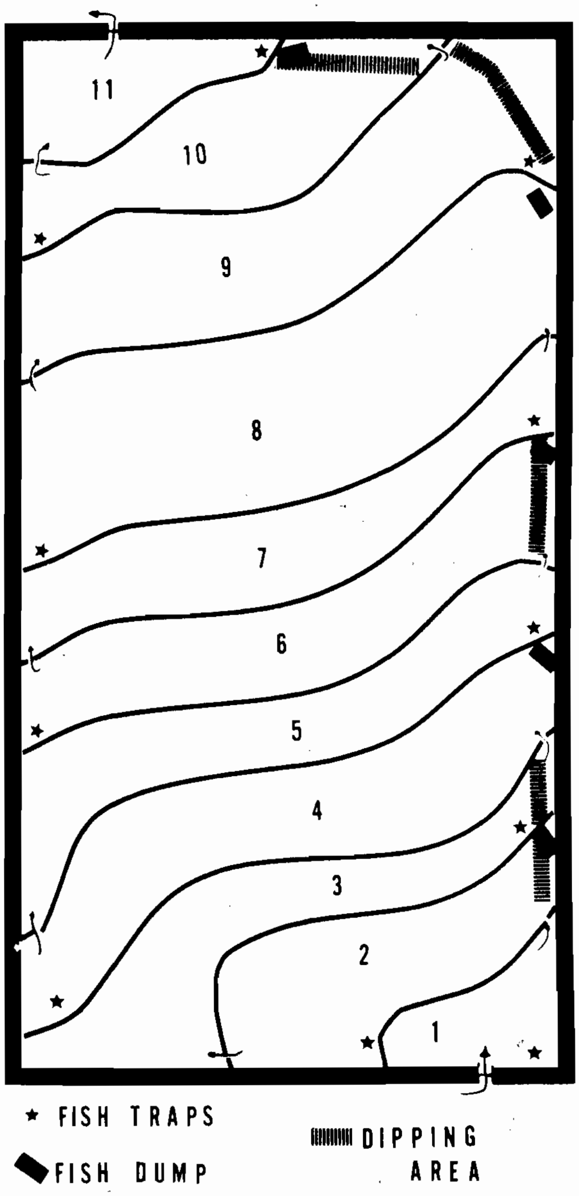

Figure 1.—Typical rice study field.

Ten dip samples were taken for mosquito larvae in 5 preselected locations in transect, across the paddy at 2-

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[1] Operationally it has been found that 100 fish to the acre will become effective in most cases when supplemented with compatible integrated chemical control.

week intervals to correspond with the fish sampling. This information is not reported in this paper.

Six experimental control rice fields were selected which were approximately the same age, physical proximity and size as the study fields. These fields were trapped for fish only once during the study. The period selected was mid July (Period IV).

RESULTS AND DISCUSSION.–The season-long results as shown in Figure 2 were not too surprising because a large increase in the population of fish is expected during this period. What was most surprising was the uniformity of this increase in trap captures of mature fish through the first 8 weeks of the study (Figure 2). Assuming fish mature in 4 to 6 weeks in the field during the early season, and can reproduce young from 4 to 10 weeks, (Krumholz 1948) it would be expected that the counts of gravid females would remain virtually unchanged in the traps through the first 4 to 6 weeks.

Twenty-four hour checks of the traps prior to the introduction of fish netted no *Gambusia*. Two perch were captured in one trap in field 24-9. Twenty-four hours after *Gambusia* were introduced into the rice fields, traps collected an average of 0.27 mature females per trap. The capture range was 0-.6 fish per trap with almost 90% of the captures in the stocked paddies, thus indicating movement of fish the length of the paddy (movement downstream) (See Figure 1). Three days later 59% of captures were made in the stocked paddies indicating further movement downstream – and some possible movement upstream. The number trapped at the 72-hour (3 day) period following the fish stocking in alternate paddies ranged from .07 to .86 fish per trap with an average of .41 fish per trap. At this time, 15 of the 63 traps placed in these fields (29-1 is excluded from this evaluation) contained mature females (7 of these traps were located in non-stocked paddies).

Table 1 summarizes the collections made in minnow traps throughout the season. Fields 21-1, 22-3 and 24-9 were subject to great fluctuations of water level. In fact, during mid June the first two fields were almost drained for application of the weedicide propanil. Predator fish may have had a decided effect on population growth, and – if not that – the capture of *Gambusia* in some fields. For example, in field 14-9 we recorded relatively high catches of perch and small bass in traps during the 5-day period following *Gambusia* introduction. No record of the catch of these fish was kept through the season.

The changing environment in rice fields and the behavior of *Gambusia* are believed to be primarily responsible for the progressive increase in catches of matures through period II of the study. Natural stocking via field inlet was considered to be miminal. It is our opinion that natural stocking would be offset by the potentially much larger fish losses via the field outlet.

Trapping at the 72-hour period was done in rice fields that were void of emergent vegetation. Period I trapping (3 to 4 weeks later) represents samples taken in considerable emergent rice growth and by period II (5 to 6 weeks) rice and many weeds were well established in the field. Normally fields are being treated for weeds at this rice development stage. These changes, we are certain, have a decided effect upon trapping results.

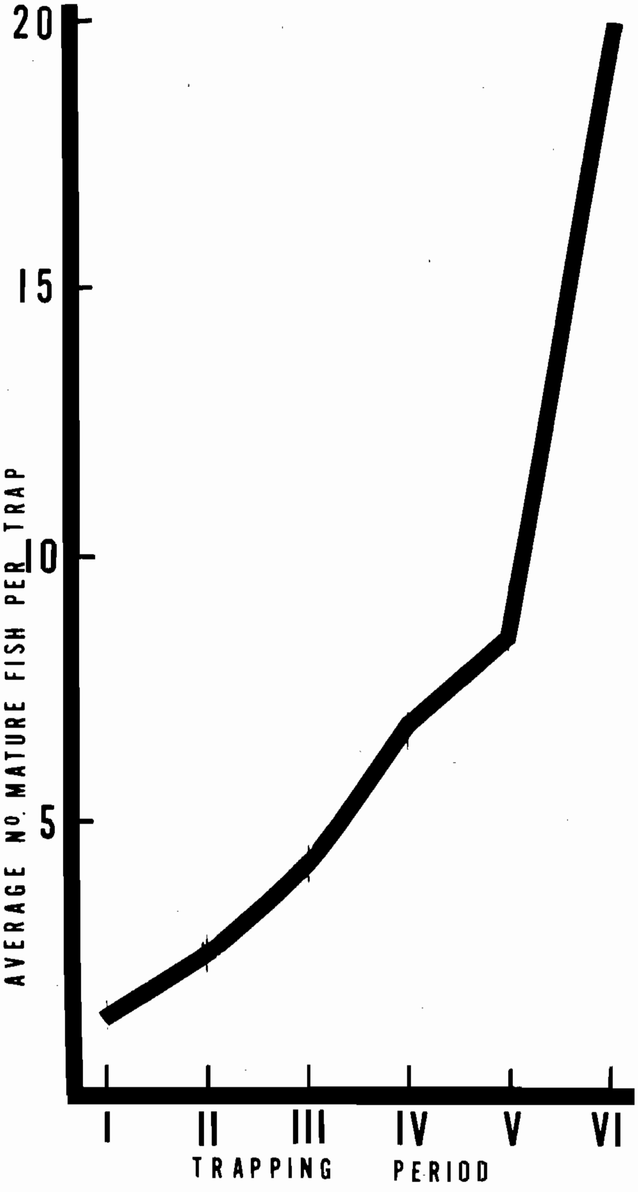

Figure 2.–Fish trapping in rice, 1972.

Table 2 shows the total *Gambusia* captures by period. Capture totals for all fields are averaged on a per trap basis and a comparison is made of mature females with combined male and immature totals. The male and immature captures for the season appear to follow similar pattern as the mature females. There appears to be a leveling off between period II and III which may reflect a generation interim. At this time the progeny of the first brood is maturing and becoming pregnant and this is reflected in the data by the steady increase in the capture of mature females.

The question of relating fish captures as indices of per acre population has not been answered. However, by using the 72-hour average trap count of mature females as the index for the initial planting of 100 fish to the acre, an estimated total of 4,868 mature female fish to the acre can

Table 1.—1972 summary of mature fish catches in six rice fields.

Field Number	Number of Traps	24 Hours	72 Hours	I 3-4 Weeks	II 5-6 Weeks	III 7-8 Weeks	IV 9-10 Weeks	V 11-12 Weeks	VI 13-14 Weeks
21- 1	14	3	4	12	29	66	68	61	146[2]
22- 3	6	2	9	7	18	12	224	22	23
16-13	15	9	13	14	58	53	94	128	255
16- 3	14	5	3	25	9	62	90	128	189
24- 9	14	0	1	3	12	32	52	81	73[3]
[1]29- 1	20	4	4	44	86	145	246	280	871
TOTALS	83	23	34	105	212	370	574	700	1557
AVERAGE		.28	.41	1.27	2.55	4.46	6.92	8.43	19.96

[1] Stocked in center of field only.

[2] 13 traps only.

[3] 10 traps only.

Table 2.—1972 total trapping counts in six rice fields.

	24 Hours	72 Hours	I	II	III	IV	V	VI
Mature	23	34	105	212	370	574	700	1557
Males & Immature		35	380	750	862	1217	1412	2725
TOTALS	23	69	485	962	1232	1791	2112	4282
Number of Traps	83	83	83	83	83	83	83	78
Average of Males & Immature		.42	4.58	9.04	10.39	14.67	17.01	34.04
Average Totals		.83	5.84	11.59	14.85	21.58	25.45	54.90

be used as the field average for period VI. Assuming the males and immatures are attracted to the trap equally as the gravid females, the per acre total for period VI was 12,961. These assumptions need further testing on a larger scale.

The main difficulty in evaluating the results of the study was the mobility of the fish and the absence of data on losses of fish leaving the field. Meaningful data are also needed on trap placement in the paddy and the relationship of its catch to the relative fish population in the field. These catches should also be related to mosquito populations.

In conclusion, the minnow trap can be an important tool for mosquito abatement districts which will provide a method of obtaining an estimate of the relative populations of mosquito fish in rice fields. When this information is correlated with mosquito larval counts it can help materially in evaluating the efficacy of *Gambusia* in these sources.

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COMPETITION FOR ENERGY IN LARVAL POPULATIONS OF *AEDES AEGYPTI*

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ABSTRACT

Aedes aegypti in Africa comprises two distinct populations, one of mainly dark-colored individuals dependent on rainfall for breeding, and a synanthropic population of paler individuals that breeds in water stored in houses. During the rainy season, both populations coexist yet seldom mix except close to houses. Exploitation competition by larvae for limited food-energy was studied as one possible mechanism of competitive exclusion of each population from the other's habitat.

In our experiments, different numbers of newly hatched larvae from both populations and different amounts of liver powder were combined in plastic tubs containing 200 ml water at 26°C to give various food-to-larvae ratios. Data on times to pupation, numbers, and weights of both sexes of pupae were collected.

The results of the study, presently continuing, suggest the following conceptual model that envisions both a time threshold--before which no pupation can occur, and a weight threshold--below which no pupation can occur. This creates a "pupation window". A population of larvae with a variance in initial (i.e. egg) size and growth-rate grows under food-limited conditions. The larvae that start biggest, or grow fastest, pupate when they reach the time threshold at a weight depending upon their growth-rate.

Slower-growing larvae pupate a little later at the minimal weight, but others fail to reach the "window" before energy is depleted and their growth rate becomes negative.

Aedes aegypti lay eggs which, in nature, may accumulate between rainfalls. High larval densities inevitably will lead to food-energy depletion in all but those breeding sites providing high energy inputs such as tree-holes.

Southwood et al. give field data from Thailand on larvae in water jars that indicates energy depletion. Thus conditions leading to exploitation competition may be frequent. If so, any factors such as larger egg size, more rapid egg hatch and faster larval growth, however slight, will give their possessors a competitive advantage. We have shown recently that even a 6-hour head start is sufficient.

Although the differences between the indoor and outdoor strains, in the parameters we have measured, have been relatively slight, they are enough to give the outdoor strain a very strong competitive advantage in direct competition experiments. Therefore, we stress that with exploitation competition for a limited niche resource subtle differences are of some importance and should not be overlooked.

Partial adulticidal control directed against a complex of pest species, by reducing exploitation competition, may allow greater survival of a normally out-competed species. If such a species were the more potent vector, as is the synanthropic *A. aegypti*, the results of partial control might be worse than none. Exploitation competition is probably the most important density-dependent regulating factor in larval *A. aegypti*.

We hope soon to extend our studies to California pest mosquitoes.

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INSECTICIDE AVOIDANCE BY OVIPOSITING *AEDES AEGYPTI*

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ABSTRACT

Observations on *Aedes aegypti* control during the 1972 dengue virus outbreak in southwestern Puerto Rico suggested that gravid females might be ovipositing preferentially in un-sprayed containers.

To test this hypothesis, laboratory and field experiments were performed using various concentrations of malathion and Abate larvicides. Although inconclusive due to technical problems, laboratory studies indicated that malathion and Abate were both repellent. Further studies in the field confirmed repellent action for both compounds. Malathion was repellent at concentrations above 125 ppm and Abate was repellent at 50 ppm, the lowest concentration tested.

The concentrations needed to prevent oviposition appear to be considerably greater than lethal concentrations for larvae, so that the practical significance of this discovery remains to be determined.

ANNUAL FISH OF THE GENUS *CYNOLEBIAS* FOR MOSQUITO CONTROL

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This is a report of research by the Butte County Mosquito Abatement District on annual fish and their potential use as mosquito predators.

The District has more than 70,000 of 400,000 acres of rice fields in Northern California. Besides a good rice crop, rice water produces large numbers of mosquitoes. Therefore, we have pointed our research in this direction.

The mosquito populations of rice fields are fairly predictable. Flood water mosquitoes, *Aedes melanimon* or *A. nigromaculis*, develop first and are univoltine except when the water level is lowered to encourage sprouting or to expose plants for pest control operations. *Culex* mosquito populations increase a little later when the rice plants grow through the surface of the water and the fields become stabilized. About midseason, when the rice shades the water, *Culex* are replaced by *Anopheles* (mostly *A. freeborni*) which do well until the rice is drained in late summer. By contrast, in rice fields of gulf coast areas of Texas and Louisiana, where rice is repeatedly drained and flooded, *Aedes* are encouraged, while *Culex* and *Anopheles* mosquitoes as well as mosquito fish are discouraged.

The mosquito fish (*Gambusia affinis*) has been the subject of lots of experimentation and manipulation (Gerberich and Laird 1968). The Sutter-Yuba Mosquito Abatement District has two fisheries biologists studying *Gambusia* rearing and use. Our efforts are complementary. When we can compare the effects of annual fish and of *Gambusia* we should be able to determine where and how well each fits in a control program.

We have used and continue to use mosquito fish in a continuing integrated approach to control, but believe that there should be more research on other methods of biological control. With diminishing numbers of effective chemicals and increasing resistance, a higher level of tolerated numbers of mosquitoes (i. e. a higher nuisance or economic threshold) has been necessary because of fixed buying power budgets and reduced return on the dollars spent. The biological control agents which were not effective enough to compete a year or two ago now appear more acceptable.

Annual fish offer a developmental cycle similar to the *Aedes* mosquitoes (Bay 1967). The fish eggs go through a drying sequence and can lie dormant on the soil until covered by irrigation water. Add to this the rapid growth rate of the fish where sexual maturity is reached in 6 to 8 weeks (Bay 1965), and the annual fish seem ideally suited to our needs. They also are voracious feeders on mosquito larvae.

The theoretical considerations appear excellent and our goal now is to see if any of the candidate species will survive and reproduce in our area, and whether we can get necessary clearance to introduce another alien species of fish into our environment. Actually, we agree with the exercise of extreme caution in introductions; once established, a reproducing organism may be more persistent than any of the persistent pesticides.

The obvious advantage of annual fish is the egg production and storage potential. We envision producing large numbers of eggs in aquaria which can then be stored for several months. Our present storage system allows us to keep eggs five months with little or no loss of viability. The viability of *Cynolebias nigripinnis* eggs decreases rapidly after five months of storage. In our studies, only one fry hatched from any of the eggs kept in damp peat an additional month and a half. Stored *C. whitei* and *C. bellottii* eggs show a more gradual decline in hatch. Peat held without flooding nearly seven months hatched 20 fry in one experiment. There is one case of *C. whitei* fry emerging from eggs kept dry for two years (Milgram, pers. commun.). We plan to place stored and embryonated eggs in the potential mosquito sources, and they should begin to hatch in a matter of hours after entering the water. Annual fish are sometimes called "instant fish" because of this unusual part of their life cycle.

Our laboratory work with *C. nigripinnis* shows that its eggs do not need to be dried for viable fry to be produced. Eggs which were conditioned by holding in damp peat moss produced higher numbers of young fish than those which were kept continuously flooded. Further experiments revealed that a dry period is required for *C. whitei* eggs to embryonate and hatch.

The genus *Cynolebias* was chosen for our research for several reasons. Dr. Ernest Bay had previously worked with *C. bellottii* in California and had shown they could withstand the stresses of our rice fields (Bay 1956a, 1965b), so to start this project, we obtained some of these fish from Dr. E. Fred Legner at the University of California, Riverside.

The *Cynolebias* spp. are "peat divers" (Turner and Pafenyk 1967) thereby differing from some annual fish which scatter their eggs on the soil surface or lay them in shallow troughs instead of burying them as do "divers". *C. bellottii* is difficult to maintain in aquaria so we chose to work with *C. whitei* and *C. nigripinnis*. These species reproduce readily and were easily obtained.

We built and stocked small rice plots provided with safeguards to prevent the escape of fish. The inadvertent introduction of *Gambusia* into one pond showed that *G. affinis* and *C. whitei* are not compatible; all *C. whitei* in the contaminated pond were soon eliminated. The incompatibility of annual and live bearing fishes was corroborated by Allen Semit, a South American killifish collector (pers. commun.). This apparent drawback may have some advantages such as natural protection from establishment of *Cynolebias* in permanent or natural waters. Each female produces a large number of eggs so the survival of a small number of reproductives should maintain the population from year to year.

Cynolebias whitei and *C. nigripinnis* can survive summer temperatures in California rice fields. In their native habitat these fish are winter breeders, thriving in water temperatures often below 40°F (Castelli 1973). In our rice paddies they survived and developed in water at temperatures ranging to 99°F. South American annual fish usually are found in acid water but our rice paddies are slightly alkaline with a pH close to 8.5. In the laboratory they thrive at pH of 6 to 7.5, showing that fish can withstand a wide range of conditions.

Cynolebias whitei and *C. nigripinnis* were observed mating and burying eggs in the rice paddies. Survival and hatchings, as the true measure of field egg production, will not be measured until the experimental rice paddies are flooded later this Spring.

To obtain information wanted by the District and by the California Fish and Game Department, we are developing a competition test between *Cynolebias*, bluegills, red-eared sunfish and large mouth black bass. We hope to gain more information on what to expect when rice fields are drained into the Sacramento River drainage, letting field raised *Cynolebias* loose in this ecosystem.

Field trials are underway with both *C. whitei* and *C. nigripinnis* in winter vernal pools. The mosquito control possibilities of annual fish were first observed in South American vernal pools (Hildemann and Walford 1963).

We are trying to maintain a colony of *C. bellottii* in the laboratory, and are attempting to develop a laboratory colony of *C. antenori*. We hope to produce enough fish of these species for field tests. *C. Bellottii* produces rather large healthy fry from eggs which store well and have already shown that they can reproduce under rice field conditions (Bay 1965a and Bay 1965b). The advantage of *C. antenori* lies in a preference for warmer temperatures and a necessary egg drying period of five months (Tulipano 1973) as compared to two months for *C. nigripinnis* and three to four months for *C. whitei*. The longer minimum period will lessen the chance of eggs hatching due to winter rains during the six or seven months between rice crops.

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A PILOT PROGRAM FOR THE INTENSIVE CULTURE OF *GAMBUSIA AFFINIS* (BAIRD AND GIRARD) AND *TILAPIA ZILLII* (GERVAIS)

PART II: INITIAL OPERATION AND PRODUCTION EFFICIENCIES

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INTRODUCTION.–The initial stages of the Orange County Mosquito Abatement District project on intensive culture of *Gambusia affinis* and *Tilapia zillii* were reported by Challet and Rohe (1974). That paper described the specialized facilities and environment maintenance and data collection systems that were used. Construction of the two ponds (80 ft. L x 4 ft. W x 2 ft. D) and their environment control systems was begun in December, 1972. The program was put into operation on January 15, 1973. The total cost for materials and supplies was $4,725.18 (Table 1).

INITIAL OPERATIONS.–*Gambusia*--Intensive culture began on April 2, 1973 after minor mechanical and operational problems of the system were overcome. Several experimental techniques were tried and found to be inadequate for mass production. The first technique employed a 40 foot section of Pond No. 1 as a nursery area. Green vinyl strips were suspended above and extended downward into the water from three 4 x 4 frames to simulate vegetation and to provide protection for the newborn fry. Adult *Gambusia* are notoriously cannibalistic on the young fry.

From April 3-6, 1,000 gravid females weighing 962 grams were transferred from a golf course pond to the nursery section. On April 4 a random sample of 40 gravid females from this group showed an average length of 44.6 mm and average weight of 1.3 grams, while 89 non-gravid females from another 40 foot holding section of Pond No. 1 had an average length of 36.7 mm and average weight of 0.6 grams.

Gravid or sexually mature females are distinguished by their large distended abdomens and by a black area in front of the anal fin, known as the "gravid spot". This reaches its maximum size just before the female drops her young.

The females in the nursery section were fed Purina Trout Chow No. 1 at 2% of their body weight or 19.2 grams daily. When available, live Daphnia from the field were fed to supplement the diet.

On May 9, 50 fry were observed in the nursery area. A random sample indicated 50% of the gravid females had dropped their young. Apparently, the rest of the fry were eaten by females wandering between the vinyl strips or were preyed upon as they left the protection of the strips.

A decision was made on May 14 to experiment with culture cages as a possible alternate to the nursery rearing technique. Three cages were built, each having a one-cubic-foot wood frame with 1/8 in. mesh plastic screen on five sides.

The nursery section was reduced to 20 feet and an additional section of 20 feet was provided for culture cages. The three culture cages were placed in a row and suspended in the pond. Water level inside the cages ranged from 1-3 inches in depth. Ten gravid females were introduced into each cage and as the young fry were born, they dropped through the screen and swam away. Thus few fry were lost by cannibalism. The same principle is applied for plastic live bearing traps used in aquariums.

On May 26 vandals damaged the three culture cages and killed the gravid females. On June 1 nine new cages, with a total of 90 gravid females were placed in Pond No. 1. By June 11 production of fry in the cage culture section was greater than the nursery section (Table 2). On June 18 a decision was made to change to culture cages for *Gambusia* production. On June 22, after moving the fry to a new section, nine cages were again placed in three rows of three each. Each row had cages with 5, 10 and 15 gravid females, for a total of 90 fish. Cages were examined daily and all females that had dropped their young were recorded and replaced with other gravid females. Fry were moved from the culture cage section at two week intervals but were not weighed, counted and harvested until they were 4-6 weeks old. Small batches of fry were counted by hand while large groups were weighed and estimated by the scale method used in California Department of Fish and Game hatcheries (Leitritz 1959). Finally, the cages were returned to the pond and filled with gravid females to begin a new culture. On August 3 the number of gravid females in each row was increased to 15, 20 and 25 for a total of 180 fish in the culture cage section. The purpose was to monitor the effect of crowding on drop rate. Beginning September 14 a noticeable drop in production occurred, which may have been due to decreased photoperiod. On September 20 lights were installed above the pond to extend the photoperiod to encourage continued reproduction through the winter. A photoperiod of 15 hours was reported to be optimum for reproduction (Richard Husbands, pers. commun.).

On October 12 a new type of culture cage was installed, which measured four feet long, two feet wide and one foot deep. Sixty gravid females were introduced into this cage, equal to the number of females in a row of the one-cubic-foot cages. However, the number of gravid females that had dropped fry were recorded only at the end of the two week rearing period.

Tilapia Operations--Intensive culture of *Tilapia mossambica* and *T. zillii* began on April 2, 1973, in Pond No. 2. Primary emphasis was upon the culture of *T. mossambica* due to its mouthbreeding characteristic which requires no special bottom material or extended nesting period. Although *T. zillii* is more tolerant of low temperature and should provide superior chironomid reduction (Legner et al. 1973), we believed that this fish would not reproduce as well as *T. mossambica* in the plywood ponds. *T. zillii* is not a true mouthbreeder and was presumed to require small tubes at the bottom of the nest for successful reproduction.

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Table 1.–Cost estimate for 1973.

I. CAPITAL COSTS:			
A. Material and Supplies to Construct Ponds From December 1972 to December 1973[a]			$ 4,725.18
II. OPERATIONAL COSTS:			
A. Utility Costs From January 1, to December 31, 1973			
Utility	Units Used	Cost	
Gas	5,086 Thermal	$ 460.04	
Electricity	12,423 Kilowatt hrs.	310.61	
Water	0.704 Acre Feet	9.40[b]	
		$ 780.14	
B. Water Softening Tanks (12ea.)			41.50
C. Purina Trout Chow (150lbs.)			42.00
D. Salary for Seasonal Fisheries Biologist (9 mos.)			5,495.35
TOTAL Operational Costs			6,358.99
TOTAL COST			$11,084.17

[a] Cost of District labor not included.

[b] Our water is from a well on District property – A more realistic cost of water from Garden Grove Water Dept. would be $62.85.

On April 6 eight male and three female *T. zillii* were introduced into one section of Pond No. 2 while ten male and seven female *T. mossambica* were introduced in another. On April 11 fry were observed in both sections. A decision was made on May 25 to stock the remaining *T. mossambica* in the field and culture only *T. zillii*. On June 18 Pond No. 2 was divided into three sections and 15 adult *T. zillii* were placed in each. On September 13 the number of adult *T. zillii* in Pond No. 2 was increased to 80. The most recent hatch of *T. zillii* fry was December 20, 1973.

Two methods were employed to harvest *Tilapia* fry from the pond: (1) a fine mesh net was used to remove the entire cluster of fry as they were guarded by their parents. This resulted in occasional injury to the young and once they reached one-half inch in size or were no longer protected by the parents, they became extremely difficult to catch; (2) the parents were removed after the fry were released from the mouth of the female or were no longer protected. These fry were moved to another section and their parents were returned to begin a new cycle.

RESULTS AND DISCUSSION.--General Operations--The facility operated satisfactorily with only minor mechanical problems during the year. High alkalinity and hardness of the water caused lime deposits on the heater coils and maintenance was required every six months. A water softening unit was installed on June 8. Water from our well has a hardness of 23, measured in grains of calcium carbonate per gallon. Hardness of the water in our ponds ranged from 7-15 grains per gallon.

Alkalinity is a problem, with a recorded low of 90 ppm in July and a high of 180 ppm in December. We are considering use of acetic or muriatic acid in concentrations that will reduce alkalinity without endangering the fish. It was necessary to replace the sand in the filter every six months, probably due to high alkalinity and the rapid buildup of plankton during the summer. We began monitoring water quality on a limited basis in June. Average readings for the year were: pH 8, hardness 10 grains per gallon, alkalinity 120 ppm, and water temperature 27°C.

Costs--Expenditures on this project for the first year are given below. The total operational and capital costs are divided equally between the two ponds. Future costs will be much less since the entire cost of the physical plant is included in the first year figures.

Cost for the production of *Gambusia*:

a) One-half of total operational and capital costs (Dec. 1972 – Dec. 1973) $5,542.08

b) Fish production (April – Dec. 1973) . . 25,826 fish

c) Cost per fish $0.22

Cost for the production of *Tilapia*:

a) One-half of total operational and capital costs (Dec. 1972 – Dec. 1973) $5,542.08

b) Fish production (April – Dec. 1973) . . . 7,168 fish

c) Cost per fish $0.77

Production Efficiencies--*Gambusia*: Fish production data were recorded from May 14 to December 31, 1973 (Table 2). During this period 25,826 *Gambusia* were produced. Peak production occurred in July with nearly 7,000 fry harvested. A low point was reached in September with only 2,000 fish harvested. With the present number of four culture cages and 240 gravid females, it appears that production during the winter will range from 2,000-4,000 fry per month. The decision to convert to culture cage production on June 18 was supported by comparing the production of 50 fry in the nursery section from April 3 - June 22 with culture cage production of 2,172 fry during a similar period.

There was no significant difference in the relative number of females that dropped young in rows 1, 2 and 3 with 60 gravid females in each row. However, there appeared to be an increase in the number of females that dropped young as the number of gravid females per cage was increased. This indicates an increase in drop rate due to population pressure, but more data are required before firm conclusions can be drawn.

The number of fry a gravid female will produce per brood may average from 40 (Hildebrand 1921) to 104 (Hoy and Reed 1970). Our observations with a small number of females show a range from 25-60 fry per brood. Table 2 shows that we can expect 10.7 fry after 4-6 weeks for every female that has dropped in our system. Using 40 fry as an average brood, a survival rate near 25% is achieved. In nature survival would probably be lower due to predation and other environmental factors.

Tilapia: From April 2 – December 31, 1973, there were 7,168 *Tilapia* harvested. We planted 426 *T. mossambica* and 4,742 *T. zillii*, while 2,000 *T. zillii* are being held for planting. Several papers were helpful during the initial organization of our ponds for the intensive culture of *Tilapia*. Information was obtained regarding identification (Lowe 1955), culture (St. Amant 1966) and history of *Tilapia* in southern California (Hoover 1971).

Table 2.–*Gambusia affinis* production for 1973 – Rearing Tank No. 1.

Batch Number	Rearing Record					No. Gravid Females that Dropped	Batch Production			Cumulative Production	
	Female Drop Period			Rearing Period							
	Start Date	End Date	Total Days for female Drop	Harvest Date	Total Days Drop to Harvest		Number of Fry	Total Weight of Fry	Weight per Fish (grams)	Total No. of Fry	Total Weight
1	May 14	June 1	18	July 12	41-59	30±	382	100.0	0.26	382	100.0
2	June 1	June 22	21	July 19	29-48	90±	1790	179.8	0.10	2172	279.8
3	June 22	July 9	17	Aug. 13	35-52	134	2195	292.4	0.13	4367	572.2
4	July 9	July 20	11	Aug. 16	27-38	178	3288	256.8	0.08	7655	829.0
5	July 20	Aug. 3	14	Aug. 30	27-41	176	3689	284.2	0.08	11344	1113.2
6	Aug. 3	Aug. 17	14	Sept. 14	28-42	352	1400	107.8	0.08	12744	1221.0
7	Aug. 17	Aug. 31	14	Sept. 28	28-42	290	3462	266.6	0.08	16206	1487.6
8	Aug. 31	Sept. 14	14	Oct. 10	26-40	259	1278	204.5	0.16	17484	1692.1
9	Sept. 14	Sept. 28	14	Oct. 24	26-40	134	731	161.0	0.22	18215	1853.1
10	Sept. 28	Oct. 12	14	Nov. 8	27-41	178	1066	234.5	0.22	19281	2087.6
11	Oct. 12	Oct. 26	14	Nov. 23	27-42	153	1061	191.0	0.18	20342	2278.6
12	Oct. 26	Nov. 9	14	Dec. 6	27-41	144	1882	207.0	0.11	22224	2485.6
13	Nov. 9	Nov. 23	14	Dec. 21	28-42	134	2110	211.0	0.10	24334	2696.6
14	Nov. 23	Dec. 7	14	Dec. 31	24-38	167	1492	194.0	0.13	25826	2890.6

CONCLUSION.–The system is operating well and the ability to produce *Gambusia* and *Tilapia* throughout the year by artificial means has been established.

The following recommendations are made for the reduction of utility and labor costs and increased efficiency of operations:

1. Reduce water temperature from 27°C. to 24°C.
2. Reduce light bulb wattage from 100w to 25w.
3. Harvest *Gambusia* fry on a weekly basis rather than biweekly.
4. Count the number of *Gambusia* females that have dropped young on a weekly basis rather than daily.

We are also considering the use of solar heaters and pond covers to conserve energy.

Monitoring capability for water quality will be increased. This may include counts of dissolved oxygen, chemical oxygen demand and ammonia-nitrogen.

The more difficult problems with *Gambusia* are encountered during the winter months when no production is taking place in nature. Production was limited during the winter months by a lack of gravid females to stock the culture cages. Limited space prevented the production of sufficient gravid females and none were available from the field. This situation will be corrected by using a section of the *Tilapia* pond (number 2) for rearing *Gambusia* to maturity.

Tilapia were planted primarily in flood control channels, and in golf course water hazards, ponds, and reservoirs. Agencies planning to use *Tilapia* should contact the California State Department of Fish and Game and obtain a permit to transport live fish. Observations of *Tilapia* under field conditions will be continued to determine their adaptability and effectiveness in chironomid reduction and algae control (Legner and Medved 1972, 1973).

Future efforts will concentrate on achieving maximum production of both species. We believe the experience gained and information produced during this first year of operation is valuable. It should be possible to double the production of both species of fish during 1974.

Other agencies are also interested in this problem. The University-Wide Advisory Committee on Mosquito Research of the University of California recommended that high priority be given to funding and implementing a study on the development of mass-rearing of the mosquito fish, *Gambusia affinis*, and $25,000 was set aside for this. We expect information from this study to relate directly to our project and to assist in solving problems such as photoperiod light intensity, disease, water quality, population pressure, etc. As we gain more information concerning these factors we may be able to approach the goal of maximum production.

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THE NATIVE DESERT PUPFISH, *CYPRINODON MACULARIUS* BAIRD AND GIRARD, A SUBSTITUTE FOR *GAMBUSIA* IN MOSQUITO CONTROL?[1]

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The most widely distributed and extensively used biological mosquito control organism is the top minnow, *Gambusia affinis affinis* Baird and Girard. Renewed emphasis is being given *Gambusia* as mosquito resistance to insecticides continues to mount and suitable alternative controls are not forthcoming. It is becoming recognized, however, that *Gambusia* possesses several undesirable traits which makes its indiscriminate use questionable. Problems of selective predation on desirable invertebrate mosquito predators, its poor foraging ability in emergent weed habitats, and its destruction of other native fishes are becoming more widely associated with *Gambusia* (Danielson 1968; Hoy et al. 1972; Hurlbert et al. 1972; A. Calhoun, pers. commun.).

Nevertheless, the soaring human population demands more thorough mosquito control, even though such be accomplished with a disregard of possible undesirable side effects. Increases in the mosquito density due to the elimination of native predators that are capable of foraging more effectively in covered habitats are ignored. Investigations of means to manipulate *Gambusia* to maximum control advantage have taken precedence to intensive studies on alternative predators. Several native fish species of the genus *Cyprinodon*, or pupfishes, deserve consideration as possible *Gambusia* substitutes.

One widespread and well established species in southern California, *C. macularius*, was chosen for intensive study. Although there is also a lack of information on undesirable traits, this species' cohabitation with many different kinds of fish and invertebrates in the irrigation drains, canals and estuaries around the Salton Sea in Riverside and Imperial Counties indicates that it might be a desirable fish to disseminate for mosquito control.

Data from two different pond habitats at Riverside, California in 1973 confirm the value of *C. macularius* in mosquito control (Table 1, Legner and Medved 1974) and chironomid midge suppression (Legner and Medved 1974). Unlike *Gambusia*, *C. macularius* was found to disseminate throughout the volume of water where it exhibited frequent characteristic darting motions. Foraging for arthropod food appeared continuous in the benthos and at the water surface, even extending effectively into floating algal mats and into non-rigid emergent vegetation such as grasses. In this way, *C. macularius* differed from *C. nevadensis* (Danielson 1968) which did not range effectively as a predator under conditions of cover. However, it may be that the rigid cover in the form of bamboo stakes used by Danielson (1968) did not approximate the natural cover afforded by native grasses in our investigation. Shoaling behavior was also observed in *C. macularius* populations, especially among younger aged individuals.

In the absence of mosquitoes and other insects that happen to drop into the water, *C. macularius* utilized benthic chironomids effectively for food (Legner and Medved 1974). Surface foraging gradually ceased as water temperatures dropped below 21°C. A range of 8° - 44°C. has been reported for this species from desert pools in the southwestern U.S.A. (Lowe and Heath 1969, Taylor and Markley 1966). It is also widely tolerant of extremes in water salinity (Barlow 1958).

The desert pupfish deserves increased attention in the integrated control of mosquitoes in the Southwest. Indiscriminate utilization of imported *Gambusia* is not justified when potentially equal or better adapted native species exist. *Cyprinodon macularius* has been observed to occur naturally at high densities in the absence of mosquito prey which is thought to be due in part to its ability to consume benthic chironomid midges (Barlow 1961, Legner and Medved 1974). The apparent inability of *Gambusia* to utilize benthic chironomids for food (Bay and Anderson 1965) probably forces these fish to seek beneficial native arthropod mosquito predators such as hydrophylids, notonectids

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Table 1.—Average number of 4th instar mosquito larvae[1] sampled from 5.5 x 7.6 x 0.41 m ponds containing an initial 25 *Cyprinodon macularius* (ca. 1♂/4♀♀ sex ratio and 5 weeks old) compared to a control. Riverside, California, 1973.

	Avg. No. 4th instar/500 ml dip[2]		
Sample Date	*C. macularius*	Control	Percent Destruction
6/15/73	3.00	4.88	38.52
6/22	2.30	5.13	55.17
6/29	0.65	4.50	85.56
7/13	0	2.81	100
7/20	0	2.94	100
8/3	0	1.56	100

[1] Mixture of *Culex peus* Speiser, *C. tarsalis* Coquillett, and *C. pipiens quinquefasciatus* (Say).

[2] Five replicates.

and dytiscids (Hoy et al. 1972) and zooplankton (Hurlbert et al. 1972), resulting in unbalanced conditions that favor mosquito survival and phytoplanktonic blooms. *Cyprinodon* has not been associated with such imbalances and, therefore, may be a superior biological control alternative to *Gambusia.*

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NOTONECTA UNIFASCIATA AS PREDATORS OF MOSQUITO LARVAE IN SIMULATED FIELD HABITATS

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INTRODUCTION.–Realization that insecticides have not provided lasting solutions to continuing mosquito problems (Kellen 1962) has generated interest in biological control agents other than larvivorous fish as an additional approach to mosquito control. The fiercely predaceous *Notonecta* species and related backswimmers have been cited as important predators of immature mosquitoes and are believed to be effective in reducing natural populations of these insects (Hamlyn-Harris 1929; Hinman 1934; Rice 1942; Laird 1947; and Usinger 1963). Recent studies by Lee (1967), who used *Notonecta shooteri* Uhler, and Ellis and Borden (1970), who used *Notonecta undulata* Say, produced laboratory data reports of backswimmer effectiveness in lowering mosquito populations. The latter authors believed their results warranted further consideration of *N. undulata* as a biological control agent for mosquitoes. Despite the finding of Lee and Ellis and Borden, there are little field data supportive of effective mosquito predation by backswimmers.

This study was performed as an attempt to evaluate the efficacy of *Notonecta unifasciata* Guerin in reducing mosquito populations occurring in semi-natural habitats. This backswimmer was selected because of its availability and earlier observations of its habitat and niche, the latter indicating mosquito control potential (Bay, unpubl. data). The mosquito species employed was the ornithophilic *Culex peus* Speiser. Two experiments were performed, one involving the introduction of backswimmer adults and the other the introduction of backswimmer eggs into simulated natural habitats containing well-established populations of *C. peus.*

MATERIAL AND METHODS.–Introduction of backswimmer adults.–This experiment was conducted from 25 July to 17 September. One and two sexual pairs[1] of laboratory-reared[2] virgin adults of *N. unifasciata* were introduced into habitats containing recruited populations of *C. peus.* The experiments were performed according to the following procedures: Twelve simulated natural habitats (Figure 1) were established, each consisting of a one-yard square redwood framed soil reservoir filled about four inches deep with local irrigation water of pH 8.5 ± .5 and total hardness of 171 ± ppm $CaCO_3$. To exclude predator and prey species other than *N. unifasciata* and *C. peus,* each reservoir was canopied with 14-mesh fiberglas screen attached to a frame of aluminum uprights on a wood base. The screens were partially covered with opaque polyethylene to reduce algae growth within the reservoirs. An emergence receptacle was placed atop each canopy and used to monitor the number of adult *C. peus* emerging. Each receptacle was made from a joined polyethylene lid and funnel capped to a polystyrene quart container having a vented bottom covered with 50-mesh brass screen.

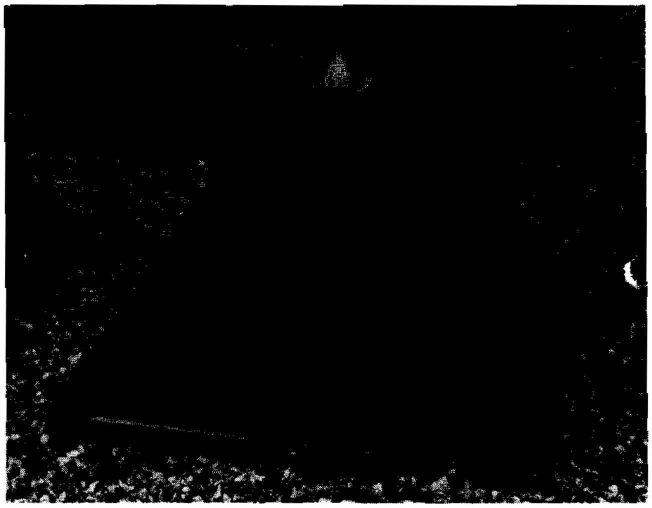

Figure 1.–A canopied, redwood-framed soil reservoir typical of the semi-natural habitats used in the adult inoculation study of *Notonecta unifasciata.*

Figure 2.–A yd^2 redwood-framed soil reservoir typical of the semi-natural habitats used in the egg inundation study of *Notonecta unifasciata.*

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[1] The words "sexual pairs" refer to male-female pairs.

[2] Procedure patterned after Ellis and Borden (1969a).

The twelve reservoirs were arranged in three random groups, each consisting of four replicates. The number of sexual pairs of backswimmers and the rate-density of mosquitoes introduced were as follows:

Group	Number of *N. unifasciata*	Rate-density of *C. peus*
1	1 sexual pair	2 egg rafts/day
2	2 sexual pairs	2 egg rafts/day
3 (control)	none introduced	2 egg rafts/day

Mosquito egg rafts were introduced from 25 July to 16 September. All rafts were collected from a mosquito mass-rearing facility (Bay, unpubl. data) and were about the same size, each containing approximately 250 eggs. To sustain the developing mosquito larvae, 2.5 gms of powdered, autoclaved Chick-starter® (a commercial poultry food obtained from the California Poultrymen's Cooperative Association) were also added daily. The sexual pairs of *N. unifasciata* were introduced into groups 1 and 2 August 12, one week after initial adult emergence of *C. peus*. Introduction of the backswimmers was made at night to discourage them from flying, a response which occurs when they are transported from the laboratory to the field and exposed to sunlight.

Adult emergence of *C. peus* was monitored at 24-hour intervals throughout this experiment. The predation efficiency of *N. unifasciata* was evaluated by comparing the mean total numbers of emerged mosquitoes trapped daily from the treated groups with those caught from the control. In addition, a comparison was made between the numbers of mosquitoes emerged from treatments 1 and 2 to determine the effect of the introduced predator densities on the rate of prey reduction. Upon termination of the experiment, the live nymphs and adults of *N. unifasciata* were recovered from the treated reservoirs. The head-widths of the nymphs were measured to determine the instar phase of development of these individuals[3].

Introduction of backswimmer eggs. Fully-embryonated eggs of *N. unifasciata* were introduced into simulated natural habitats containing natural populations of various insect predators and prey species, including recruited immatures of *C. peus* developed from introduced egg rafts. Four rafts of *C. peus* and 5.0 gms of powdered, autoclaved Chick-starter® were added daily from 8 September to 25 November to each of nine one-yard square redwood-framed soil reservoirs (Figure 2) in a 3 by 3 array having a total included area of 121 ft^2. The reservoirs had been filled and continually supplied with unfiltered irrigation water and left undisturbed since the end of the previous summer. Nine to eleven days after mosquito egg rafts were introduced, three replicates of 50 and 100 eggs of *N. unifasciata*, developed to approximately 24 to 48 hours from eclosion, were introduced into the reservoirs, as diagrammed below:

100	50	Control
50	Control	100
Control	100	50

The populations of fourth-instar mosquito larvae were monitored at 24-hour intervals by recording the mean of one-pint dip samples taken at each corner of each reservoir. To evaluate predation efficacy, the mean of the sum of the daily means (daily grand mean) of fourth-instar mosquito larvae from the reservoirs seeded with backswimmer eggs was compared with the daily grand mean of larvae from the controls. Also, the daily grand means of fourth-instar mosquito larvae from the 50 and 100 egg replicates were compared to determine if the differing egg densities influenced the rate of mosquito reduction.

The kinds of predator and prey species of aquatic insects other than *N. unifasciata* and *C. peus*, and other macroscopic organisms cohabiting the reservoirs, were determined weekly by visual inspection. No attempt was made to evaluate densities. However, *N. unifasciata* in these habitats were counted after ecdysis of third, fourth, and fifth-instar nymphs. When all the fifth-instar backswimmer nymphs molted, the resulting adults were counted and the sexes determined.

RESULTS. The number of experiments that could be performed in the limited number of reservoirs available was considered to be statistically insufficient to support firm conclusions. However, the gross results obtained were quite impressive.

Introduction of backswimmer adults.—The adult sexual pairs introduced into these reservoirs produced offspring (Table 1). The numbers of young were not proportionate to the numbers introduced. This unexpected result could have been due to the use of too few replicates. However, cannibalism following mosquito reduction, or presumably higher incidence of internecine competition among progeny at the greater backswimmer density could be involved.

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[3] The stage of development of *N. unifasciata* can be determined from head-width dimensions, the sizes of which are independent of development (Hazelrigg unpubl. data).

Table 1.–Surviving numbers and growth stages of *N. unifasciata* following termination of field release experiments involving introduction of single and double adult male-female pairs of this backswimmer into yd^2, redwood-framed soil reservoirs harboring immature populations of *C. peus*.

Predator Density	Prey Density	Replicate Number	No. of Surviving Nymphs per Stadia and Introduced Adults 1	2	3	4	5	Adults Male	Adults Female	Total Immature Stadia
One Male-female Pair	Two Egg Rafts per Day	1	2	1	4	2	0	1	1	9
		2	0	0	0	44	8		1	62
		3	0	13	21	10	0	1		44
		4	0	3	22	35	0			60
Two Male-female Pairs	Two Egg Rafts per Day	1	0	0	1	0	0	1	1	1
		2	1	20	10	0	0	1		31
		3	18	29	41	14	1	1	1	103
		4	0	0	1	0	0		1	1

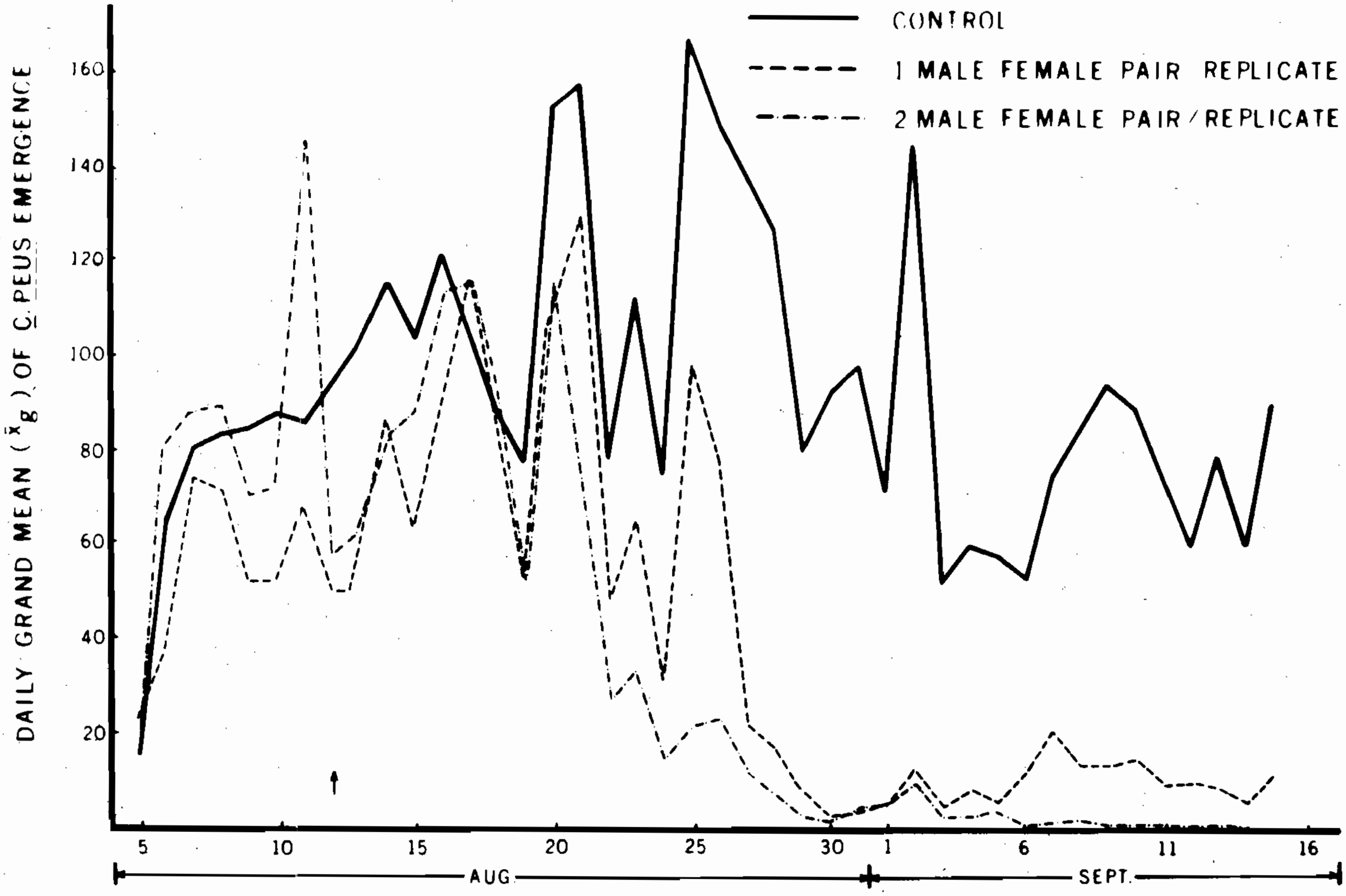

Figure 3.–Average daily emergence of *C. peus* after one and two sexual pairs of *N. unifasciata* were inoculated into canopied, yd^2, redwood-framed soil reservoirs. The reservoirs were supplied daily with two egg rafts of *C. peus* and 2.5 gms Chick-starter®. Arrow denotes introduction of backswimmers.

Figure 3 shows that at both predator densities mean mosquito emergence was noticeably reduced about ten days after the introduction of backswimmers (22 August). Dramatic reduction in mosquito emergence occurred 16 to 17 days after backswimmer introduction (28 and 29 August) and coincided with backswimmer development up to second and third-instar. From then on (29 August to 17 September), minimum mosquito emergence continued from the treated reservoirs, the controls having stabilized at an average carrying capacity of about 75 *C. peus* larvae per sampling day. (A peak count on 2 September in the control is probably a sampling error). During this period, the average apparent difference in mean mosquito emergence between the treatments and control was approximately 65 to 70 adults per day.

Figure 4, shows that the two sexual pairs of backswim-

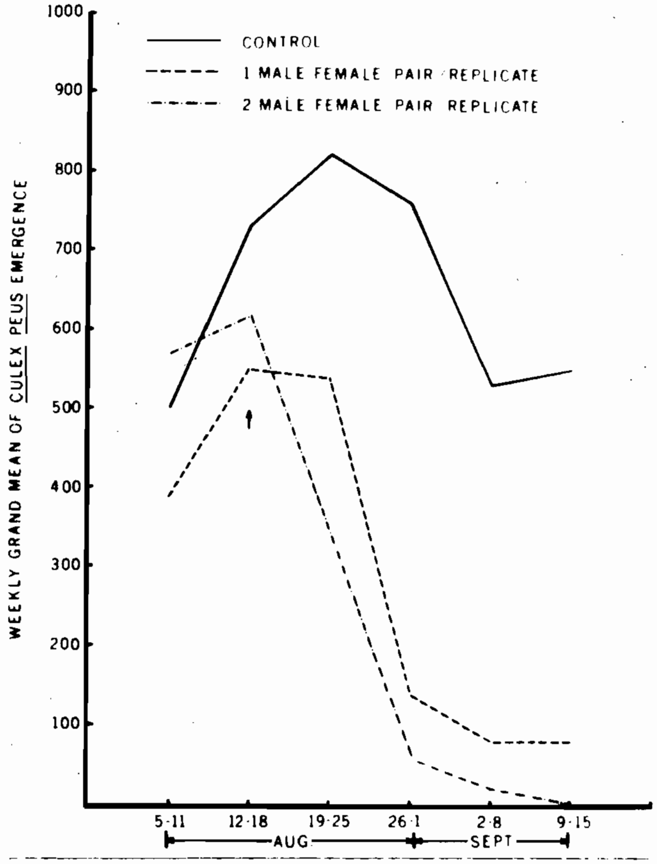

Figure 4.—Average weekly emergence of *C. peus* after inoculation of one and two pairs of *N. unifasciata* into canopied, yd^2, redwood-framed soil reservoirs. The reservoirs were supplied daily with two egg rafts of *C. peus* and 2.5 gms Chick-starter®. Arrow denotes introduction of backswimmers.

mers tended to more rapidly reduce the immature *C. peus* populations than the single sexual pairs. However, both predator groups were near equally effective. The delayed and somewhat slower rate mosquito reduction achieved by the single sexual pairs may have been due to chance variation, or to the fewer adults initially present and the production of fewer progeny.

Introduction of backswimmer eggs.—Results of the egg study are summarized in Table 2 and Figure 5. Egg introductions were made on different days, since sufficient eggs were not available for simultaneous introduction into both treatments.

At both egg densities, the number of fourth-instar larvae was reduced beginning approximately two or three weeks after backswimmer egg introduction and coincident with the development of third and fourth-instar backswimmer nymphs (Figure 5). At 50 backswimmer eggs per reservoir, reduction of mosquito larvae continued after 1 October, reaching a minimum on 7 October, shortly after first appearance of fourth-instar nymphs about 20 days after egg introduction. A slight, fluctuating increase in mosquito larvae ensued from 14 to 31 October due to an unexplained high backswimmer mortality in two of the three replicates (Table 2). After 1 November, the mosquito larval populations decreased in treatments and in controls, although a distinct difference continued until late November (Figure 5)[4]. With 100 backswimmer eggs per reservoir, reduction of mosquito larvae continued after 9 October, reaching a minimum level on 15 October, remaining at that level for the rest of the experiment. Minimum mosquito densities occurred shortly before the earliest appearance of fifth-instar nymphs or about 26 days after egg introduction.

Other predators cohabiting all reservoirs included both adults and larvae of *Laccophilus* sp. Leach and *Rhantus* sp. Dejean, two species of dytiscid beetles; none or several odonate naiads of the genus *Pantella* sp. Hagen; and many veliids (Hemiptera) of the genus *Microvelia* Westwood. Alternative prey included one or more species of chironomid larvae, *Ceriodaphnia* (cladocera), and ephemeropteran nymphs of the genus *Centroptilum* Eaton.

The percentage of notonectid progeny which survived following the first appearance of fourth-instar nymphs in each of the replicates supplied with 50 eggs was 22, 26, and 54, and that of replicates supplied with 100 eggs was 47, 48, and 54 (Table 2). Few backswimmers molted to adults, presumably due to low water temperatures associated with November. Only adult backswimmers were found to successfully overwinter. The resultant adult population of the reservoirs with 50 eggs each was two (one male and one female), one (female), and none. That of reservoirs with 100 eggs was four (two females and two males), two (females), and one (male).

DISCUSSION.—The evidence obtained indicates that in limited-area habitats such as those used in this study one or more sexual pairs of laboratory-reared virgin adults of *N. unifasciata* will establish and successfully mate, producing viable offspring. Although Bay (unpubl. data) has shown that nine adults of *N. unifasciata* kept in 100-gallon aquaria can maintain 90 to 98 percent mosquito reduction, the younger backswimmer progeny resulting from the adult introductions were presumed to be responsible for most of the observed mosquito reductions. For example, the period of dramatic mosquito reduction and concomitant appearance of older nymphs (fourth and fifth-instar) presumably occurred as a consequence of younger (second and third-instar) predators reducing mosquito immatures. Also, the marked reduction of immature mosquitoes occurred with the development up to third-instar nymphs during the egg introductions study. Ellis and Borden (1970) have shown (1) that backswimmer nymphs become increasingly efficient in killing mosquito larvae as they develop from first to fifth-instar; (2) that younger mosquito larvae are preferred over older larvae; and (3) that fourth and fifth-instar nymphs consume more prey than other predator age groups, first-instars and adults having the lowest rate of predation.

Results of the egg study indicate that mass releases of eggs of *N. unifasciata* into predator-prey complexes containing abundant mosquitoes and few alternative prey can result in effective mosquito reduction. This suggests that *N. unifasciata* prefer mosquito immatures to other prey such as substrate dwelling midge larvae, swift-swimming ephem-

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[4] In the area where this experiment was conducted, natural larval populations of *C. peus* are known to diminish to a minimum in late October and early November due to the onset of lower temperatures associated with seasonal change.

Table 2.—Dates (1971) and population counts associated with the development of *N. unifasciata*, following field release of its eggs into seminatural habitats harboring well-established mosquito populations, other insect predators, and alternative prey species. Dates listed under the heading "Hatching", headings "1st, 2nd, 3rd and 4th Molt" and heading "Adult Emergence" refer to the first appearance of each event described. Population counts of *N. unifasciata* were not begun until initial appearance of the fourth-instar nymphs (after 3rd molt).

No. of Eggs per Replicate	Replicate Number	Egg Introduction	Hatching	1st Molt	2nd Molt	3rd Molt	No. of Nymphs/Stadia Following 3rd Molt					Molt	No. of Nymphs/Stadia Following 4th Molt					Adult Emergence	No. of Nymphs and Adults per Stadia Following Adult Emergence						Earliest Completion of Developmental History Following Eclosion
							1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	A	Total Days
5	1	9/17	9/18	9/24	9/29	10/5			7	4		10/12				7	44	11/13					4	1	62
50	2	9/17	9/18	9/24	10/03	10/08			9	4		10/14				9	2	-							- -
	3	9/17	9/18	9/24	9/30	10/06			25	2		10/12				26	1	11/13					22	1	62
	1	9/19	9/20	9/27	10/04	10/09			44	4		10/16			1	35	2	11/23				2	21	2	74
100	2	9/19	9/20	9/27	10/04	10/09		2	51	1		10/17				46	1	11/25					38	1	76
	3	9/19	9/20	9/26	10/04	10/09		3	43	1		10/17			1	31	4	11/26				3	23	1	77

eropteran nymphs, or minute entomostraca. Several predator Notonectids are reported to discriminate between cohabiting species of mosquito larvae, as well as between mosquito larvae and alternative prey (Laird 1947; and Ellis and Bordon 1970). Discrimination among prey by backswimmers is determined by the interaction of several factors, including the size, speed of mobility, niche, and the degree of difference of these factors occurring between the prey species (Laird 1947; and Ellis and Borden 1970).

The value of cohabiting alternative prey should be viewed favorably if they do not interfere with mosquito reduction while providing additional support for backswimmers during their early nymphal development or during periods of mosquito scarcity. It is possible that the abundant alternative prey *Ceriodaphnia* (cladocera in particular) initially may have been preferred by younger backswimmer nymphs, helping to sustain them until older nymphs outgrew them.

From the egg study, it is apparent that *N. unifasciata* can compete with other common insect predators in habitats harbouring abundant mosquitoes. It is not known what success to expect from *N. unifasciata* in natural predator-prey complexes containing *Gambusia affinis*. The author has seen those two predators coexisting in large numbers in habitats when prey (primarily corixid nymphs) were abundant. However, to introduce eggs of *N. unifasciata* into habitats dominated by mosquito fish, *G. affinis*, would probably serve little purpose other than to provide the mosquito fish with additional prey; it is doubtful that such an effort would result in the establishment of backswimmers.

The findings of these studies suggest that *N. unifasciata* can be employed as a successful biological control agent in limited mosquito habitats. Control programs could involve the introduction of mated pairs of adults, nymphs, or eggs. The author suggests inoculation or massive releases of adults only in habitats believed stressful to *N. unifasciata* and when colonization appears unlikely; adults are more durable and adaptable than nymphs, yet they are likely to fly away. Under other conditions, the author suggests the periodic mass release of eggs nearing eclosion. Use of eggs assures maximum economy in a mass-rearing program, ultimately supplies to the environment the important prey-consuming developmental backswimmer stages, precludes backswimmer migration, and may yield potentially adaptable individuals capable of permanent habitat colonization. The author believes that field releases into habitats should be avoided if the habitats (1) contain abundant alternative prey that have a size, mobility, and niche similar to mosquito larvae; and/or (2) contain floating algae or debris and profuse emergent vegetation that obstructs most of the nectonic zone where prey and predator most often interact. To assure the greatest degree of success, field releases should occur during the seasonal range of this predator. Releases could probably be made into a wide variety of year-round or seasonally permanent aquatic habitats varying widely in degrees of water quality without seriously jeopardizing control effectiveness. Finally, the judicious use of *N. unifasciata* with other naturally occurring predators may prove to be a practical approach in reducing immature mosquito populations.

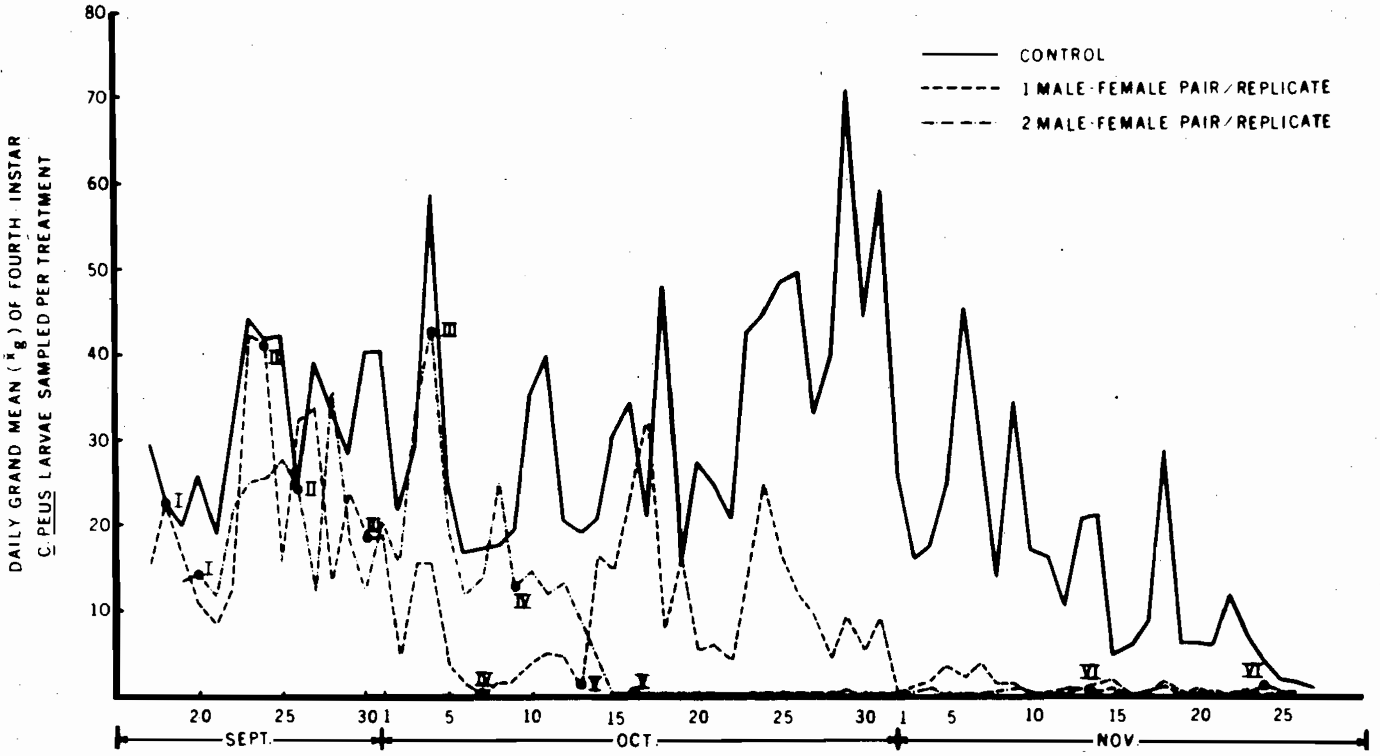

Figure 5.—Grand average daily emergence of mosquitoes following introduction of viable eggs of *N. unifasciata* into yd^2, redwood-framed soil reservoirs. Reading left to right, the Roman Numeral and symbol ● denote sequence of developmental stages (egg eclosion [I] to adult emergence [VI] first observed at both egg densities.

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THE EFFECT OF LIGHT AND TEMPERATURE ON EGG HATCH IN *CULEX PIPIENS*

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The concept of internal biological clocks controlling various observed biological functions and activities has been of interest in recent years in understanding the life history and ecology of various insects. These biological clocks can be long term (annual, seasonal, etc.) or short term (circadian). Using light as the variable factor, regular 24-hour circadian cycles have been demonstrated for such activities as oviposition in *Aedes aegypti* (Haddow and Gillett 1957), adult emergence in *Drosophila pseudoobscura* (Skopik and Pittendrigh 1967) and egg hatch in *Pectinophora gossypiella* (Minis and Pittendrigh 1968).

We are presently investigating the effect of light and temperature regimes on egg hatch in *Culex pipiens*. The first experiment was designed to compare average hatch time of *C. pipiens* eggs subjected to continuous dark (control) with that of eggs exposed to a 12-hour light period (experimental) at various times during their embryonic development.

Three replicates in each consecutive 3-hour time period for 24 hours were observed. The experimental eggs hatched later than the control eggs in all cases except one. The average hatch time for the control and experimental eggs was 36.72 and 38.29 hours respectively. A one-way analysis of variance showed significance at the 1% level, suggesting that light may affect egg hatch in *C. pipiens*. A more extensive study has therefore been started.

It was noted that egg hatch occurred as early as 30 hours after oviposition with an increase in temperature of about 10°F above that of the control eggs. It was also noted that oviposition tended to show a 24-hour periodicity under constant light and a temperature cycle with an amplitude of 10°F.

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STUDIES OF THE PREDATORY BEHAVIOR OF NOTONECTIDS ON MOSQUITO LARVAE

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Investigations of the predatory behavior of several species of the genus *Notonecta* were conducted during the past year in relation to evaluation of these aquatic insects for use in mosquito control programs. The research reported in this paper concentrates on attack behavior of the following 4 species: *Notonecta kirbyi, N. shooterii, N. undulata* and *N. unifasciata.* In addition, studies are reported on predatory behavior of the nymphal instars of *N. unifasciata* against *Culex tarsalis* and *Culex pipiens.* All species on which studies were conducted are common to a 50 mile radius of the San Francisco Bay Area, and all are potential candidates for use in mosquito control programs. The following literature citations are relevant to studies reported in this paper (Clark 1928; Wolda 1961; Ellis and Borden 1970; Toth and Chew 1972a and Toth and Chew 1972b).

All tests were run in glass battery jars (6″ diameter) or in white plastic containers (6″ diameter) each filled with 2300 ml. of tap water. Except where specified, tests were conducted at room temperature (68 ± 2°F.) under a 10 hr. light, 14 hr. dark cycle. Larval *Culiseta incidens* (wild caught), *Culex pipiens* (laboratory strain), *C. tarsalis* (wild caught) and *Anopheles freeborni* (laboratory strain) were used as prey species for various parts of the experiment.

Feeding rates of the adults of four species of *Notonecta* were measured in Percival® constant temperature cabinets maintained at 40, 43, 47, 53, 59 and 65°F. *Culiseta incidens* 4th instar larvae were used as prey because they remained relatively active at all temperatures. Prior to each test at any particular temperature the adult notonectids were allowed 48 hrs. to acclimate to the temperature and were continuously fed mosquito larvae. Each container contained three adults of a particular species and ten prey. After 24 hrs. dead prey were counted and removed and replaced with live prey. Each experiment was run for at least 48 hrs. with two replicates for each species.

Various stages of *N. unifasciata* were given 2nd, 4th instar and pupae of *C. tarsalis* to study prey selection by the nymphal instars. Two predators of each instar (2nd instar through adult) were placed in plastic containers along with ten of each stage of prey (*C. tarsalis*). Counts of dead or moulted prey were made at 2, 4, 6 and 25 hrs. after addition of the predators. After each count, dead bodies and cast skins were removed but not replaced. Two controls without predators were run simultaneously.

The effect of floating vegetation (*Lemna* sp.) on the predatory efficacy of the various stages of *N. unifasciata* was studied in each of seven containers. Five grams of duckweed, drained and blotted dry on paper toweling, were placed on the water surface (154 cm^2) and spread out. To each container 20 each of 1st, 2nd, 4th instar larvae and pupae of *C. pipiens* were added. A single predator (all six stages represented) was added to each container. The remaining container served as a control. Three replicates were run concurrently and a count was made after 48 hrs. Live and dead larvae were counted as well as shed skins. When all larvae could not be accounted for, it was assumed that they had been eaten. Results were compared to an identical experiment run without duckweed.

To compare feeding preferences of adult *Notonecta* spp., a single adult male was placed in a plastic container with 10 fourth instar *Anopheles freeborni* and 10 fourth instar *Culex pipiens.* Dead larvae were counted and removed after 2, 4, 6 and 24 hours. Two replicates were run simultaneously.

Temperature studies indicated that all species of adults tested killed mosquito larvae at relatively low temperatures (Figure 1). *N. shooterii* demonstrated the greatest overall rate of predation as temperatures increased. All species had approximately the same attack rate at 70°F. Further tests are necessary to eliminate bias due to the physiological state of the species.

Comparative attack behavior of four species of *Notonecta* at room temperature with *Culex pipiens* and *Anopheles freeborni* as prey did not show any major differences in selection with the exception of *N. shooterii.* This species readily attacked *Culex* sp. over the *Anopheles* sp. (Figure 2).

Immature stages of *N. unifasciata* were compared as to preference of prey size by offering them equal numbers of 2nd and 4th instar larvae and pupae of *Culex tarsalis.* As suspected there was a general relationship between the size of the predator and size of the prey seized. Pupae were less preferred by all instars, however, adults and later instar nymphs will take them eventually (Figure 3 and 4). This underlines the necessity of feeding early instar mosquitoes

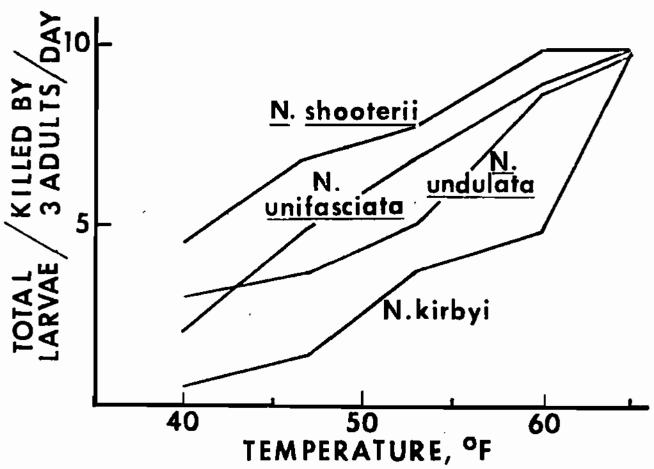


Figure 1.–Comparative predation rates of adult *Notonecta* spp. on 4th instar *Culiseta incidens* at various temperatures.

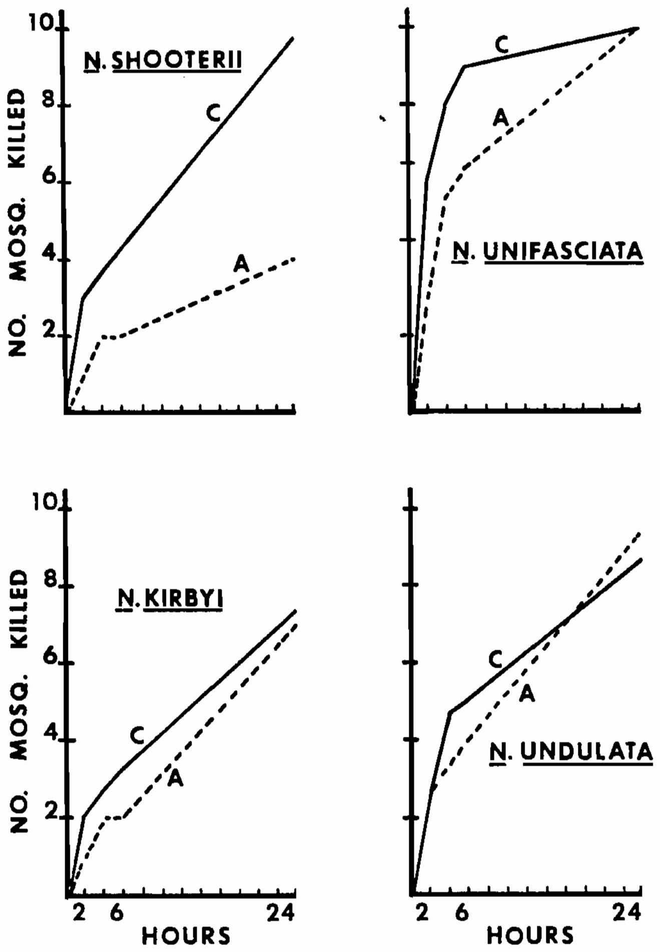


Figure 2.–Comparative feeding behavior of male *Notonecta* spp. on *Culex pipiens* (C) and *Anopheles freeborni* (A)

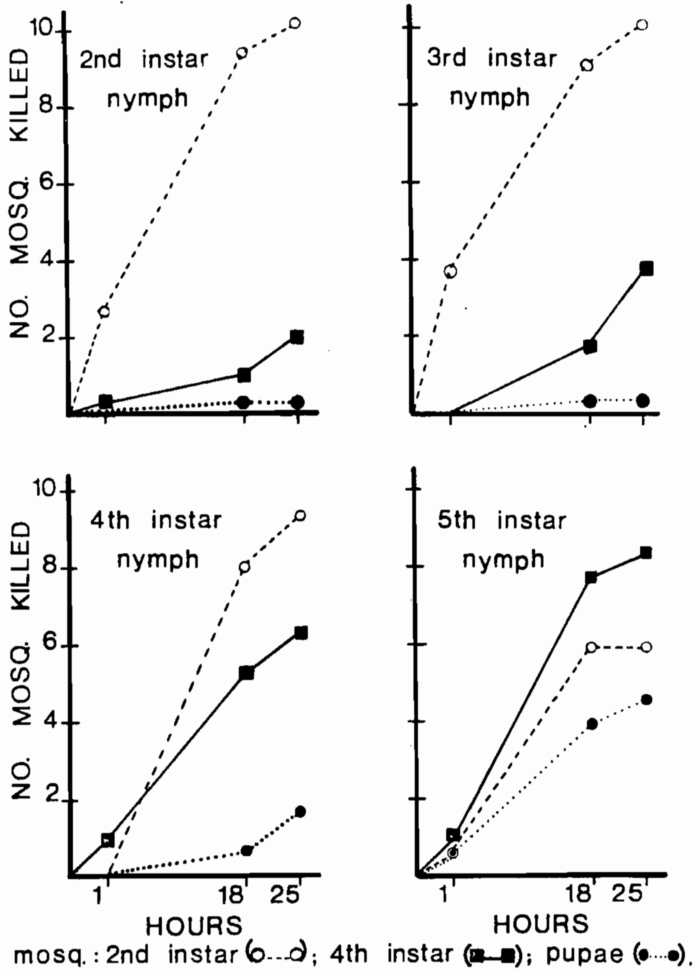
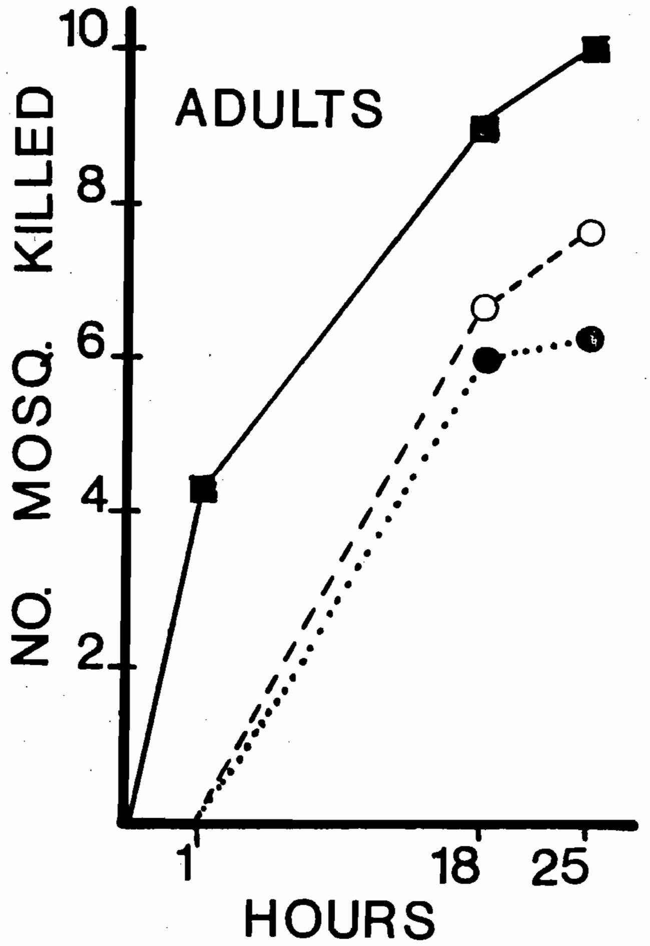


Figure 3.–Predation of *N. unifasciata* nymphs on *Culex tarsalis* immatures.

or some other small prey such as *Daphnia* to early stage nymphs for rearing purposes. It also suggests for optimum control possibilities in the field that partially developed eggs must be released early in the mosquito season.

Figure 5 shows the attack behavior of all stages of *N. unifasciata* against most stages of *C. pipiens* and the interference effect of duckweed (*Lemna* sp.), a floating plant sometimes found in association with this mosquito. A rather strong correlation was again noted for size of prey in relation to size of predator. Early instar notonectids restricted their attack essentially to early instar mosquitoes. Fifth instar nymphs took very few first instar larvae while adults failed to feed on this stage at all. Some pupae were consumed by later instar nymphs and adults but the late instar larvae were preferred. Fourth instar nymphs appear to have the broadest selection proportionately as well as attacking the greatest number of individuals. *Lemna* sp., with its trailing roots, reduced attack effectiveness by about 50% in the first 4 nymphal instars. Differences were less pronounced in the last instar and adult.



mosq. : 2nd instar (o---o); 4th instar (■—■); pupae (●---●)

Figure 4.–Predation of *N. unifasciata* adults on *C. tarsalis* immatures.

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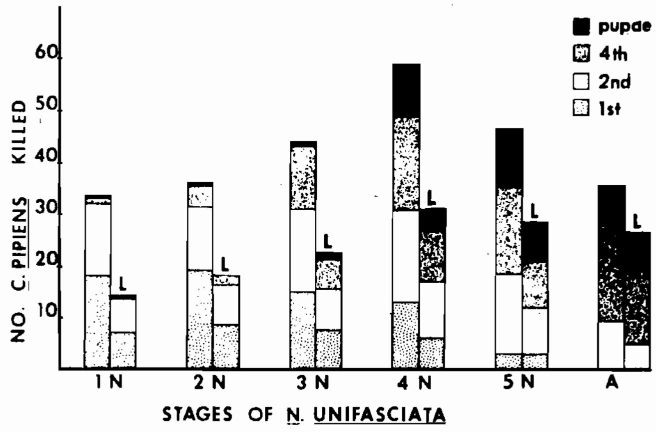

Figure 5. Attack behavior of *N. unifasciata* stages on *Culex pipiens* with and without *Lemna* after 48 hours.

MUCILAGINOUS PLANT SEEDS: POTENTIAL AGENTS FOR BIOLOGICAL CONTROL OF MOSQUITO LARVAE

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Reeves and Garcia (1969) observed that mosquito larvae subsequently die when they become attached by their oral brushes to the mucilage halo which surrounds certain immersed plant seeds. We have confirmed this phenomenon of mucilaginous seeds entrapping *Culex quinquefasciatus* larvae (3rd - 4th instar). The attachment of larvae to certain mucilaginous seeds occurred at a much higher rate than could be accounted for by random location and suggested that these seeds might be exerting a positive attraction for mosquito larvae. This paper presents the results of an investigation which pertains to the chemo-attraction of plant seeds for mosquito larvae and their interaction following attachment.

The mucilaginous seeds used in this study were *Capsella bursa-pastoris* (Shepherd's Purse), *Descurainia sophia* (Tansy-mustard), *Linum usitatissimum* (Common flax), *Plantago insularis* (Plantain), and *Sporobolus airoides* (Fine-top salt grass). The non-mucilaginous seeds used were *Brassica* sp. (Mustard), *Magnolia grandiflora* (Evergreen magnolia), *Phaseolus* sp. (Bean), and three varieties of *Pisum sativum* (Early Alaskan Pea, Mammoth Melting Sugar Pea, Improved Telephone Pea). *Culex quinquefasciatus* larvae (3rd - 4th instar) were used in all of the experiments.

A plexiglass experimental chamber (43 x 6 x 5 cm) with a permeable barrier of fine grade copper screen located 2.5 cm from one end of the chamber separated the seed area from the remaining chamber area. Starting from the permeable barrier, five zones were located 7.5 cm apart. The chamber was filled with 700 ml of charcoal-filtered deionized water (24°C). Two grams of seeds were completely submerged in the seed area. Approximately 100 mosquito larvae were released in the middle of the chamber (zone 0) at the start of the experiment. At least three trials were run for each seed studied. The entire chamber was placed in a light-tight box. At the termination of an experiment (1 hr), the zone divider release string was pulled from outside the box, allowing the zone divider to fall into place and divide the chamber into five areas without disturbing the larval distribution. The chamber was removed from the dark and the numbers of larvae in each zone [+2(closest zone to seed area), +1, 0, -1, -2] were recorded. If the number of larvae remaining in zone 0 was divided equally between the positive and negative groups, a positive attraction percent was recorded when 66% of the larvae were positive.

All mucilaginous seeds showed a positive chemo-attraction for mosquito larvae. Non-mucilaginous seeds did not attract mosquito larvae, with the exception of *Pisum sativum* (Early Alaskan). The seeds that produce mucilage and cause larvae attachment (*C. bursa-pastoris* and *D. sophia*) were strongly attractive after one hour, while the other mucilaginous seeds to which larvae did not attach were not attractive until after 2 - 4 hours of incubation. The attraction of *C. bursa-pastoris* seeds for the larvae following heating (155° for 30 min.) and freezing (0° for 4 hr.) was unaltered and was also present following dialysis.

The mucilaginous seeds appear to emit a substance(s) that is toxic for the mosquito larvae when both are incubated together. This toxic factor can be demonstrated when the larvae are separated from *C. bursa-pastoris* seeds by a permeable barrier which prevents attachment. The mosquito larvae may be dying from this toxic substance after they have become entrapped by the mucilaginous seed coat.

Capsella bursa-pastoris seeds are capable of entrapping and killing the mosquito larvae even after being soaked in deionized water for five months. After several days of incubating seeds and larvae together, the dead larvae attached to seeds begin to undergo rapid breakdown. It was determined that *C. bursa-pastoris* seeds secrete a proteolytic enzyme that may be responsible for the breakdown of the attached mosquito larvae.

In simulated field conditions in the laboratory with field water and soil, 86% of the larvae were entrapped by *C. bursa-pastoris* seeds after 48 hours. Results from 24-hour field experiments show that as in the laboratory, mucilaginous seeds are able to entrap mosquito larvae. The attachment percent in the field trials (30-58%) is dependent upon the volume of water in the test site. The field study indicates that there is a possible interaction of plant seeds and mosquito larvae in nature.

The presence of a chemo-attractant, a toxic factor, an entrapment mechanism, and a possible digestion process indicate that plant seeds do have biological control properties for mosquito larvae.

ACKNOWLEDGMENTS.—All seeds except those of *S. airoides* were kindly supplied by Dr. W. H. Tallent of the USDA, Agricultural Research Service in Peoria, Illinois. Dr. A. L. Larsen of the Colorado State University Seed Laboratory, Fort Collins, Colorado, generously provided *S. airoides.* We express our gratitude to the Jefferson and Orleans Parish Mosquito Control Districts for supplying mosquito larvae. This investigation was supported by a grant from the Louisiana Mosquito Control Association.

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STUDIES OF INSECT PREDATORS AS AGENTS TO CONTROL MOSQUITO LARVAE, WITH EMPHASIS ON STORAGE OF *NOTONECTA* EGGS

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The development of insecticide resistance has brought into perspective the importance of utilizing biological agents whenever possible in mosquito control. Predatory insects especially are receiving closer attention as their role in natural control becomes clearer (Hall et al. 1953; James 1964, 1966, 1967; Notestine 1971; Roberts et al. 1967; Veneski and Washino 1970; Washino 1969), particularly the Notonectidae (Ellis and Borden 1969a, b, 1970; Garcia 1973; Hazelrigg 1973; Toth and Chew 1972a, b). The detrimental effects of *Gambusia affinis* (Baird and Girard) on predaceous insects have been suspected when mosquito population surges followed fish introduction at low stocking rates (Hoy et al. 1972). Washino (1968) and Washino and Hokama (1967) showed that less than 1% of this predator's diet in rice fields usually consists of mosquito larvae. Such evidence has led us to consider native insect predators as additional means of control in semi-permanent waters.

The potential for manipulating invertebrate predators in seasonal mosquito habitats was brought into focus during the summer of 1973, when control of the encephalitis vector, *Culex tarsalis* Coquillett, as indicated by pupal sampling and adjacent adult natural resting site counts, was achieved on 6,000 acres of Kern River snowmelt water, without the use of insecticides (Kern Mosquito Abatement District, Entomologist's Report, June 1973). This water was spread in 12 locations, for a 17 week period, as part of a groundwater recharge program, much of the acreage being on native desert land. Densities of *C. tarsalis* pupae during this period averaged 0.05/dip or lower. It appears that agricultural activities may have affected insect predator populations in other parts of Kern County where such spectacular low counts were not obtained. A scarcity of aquatic overwintering habitats, the existence of *G. affinis* in most sites with water, and irregular irrigation schedules may have been detrimental to their survival. The lack of overwinter habitats may prevent the normal colonization of spring mosquito habitats, and irregular irrigations cause drying before completion of immature predator development. In the absence of *G. affinis*, spring insect collections from *Culex* habitats without adjacent riparian systems rarely show the number or diversity of predaceous forms common to sites in close proximity to overwintering habitats.

In the aforementioned Kern River study, a complex of 10 native insect predators is believed to have contributed significantly to the high level of control achieved although determination of the role each predator played in larval population regulation was not assessed. Better methods of quantitatively sampling the aquatic habitat are required for fast moving predators.

Mass production and storage techniques for those predators demonstrating greatest predation ability are advantageous for maximum utilization in control programs. Evidence to data points to Notonectidae as one of the most important predator groups, and research emphasis is directed to their use by way of inoculative and inundative releases each spring in favorable habitats.

In theme with the above goals, an investigation was initiated to determine the viability of *Notonecta unifasciata* Guerin eggs maintained under prolonged laboratory storage at different temperatures and periods of embryonic development (Sjogren et al. 1974). Eggs collected from adults fed mosquito larvae were incubated at 25.6°C. to achieve three embryological stages (1, 4, and 8 days old), 5 replicates of 25 eggs each were stored at 2.2°, 8.3°, and 14.4°C., for 4, 6, 8, 10, and 12 week periods. Control egg groups were held at 25.6°C.

There was a progressive loss in egg viability observed at increasing lengths of storage for each age group, particularly at the lower temperatures. However, the oldest embryos were generally able to survive most successfully. All age groups had progressively greater survival rates at the higher storage temperatures. Although maximum viability was achieved at 25.6°C., a temperature which did not inhibit development, for storage purposes greatest viability was observed with 8 day old eggs at 14.4°C.

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MOSQUITO DENSITY AND BIRD-MAMMAL FEEDING RATIOS OF *CULEX TARSALIS*[1]

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ABSTRACT

Observations were made on the blood-feeding behavior of *Culex tarsalis* attracted into stable traps, each of which was baited both with a chicken and a jackrabbit. Although most feedings were on the chicken, there was a trend toward increased jackrabbit feeding, but poorer overall feeding success, as larger numbers of females were collected; but a need for more definitive data was apparent.

Relatively small numbers of *Culex erythrothorax*, *Anopheles freeborni*, *Aedes nigromaculis*, *A. melanimon*, and *A. vexans* also were collected, and all but *C. erythrothorax* fed mainly on the jackrabbit. However, nearly 30% of the engorged *A. freeborni*, *A. nigromaculis*, *and A. vexans* fed on the chicken.

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SIMULATED-FIELD PREDATION OF SINGLE-PREY (*CULEX PEUS*) AND ALTERNATIVE-PREY (*CULEX PEUS; CHIRONOMUS* SP. 51) BY *ANAX JUNIUS* DRURY (ODONATA: AESCHNIDAE)

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ABSTRACT

Simulated-field predation tests with the predator *Anax junius* were conducted in enclosed-unit fiberglass tubs, with both single and alternative-prey available (*Culex peus; C. peus* and *Chironomus* species 51 respectively). *C. peus* egg rafts and *C.* sp. 51 egg masses were periodically introduced to sustain prey populations, and daily prey emergence was recorded. Predators were initially introduced at three densities and monitored at two week intervals for growth and population size.

Results showed *A. junius* naiads controlled mosquito populations at all three densities in the single, and the two higher densities in the alternative-prey tests. In both tests, the higher predator densities were seen to reduce prey populations in a shorter period of time. However, with subsequent mosquito reduction predator populations also declined to a few remaining large naiads. These reduced naiad populations continued to maintain control of the mosquitoes. With the presence of an alternative-prey, predator populations still declined after mosquito reduction, but remained significantly higher than in the single-prey tests. Naiads exercised no significant control over the midge populations.

INTRODUCTION.–There are almost one half million acres of rice fields in California (Hoy and Reed 1970). Each year mosquito populations become a more serious problem due to continued resistance to insecticides. Although the larvivorous fish *Gambusia affinis* is frequently utilized as a biological control agent, control agencies still rely primarily on insecticides and source reduction procedures for mosquito control. The necessity for continued investigation of both old and new biological control agents to augment control programs is clearly now at hand.

Anax junius Drury (Odonata: Aeschnidae) is found throughout North America. The immatures or naiads are found typically in small ponds and lakes with emergent vegetation, in which adult females lay their eggs. Although capable of over-wintering in the northern temperature zones, *A. junius* is more populous in warm southern regions, with adults being found year-round in Florida (Needham and Westfall 1955). Certain attributes of *A. junius* naiads indicate that they might be effective agents for biological manipulation in reducing mosquito larval populations. Attributes of the naiads include excellent vision (Pritchard 1963), voracity (Hinman 1934), and active prey search (Lee 1967).

Like most Odonata, *A. junius* is recognized as a general predator. Its potential as a mosquito predator was first recognized by Hinman (1934) and Wright (1946). Although *A. junius* has been studied occassionally, little quantitative information regarding its predatory habits is yet available.

Gut and fecal analyses indicate the general predaceous habits of both adults and immatures. Warren (1915) found chironomids and ostracods to comprise the main part of the diet of *A. junius* and *Pantala flavescens* (Libellulidae) in Hawaii. Other organisms in gut contents were mosquitoes, dytiscids, shrimp, segmented worms, tadpoles and top minnows. Kingsbury (1937) reported significant losses in fish hatcheries due to predation by large *A. junius* naiads.

Pritchard (1964) investigated the food of dragonflies (*Aeschna* spp., *Anax* spp.: Aeschnidae; *Libellula* spp., *Pantala* spp.: Libellulidae) in Alberta, Canada, and found the type of food consumed was related to the quantity of available prey rather than to quality preference. Chironomid larvae, the most numerous organisms in pond samples, were the most common naiad food. When mosquito larvae were present, they were found in *A. junius* gut contents. Pritchard (1964) and Happold (1965) have attributed any absence of mosquito larvae in the naiad diet to habitat incompatibility, for dragonflies (in Canada) typically require permanent bodies of water whereas most mosquitoes of Alberta frequent temporary ponds. Similar results have been reported on the food of damselflies in different pond habitats in the Soviet Union (Fischer 1964).

METHODS.–Outdoor field units were provided water from an irrigation reservoir (pH 8.5 ± 0.5; CaOH 171 ± 17 ppm), filtered through six feet of sand and gravel.[1] Naiads were initially collected as eggs in stems of water grass, *Echinochloa crusgalli* (Gramineae) and the sedge *Scirpus* prob. *pacificus* (Cyperaceae). These eggs were incubated in the laboratory and reared at 31°C to the second instar (2-3mm) for field release. *Culex peus* egg rafts were collected from an outdoor rearing facility to be introduced to all predation tests, described in more detail in the following pages. *Chironomus* sp. 51 egg masses were collected in the same manner for introduction to the alternative-prey tests. Larvae of both Nematocerous species were fed with sifted, autoclaved chick-starter, a commercial poultry food. Water temperature was continually recorded at mid-depth with a remote reading, recording hygrothermograph. In treatments involving single and alternative-prey, units and their compartments were chosen at random.

Enclosed, controlled predation tests involved the use of fiberglas tub units (Figure 1) 96x96x46 cm. The tubs were translucent, admitting considerable light to each treatment. Each tub was compartmentalized into a test and check half with a double saran screen divider (32 mesh). The water level in each tub was maintained at 13-15 cm by a commercial float-valve. Thirty-two bamboo sticks were arranged in four rows (8 sticks/row, Figure 2) per treatment to simulate plant stems in the natural habitat. Daily prey emergence (adult midges and mosquitoes) was recorded from each

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[1] Research conducted at the aquatic research station, University of California, Riverside: July through November, 1971.

treatment via emergence receptacles (Figure 1). Naiads were introduced as second instars to minimize natural mortality, for laboratory Life History (LH) studies indicated a large mortality factor during the egg and first instar (Beesley, Ph.D. Thesis). Predator populations were then monitored every two weeks.

Two separate tests utilized these enclosed fiberglas units. The first, a single-prey species test, evaluated naiad control of mosquito populations in the units at three predator densities: 25, 50 and 100 naiads per treatment. Depending on availability, from 1-3 egg rafts of *C. peus* were introduced daily to each test and check treatment, together with 5 gm of chick-starter. The second test, an alternative-prey study, evaluated naiad control of mosquito populations when an alternative-prey was available, i.e., midge larvae. *C. peus* egg rafts and 10 gm of chick-starter were added in the same manner as in the single prey test, together with one egg mass of *Chironomus* 51 at 7-10 day intervals. Three densities of naiads were again initially introduced; 10, 20 and 40 per treatment. Predator numbers differed because of limited time and rearing facilities. However, the densities of 25 and 50 in the single-prey test and 20 and 40 in the alternative-prey test were considered sufficiently close for director comparison of results.

RESULTS AND DISCUSSION.—Single-prey tests resulted in complete mosquito control at all three naiad densities (Figure 3). Prey regulation was affected earlier at the two higher predator densities. There was a 19-day lag in control with 25 naiads/unit, and only a 12-13 day lag with 50 and 100 naiads/unit. With subsequent prey reduction, predator populations rapidly declined (Table 1).

In the alternative-prey test, mosquito populations in the check treatments were similar to those attained in the first test (Figure 4). Midge populations were slightly lower than mosquito populations (Figure 5). At 10 naiads/unit, partial control was achieved, being apparent 16 days after introduction (Figure 4). At 20 and 40/unit, complete control was evident within 12-14 days (Figure 4). There was no significant reduction of the midge populations (Figure 5). Predator density declined with mosquito control, but not as greatly as in the first test (Table 1).

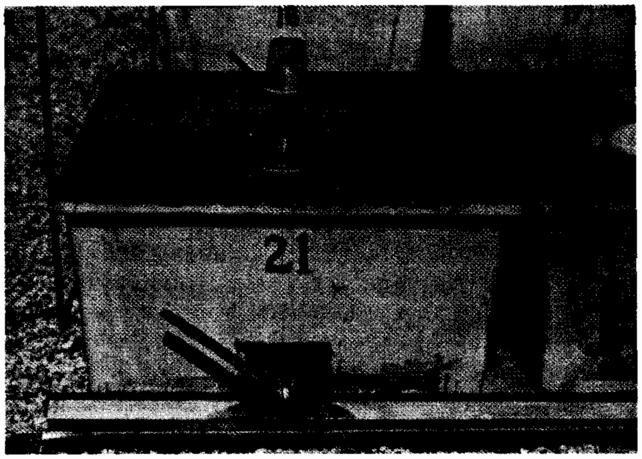

Figure 1.—Outdoor fiberglas unit used for enclosed, simulated-field predation tests. External view of tub showing receptacles for adult midges and mosquitoes.

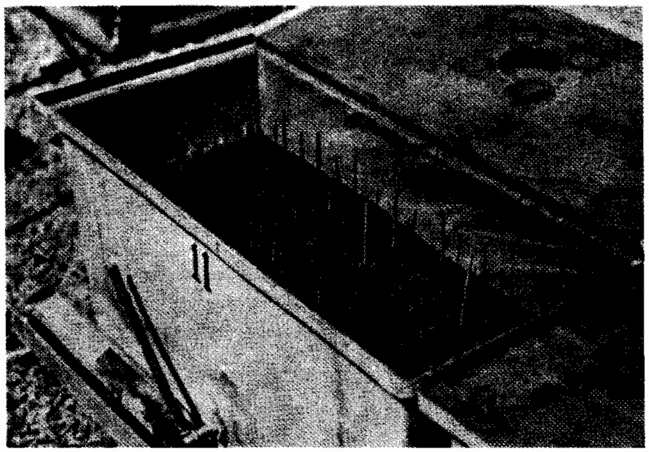

Figure 2.—Internal view of same unit, exposing vertical rows of bamboo sticks.

Table 1.—Numbers of *Anax junius* surviving in enclosed-unit predation tests.

	Single Prey: *Culex peus*				Alternative Prey: *Culex peus* *Chironomus* 51		
Days from Introduction	Initial Naiad Density			Days from Introduction	Initial Naiad Density		
	25	50	100		10	20	40
14	25[a]	19 ± 8	16 ± 5	14	8 ± 1		22 ± 6
29	20 + 3	5 + 2					
29	20 ± 3	5 ± 2	4 ± 1	21		15 ± 2[b]	
42	15 ± 7	4 ± 2	22 ± 1	30	7 ± 1	15 ± 2	17 ± 5
				44	6 ± 2	13 ± 5	13 ± 4
				60	6 ± 2	12 ± 5	13 ± 4
$X_{^oC}$ = 26 ± 1 (21-33°C)				$X_{^oC}$ = 20 ± 3 (16-28°C)			

[a] Naiads introduced two weeks late.

[b] Naiads introduced 5 days late.

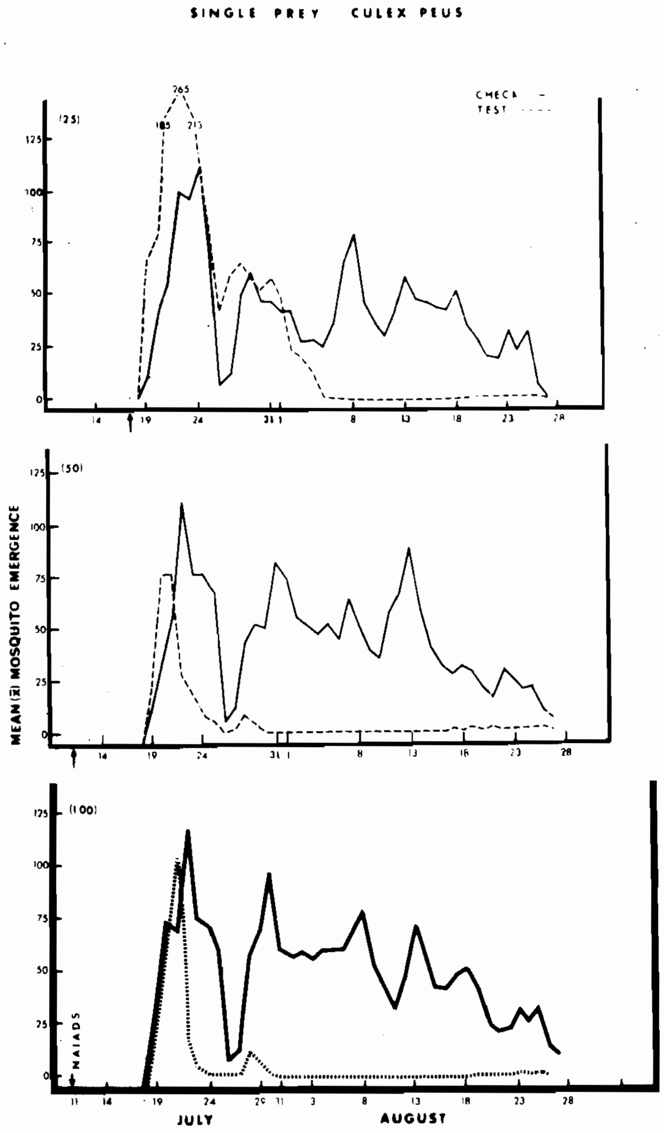

Figure 3.--Test 1. Average daily mosquito emergence in the single-prey species test. Initial naiad density is indicated ( ) in the upper left corner.

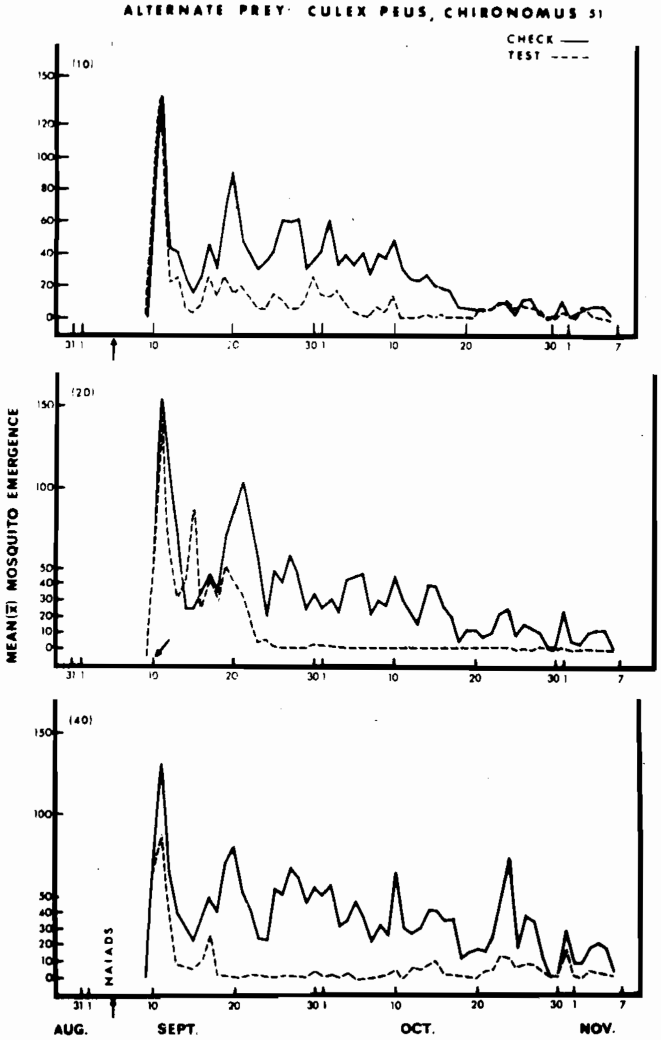

Figure 4.--Test 2. Average daily mosquito emergence in the alternative-prey species test. Initial naiad density is indicated ( ) in the upper left corner.

Comparison of the predator populations revealed an interesting trend. With *C. peus* as the sole food source, naiads decimated prey populations within 2-3 weeks, after which little food was available. As a result, naiad populations declined rapidly to a low population equilibrium, yet were still capable of regulating the mosquito populations. Within four weeks of introduction naiad densities of 25, 50 and 100 were reduced to means of 15 ± 7, 4 ± 2 and 2 ± 1 (Standard Deviation) respectively. In contrast, naiad populations in the alternative-prey tests remained considerably higher, even though mosquito populations were consumed. After four weeks naiad densities of 10, 20 and 40 maintained an equilibrium of 6 ± 2, 13 ± 5 and 13 ± 4 respectively. From an initial naiad density of 50 in the first and 40 in the second test, a significant difference in predator equilibria was apparent; i.e., a reduction from 50 to 4 ± 2 vs. 40 to 13 ± 4 within one month. Although not as obvious when comparing densities of 25 vs. 20, this trend was still apparent. Initially both populations dropped an average of 5 naiads (25: 20 vs. 20: 15) within two weeks. In the first test this trend continued for the following two weeks while an equilibrium of 15 was maintained for the duration of the second test (Table 1).

Naiad populations were related to food supply. When the initial prey population (mosquitoes) was consumed, cannibalism occurred, probably due to increased food requirements with naiad growth. Cannibalism occurred in both tests, but significantly less in the second due to the increased available food, i.e., midges. These closed systems were capable of supporting much higher (ca. 3X) predator populations than attained in the presence of a single-prey.

Many of the units were contaminated with small Cladocera. Although *A. junius*, particularly the smaller naiads, do feed on this small Crustacean, it did not act as

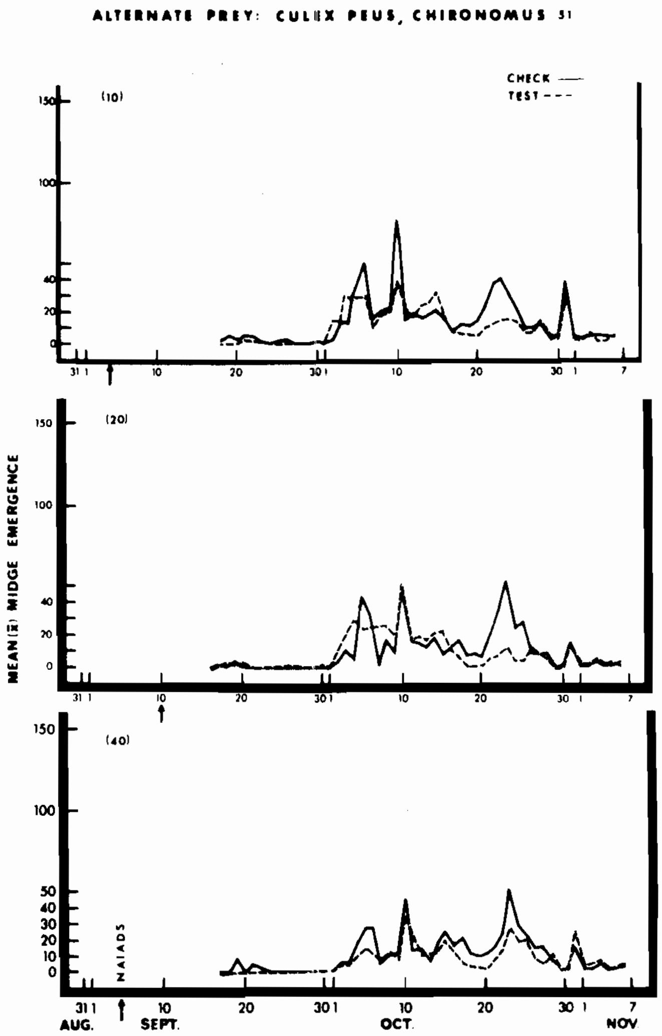

Figure 5.--Test 2. Average daily midge emergence in the alternative-prey species test. Initial naiad density is indicated ( ) in the upper left corner.

a buffer on the predator populations in the single-prey test compared to the midge larvae in the alternative-prey test. Several units were also contaminated by the very small midge *Paralauterobomiella* spp. which were also considered inconsequential to the naiad populations.

Predation tests were conducted during overlapping seasons, the first during mid-summer and the second during late summer and fall. Water temperatures were similar for most of the duration of both tests, declining in the late fall during the second test (Table 1). *Anax junius* does not diapause but simply retards development with the onset of winter conditions. Declining photoperiod and temperature did affect naiad food consumption, evident in the latter part of October with emergence of a few adult mosquitoes.

The main limitation of this predator is attributable to pond stability. Essentially, *A. junius* needs a permanent or semi-permanent pond with emergent vegetation for successful oviposition. Mosquitoes typically frequent temporary water while midges are more apt to be found in permanent or stable ponds. Previous food analysis has revealed this habitudinal incompatibility (Happold 1965; Pritchard 1963). In California rice fields this is not the case; these areas are flooded seasonally, providing suitable habitat for mosquitoes, midges, dragonflies and other biota. These fields are drained annually in the fall with crop harvest thus destroying large overwintering sites for this indigenous predator *A. junius*.

In conclusion, these simulated field tests revealed three significant findings. (1) *Anax junius* naiads qualitatively preferred *C. peus* to the benthic *Chironomus* 51 larvae. (2) Mosquito control was attained under these circumstances, indicating a potential for such under field conditions. (3) With the presence of an alternative-prey, significantly higher predator densities were sustained. These data indicate a potential for *A. junius* as an applied biological control agent. The active role of this predator may now be considered in future control programs.

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MOSQUITO CONTROL WITH EUROPEAN GREEN HYDRA IN IRRIGATED PASTURES, RIVER SEEPAGE AND DUCK CLUB PONDS IN KERN COUNTY[1]

Hyo-sok Yu[1], E. F. Legner[2], and R. D. Sjogren[3]

Chlorohydra viridissima (Pallas) was used successfully to control *Aedes nigromaculis* (Ludlow) and *Culex tarsalis* Coquillet populations in irrigated pastures, in Kern River seepage, and in duck club habitats of Kern County in 1973.

Hydra were cultured at the Kern Mosquito Abatement District under controlled temperatures of 25° + 2°C. as described by Lenhoff and Brown (1970). Inoculation experiments were conducted in both open and weed-covered habitats. In the open type, a pasture and river seepage habitat were used. Hydra were released in 1-M^2 net enclosures (11.8 mesh/cm plastic) that were placed in the midst of high density mosquito larval populations (100+ /dip). The square enclosures extended to a depth of 89 cm with redwood stakes at each of the 4 corners. From 10 plots selected at random in a 2.5 acre irrigated pasture on July 26, 5 each were inoculated at random with 500 hydra, and 5 left as controls. Four random 400 ml dip samples were taken from each plot in the pasture daily until the water dried. Daily sampling was continued at the same sites after reflooding.

In river seepage water, similar replicates were selected but each net enclosure was subdivided into 2 equal halves, with one half of each enclosure being inoculated with 250 hydra on July 2 and daily larval samples taken beginning June 28.

The second type of experiment was performed in emergent weeds (*Scirpus* and *Distichlis* spp., etc.) of a duck club habitat. Here hydra were released at selected sites without enclosures, but where *Culex tarsalis* breeding was prominent. Ten plots were chosen at random in two 4-acre ponds. Five such sites were inoculated with 1500 hydra each, with 5 being left as controls. Larval samples were taken weekly, 2 dips in each of 4 different directions from the plot center and up to and including a radius of 152 cm.

Hydra inoculations were made using the chilled volumetric method of Lenhoff and Loomis (1957). Inoculation rates in all experiments were ca. (circa = about) 1 hydra/10 cm^2 of water surface.

Hydra were sampled using 3 different methods according to conditions found in each habitat. Since the general method of field collection reported by Kanaev (1952) and Southwood (1971) was unsuitable to our experiment, the most appropriate technique employed 4 vegetation substrate samples per replicate for pastures, 4 bamboo stake samples (Yu & Legner 1973) for river seepage areas, and 160 bamboo stakes combined with 16/replicate thin vertically placed white plastic plates. 30.4 x 10.2 cm. Bamboo stakes were spaced ca. 15 cm apart and plates ca. 30.5 cm apart. Hydra that became attached to the stakes and plates were counted immediately in the field and returned to each respective plot.

Statistical differences were tested between control and treatment means using Student's "t" test (Snedecor 1946); and no transformations were required of the normally distributed data.

RESULTS AND DISCUSSION

Aedes nigromaculis Habitat.—A significant host population reduction appeared 24 hours after hydra were inoculated in pastures, with 57.8% actual destruction (Table 1). Host destruction gradually increased to 84.5% on July 30, the differences in density between inoculated plots and the controls being significant at the 95% level. The pasture mosquito population before treatment averaged 11.70 larvae/dip in the control and 11.75/dip in the treated areas. Mosquito density in the controls peaked on July 29, while areas that had been inoculated with hydra were significantly lower (95% level) (Table 1). After drying and subsequent reflooding on August 14, the *Aedes* population did not recover the high density observed in July, but a significantly lower density was recorded (95% level) in the hydra treated plots (Table 1).

The hydra population during the first irrigation interval could not be determined; however, it was confirmed through observation of vegetation substrates that hydra had

Table 1.—Average number of *Aedes nigromaculis* larvae/400-ml dip in irrigated pastures treated with *Chlorohydra viridissima* as compared to a control. Kern County, 1973.

	Avg. larvae/dip		
Sample Date	Control	Treated	% Destruction[1]
Pretreatment			
July 26	11.70	11.75	
Post Treatment			
July 27	6.28	2.65	57.80[2]
July 28	15.23	6.88	54.83[2]
July 29	21.50	6.42	70.14[2]
July 30	10.50	1.63	84.48[2]
August 14	1.05	0.43	59.05[2]
August 15	1.13	0.73	35.40[2]

[1] 100 - (treatment/control) x 100.

[2] significant at 95% level.

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survived the drying period in low numbers (about 5% of the inoculated population). Temperature conditions during the pasture period were within the tolerable range of 25-38°C. determined for this hydra strain.

Culex tarsalis Habitats - River Seepage.—The first significant *C. tarsalis* population reduction was 28.7% recorded 24 hours after hydra inoculation (Table 2). On the second day, the reduction was increased to 38.9% (Table 2). The overall population destruction was 33.9% which was lower than in the *Aedes nigromaculis* experiment (67.2%). These reductions were significantly different at the 95% level. The control *Culex* population before treatment was not significantly different from the treatment plots (Table 2). Lower efficiency of control in river seepage may be related to comparatively poor adaptability of green hydra to this habitat, as determined previously with the method of Yu and Legner (1973). Continued monitoring of this population was precluded by unexpected appearance of *Gambusia* top minnows in the test area.

Table 2.—Average number of *Culex tarsalis* larvae/400-ml dip in Kern River seepage treated with *Chlorohydra viridissima* as compared to a control. Kern County, 1973.

Sample Date	Avg. larvae/dip Control	Avg. larvae/dip Treated	% Destruction[1]
Pretreatment			
June 28	55.67	62.83	
Post Treatment			
July 5	118.21	84.25	28.73[2]
July 6	121.31	74.06	38.95[2]

[1] 100 - (treatment/control) x 100.

[2] significant at 95% level.

Duck Club Ponds.—The first significant population reduction of 49.3% was obtained one week after the hydra treatment (Table 3). Thereafter, % destruction increased progressively until October 13 to a maximum of 79.2%. After this date the degree of destruction gradually decreased to 66.7% and 50% on November 15 and December 20, respectively (Table 3). No significant differences were detected at the 90% level after taking the November 15 sample. The overall average destruction was 63.7%, significant at the 95% level.

The presence of hydra kept the *C. tarsalis* population density below the arbitrary threshold of 1/dip beginning two weeks after initial inoculation (Table 3). These results closely paralleled those obtained with *C. peus* and *C. pipiens quinquefasciatus* in 1972 (Yu and Legner 1973).

Table 3.—Average number of *Culex tarsalis* larvae/400-ml dip in duck club ponds treated with *Chlorohydra viridissima* as compared to a control. Kern County, 1973.

Sample Date	Avg. larvae/dip Control	Avg. larvae/dip Treated	% Destruction[1]
Pretreatment			
August 29	4.06	6.44	
September 8	5.81	7.03	
Post Treatment			
September 22	4.30	2.18	49.30[2]
September 29	3.40	1.12	67.06[2]
October 5	1.80	0.54	70.00[2]
October 13	0.48	0.10	79.17[2]
November 15	0.06	0.02	66.67
December 20	0.07	0.03	57.14

[1] 100 - (treatment/control) x 100.

[2] significant at 95% level.

The hydra population measured as the mean number / 20 bamboo stakes was 0.35 / row, two weeks after inoculation. This increased to 0.55 / row at the end of the third week. A measurement with plastic samplers showed hydra numbers of 22.3 / sampler one month after inoculation and an increase to 32.0 / sampler in the following month (ca. 43% increase). However, the budding index, i. e., a measure of environmental favorability, decreased slightly from 0.42 to 0.35.

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LABORATORY AND SMALL-SCALE FIELD EXPERIMENTS WITH PLANARIA (TRICLADIDA, TURBELLARIA) AS BIOLOGICAL MOSQUITO CONTROL AGENTS[1]

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The extraordinary regenerative capabilities of planaria (Turbellaria: Tricladida: Platyhelminthes) were known since the early 1800's, some investigators considering them almost immortal under the edge of a knife (Bronsted 1969). The ability of planarians to devour mosquitoes among other prey was soon discovered, and Jenkins (1964) included planaria in his list of mosquito predators, thereby pointing out the need for investigations in biological control. To date there have been few formal attempts to explore mosquito control possibilities with planaria.

The Turbellaria are nearly all free-living, non parasitic worm-like animals (Borradaile and Potts 1958). Among the 6 orders are species that have great potential as predators of mosquitoes in fresh water habitats. Some genera in the order *Rhabdocoela*, such as *Microstomum*, retain nematocysts in their ectoderm which are derived from Coelenterates on which they have fed (Kepner and Barker 1924). Such structures are useful in warding off predators, but also may be useful in killing mosquitoes.

Our attention was drawn to a contaminate culture of *Dugesia dorotocephala* (Woodworth) (Tricladida) in mosquito rearing ponds at the University of California, Riverside in 1971 (Legner and Medved 1972). Repeated subsequent observations of this species in ponds followed by eradication of *Culex peus* Speiser, *C. tarsalis* Coquillett, and *C. pipiens quinquefasciatus* Say immature stages gave startling testimony of the planarian's destructive capabilities. Studies were begun to examine *D. dorotocephala* as a predator of *C. peus* under controlled laboratory and field experimental conditions.

Laboratory studies in 1 L. polystyrene aquaria showed that reproduction of *Dugesia* by transverse fission was accelerated by higher feeding rates and crowding (Medved and Legner 1974). Loss of appetite in culture could be offset by increasing the density of *Dugesia*.

Field experiments in 1-m^2, 0.2 m deep redwood sided ponds showed that 100 inoculated *Dugesia* significantly suppressed second and third instar *Culex peus* that were provided at the rate of 200/week from July 16 through August 15 (Table 1, Medved and Legner 1974).

A comparison between a 2-year old laboratory culture of the *D. dorotocephala* planaria with 2 cultures obtained from Lytle Creek in the San Gabriel Mountains 42 km. distant on 2 separate dates in September, 1973 showed a slightly greater, but not significant rate of multiplication in the laboratory culture. There were no apparent differences in their respective feeding rates on *Culex peus* (Medved and Legner 1974).

DISCUSSION.—*Dugesia dorotocephala* is negatively phototactic and remains hidden under rocks and debris in the water during daytime. In darkness it crawls and slides over solid surfaces, and glides through the water in what appears to be a search for food. The sticky mucus is secreted by epidermal glands which aids in attachment to surfaces and in the capture of prey. Besides mosquito prey, various species of planaria are known to feed on small crustaceans and other small aquatic organisms, which they trap in their secreted mucus (Castle 1928, Hyman and Jones 1959, Storer and Usinger 1965). Dead organisms are also consumed. In the absence of food, planaria are able to resorb internal organs, thereby avoiding early death (Storer and Usinger 1965).

Planaria have never been mass produced in numbers suitable for mosquito control, but asexual reproduction is known to be enhanced by certain chemical substances by increasing planaria density (Bronsted 1969), and the temperature of the rearing medium (Castle 1928). Mechanical sectioning of individuals can result in the formation of several regenerated individuals, but the technique is laborious, does not consistently produce regenerated individuals and is probably unsuited for mass production.

Behavior studies in laboratory aquaria and in an outdoor bentho aquarium (Bay 1972) showed that *Dugesia* is capable of killing and consuming the fluid contents of all *Culex* larval instars as well as the pupa and egg rafts. Mucus secretions effectively immobilized *Culex* larvae which could then be consumed later. The secretions were also used to cement particles of sand together which served as anchors to which mosquito larvae were actively attached. The cemented particles also could serve as resting sites for *Dugesia*.

Table 1.—Average density of *Culex peus* larvae in 1-m^2 ponds in the initial presence of 100, 1.4 cm *Dugesia dorotocephala* on July 16, compared to a control. Riverside, California, 1973.

	Avg. No./40-ml dip		
Sample Date	*Dugesia* Present[1]	Control[2]	% Destruction[3]
July 20	0.25	4.50	99.44
July 27	0.37	6.75	94.52
August 2	0.63	7.00	91.00
August 10	0	2.50	100
August 15	0.12	3.75	96.80

[1] Average of 2 replicates.
[2] Average of 3 replicates.
[3] = 100 - (treatment/control) x 100.

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Dugesia deprived of food for 48 hours responded within minutes to *Culex* larval food. Larvae at the surface were ensnared in mucus by *Dugesia* gliding through the surface film of water. The fluid content of such larvae was either consumed at the surface or after larvae had sunk disabled to the bottom. Some group feeding activity was also observed, where up to 12 *Dugesia* cooperated in snaring prey. No intraspecific combat was observed, even when 2 or more *Dugesia* fed upon a single *Culex* larva.

Wiggling *Culex* larvae were invariably preferred to motionless or drowned larvae. Effective foraging on eggs, larvae and pupae of *Anopheles* and *Culex* was also observed in floating filamentous algal mats.

An optimum temperature range of 20° - 26°C. was determined for this species in 1 L. laboratory aquaria. Above 29°C., *Dugesia* ceased to feed and retired to cover under benthic rocks and debris, with mortality ensuing after 20 min. at 30°C. Although the maximum water temperature during the field predation experiment (Table 1) exceeded the 30°C. lethal level by about 3°C., average temperatures were well in the range of optimum *Dugesia* activity.

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A COMPARISON OF PARITY RATES IN MOSQUITOES CAPTURED BY LIGHT TRAP AND CO_2-SUPPLEMENTED LIGHT TRAP

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ABSTRACT

Although CO_2 in the form of dry ice suspended near a light trap increases the number of mosquitoes captured, little information exists on the parity rates of those mosquitoes. A study was designed to determine if either trapping method, light trap or CO_2-supplemented light trap, was more selective for a specific age group of mosquitoes.

Three Standard New Jersey Light Traps were each baited alternately with approximately 3 kg. of dry ice weekly. More than 15000 mosquitoes were trapped and identified during the study and 3600 or about 25% were dissected for for parity determinations. Parity was determined by Detinova's method of tracheolation. Twelve species representing six genera were collected with *Culex salinarius, Aedes canadensis, A. solicitans, A. vexans* and *A. cantator* being the predominant species.

For each species, the light trap attracted a significantly greater percentage of parous mosquitoes than did the CO_2-supplemented light trap. The difference between the trapping methods ranged from 11.3% in *Culex salinaruis* to 27.4% in *A. cantator.* Overall, 45% of all mosquitoes trapped in the unsupplemented light trap were parous compared to 31% in the CO_2-supplemented trap.

BACILLUS SPHAERICUS var. *FUSIFORMIS* AS A POTENTIAL PATHOGEN AGAINST *CULEX TARSALIS* AND *CULEX PIPIENS*

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ABSTRACT

The initial finding of Singer on the larval activity of *Bacillus sphaericus* var. *fusiformis* against *Culex pipiens* has been independently confirmed as well as extended to include *Culex tarsalis* larvae. Excellent activity under laboratory conditions, coupled with very promising leads on techniques for long term storage, suggests that this microbial pathogen may prove successful under conditions of practical field application. Unfortunately, the larval spectrum of activity has been observed to be quite limited, since no effective control was noted using this selected pathogen against *Aedes dorsalis, Aedes taeniorhynchus, Aedes aegypti,* and *Anopheles freeborni.*

Kellen, Clark, Lindegren, Ho, Rogoff and Singer (1965) demonstrated that an isolate of *Bacillus sphaericus* var. *fusiformis* (Neide) had a low but measurable larval activity against 10 species of mosquitoes. The ED_{50} dosages ranged from 10^7 - 10^8 spores/ml. In testing a series of accessions of the World Health Organization International Reference Center (WHO/IRC), which were initially obtained from Delhi, India (1321/I-X), Singer isolated *B. sphaericus*. Several such isolates demonstrated marked larval activity against *Culex pipiens* var. *quinquefasciatus*. These findings were published by Singer (1973). He was able to demonstrate a 10^4 improvement in activity over that reported by Kellen, et al. (1965). Singer's earlier work was repeated and confirmed at NBRL. Of equal importance, however, was the observation that this isolate demonstrated marked efficacy against *Culex tarsalis* larvae.

MATERIALS AND METHODS

Culture preparation and assay.—An inoculated BHI agar plate (Brain-Heart Infusion, Difco) incubated for 18-24 hrs at 30°C was used as a seed culture source. A fresh transfer was made wherever a new experimental challenge series was initiated, so that the inoculum was no more than 1-3 days old. A generous loop-full from the agar surface growth was used for a broth inoculum or to seed a selected solid nutrient agar. For plate inoculum, 0.1 ml of sterile distilled water was added to a loop-inoculated agar so that an emulsified inoculum could be easily spread over the entire agar surface with the aid of a dally rod.

The surface growth from an agar plate was removed with two 5-ml sequential washings, using 1% sterile peptone solution (pH 7.0). This provided a test suspension of 2-8 $\times 10^9$ cells/ml in the case of N2x agar (proprietary medium obtained from Nutrilite).

All incubation was at 30°C. Broth cultures were placed on a rotary shaker, using 50 ml of culture in a 250 ml Erlenmeyer flask. Viable assays were done using peptone dilutions and BHI agar plates, incubated for 24 hrs at 30°C.

Alfalfa infusion agar.—Sixteen grams of fresh frozen alfalfa in one liter of distilled water were autoclaved for 45 min. at 20 lb/sq. in., using a two-liter flask to minimize liquid loss. The hot liquid was filtered through glass wool and then 15 g of agar were added (1.5%). This liquid was re-autoclaved for an additional 15 min, after which this hot sterile fluid was aseptically dispensed into sterile petri dishes (20 ml/dish).

Larval test challenge.—All test larvae were held at 77°F. (25°C.) in 0.5 oz. plastic wells (25 wells per tray) containing 2 larvae and 4 ml of a nutrient fluid to provide for larval growth. An 18-gauge blunted hypodermic needle was used to add two drops (.04 ml) of a desired test challenge dilution to each test well, thus providing a 1:100 dilution of the initial challenge fluid for a larval "well" challenge.

Maintenance of test larvae.—All test-challenged larvae were held until adult emergence was completed (approximately 11 days). Data reported are based on healthy adult emergency; i. e., a fully formed adult mosquito. Mortality occurring during the larval stage, the pupal stage, or resulting during adult emergence was noted. Additionally, defective adults were observed and recorded. The data reported, however, are based on healthy adult emergence for ease of presentation.

RESULTS AND DISCUSSION

Data are summarized in Table I. It is important to note that the difference in susceptibility of second larval instar challenge versus fourth larval instar challenge is less than a factor of 4 (A, B) a result which was not observed using *Bacillus thuringiensis* (HD-1) (Goldberg et al. 1973). A relatively low level of activity was noted against *Aedes dorsalis, Aedes taeniorhynchus, Aedes aegypti* and against *Anopheles freeborni* in parallel tests, thus limiting the potential range of application of this larval pathogen.

It is important to define the role of the growth media for this unique larval pathogen. When this organism was grown using Brainheart infusion (agar or broth) nutrient agar or broth, trypticase soy, glucose yeast extract, and several other test media, a markedly reduced larval activity was noted, compared to the activity obtained using a synthetic growth media developed by Singer. By a bit of luck, an element in research which is rarely given proper credit, it was noted that this organism would grow seeded onto a "fresh-frozen" alfalfa infusion, producing an active or toxic culture, but unfortunately, with a ten-fold reduction in cell yield as compared to a parallel test culture using the synthetic or the N2X media (C). This result suggests, however, that this organism may become established under selected field conditions and provide for long-term control with a minimal requirement for re-seeding, if this organism can multiply in the grass-water interface.

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Table 1.–Observed response of *Culex tarsalis* (K.L.) to challenge of *B. sphaericus* (SSII-1).

A. 11 Hr. N2x broth culture, 30°C
2nd instar larval challenge
est. ED_{50} 1.6×10^3/ml est. ED_{90} 5.3×10^3/ml

B. 8, 10 Hr. N2x broth culture, 30°C
4th instar larval challenge.
est. ED_{50} 5.6×10^3/ml est. ED_{90} 1.9×10^4/ml

C. 24 Hr. alfalfa infusion agar culture, 30°C
2nd instar larval challenge.
est. ED_{50} 7.0×10^3/ml est. ED_{90} 2.4×10^4/ml

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D. 24 Hr. N2x agar culture, 30°C
2nd instar larval challenge.
est. ED_{50} 700 cells/ml (clone #6)
est. ED_{50} 1.8×10^4 cells/ml (clone #8)
ranking of 10 test clones - 6, 5, 7, 1, 2, 3, 9, 10, 8

E. 24 Hr. N2x broth, 30°C - refr. 3 days, vacuum dried at 23" H_g
for 18 Hr. at 45°C
2nd instar larval challenge.
est. ED_{50} 1.4×10^3/ml est. ED_{90} 5.0×10^3/ml

For practical field utilization, it is almost essential that the spore phase of this organism be utilized. Currently, optimal activity is associated with the late vegetative phase of growth. By careful clonal selection (D), however, we are currently able to demonstrate high activity in pure spore preparations, as is evidenced by a ED_{50} value of 700 cells for clone no. 6. All of the test culture results are summarized in Table 1 (D) were grown to produce nominal pure spore preparations, but on comparing the activity of clone no. 8 vs. no. 6, a considerable range in the ED_{50} dose is evident, thus demonstrating both the requirement and the feasibility of clonal selection.

Nutrilite Products, Inc., of Buena Park, California, tested the growth of *B. sphaericus* (SSII-I) in a medium used for the commercial production of *B. thuringiensis.* Cells grown in this medium were equally as effective in larval control as those grown using a synthetic medium developed by Singer. More important, however, a vacuum-dried preparation was found to have an activity equal to a fresh broth culture (E) following a storage of one week at room temperature. We are currently attempting to standardize the preparation of a dry culture for subsequent field testing. It is projected that 0.1 lbs/acre, a figure based on our current laboratory data, may prove effective for field control; (i. e., 10^4 cell/ml = ED_{90}; 10" cells/gm technical: 1 lb/acre 1 µg/ml in 6" of water).

Following EPA approval, we are planning to initiate field studies in Kern County Mosquito Abatement District.

The current emphasis on narrow spectrum control, which should minimize ecological disturbances has, however, resulted in an order of magnitude increase in costs. As a result of these factors, direct government financial support in relation to (1) basic research, (2) EPA approval for field use and (3) commercial product development, requires new funding concepts. We sincerely hope that the EPA workshops which are currently being formulated will place these problems on their agenda for immediate priority action.

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REPRODUCTIVE CAPACITY OF *AEDES NIGROMACULIS* AND DENSITY OF EGGS IN OVIPOSITION SITES

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The pasture mosquito, *Aedes nigromaculis* (Ludlow), is one of the most abundant and noxious mosquitoes occurring in irrigated pastures in the Central Valley of California; however, very little is known about its reproductive capacity and the density of eggs in natural oviposition sites. According to Husbands and Rosay (1952), field-collected mosquitoes fed on human blood produced 0 to 110 eggs per female in the laboratory. Barr and Al-Azawi (1958) reported that a female of this species laid 156 eggs on the surface of tap water in a test tube. Miura and Takahashi (1972a) reported that the mean number of eggs laid by females fed on rabbit blood was 116 with a range of 55 to 218. Females of this species preferably laid their eggs in bunch grass clumps in irrigated pastures, especially the clumps in depressions where moisture content was high and could be retained for a long period (Miura and Takahashi 1972b). Nothing has been reported on the density of eggs in natural oviposition sites.

The objective of the study was to determine the reproductive capacity of pasture mosquito females and to estimate the number of eggs laid per unit of bunch grass clumps in irrigated pastures.

MATERIALS AND METHODS

Reproductive Capacity.—Mosquitoes used in this study were either laboratory-reared or collected in the field as late stage larvae or adults. The methods used for rearing and handling were those of Miura (1967) unless otherwise stated. The reproductive capacity was estimated by two different procedures: (1) the actual mean number of eggs laid per female, (2) the mean number of oocytes per female at each developmental stage of the ovarian follicles. The number of eggs laid per female was determined from 2-3 day old, well fed, field-collected mosquitoes individually isolated in 15 x 125mm test tubes each of which contained a moistened strip of absorbent paper for oviposition. Dixie cups (4 oz) were also used as oviposition chambers. The reproductive capacity of the field-collected adults described above was also estimated by allowing groups of ten to oviposit in 2-quart cartons with net tops.

The reproductive potential of this species was determined by counting oocytes in the ovarian follicles. The technique used was described by Rosay (1969).

The reproductive capacities of the OP-R and S-strains of this species were determined from females maintained by the induced mating technique (Miura 1969).

Density of Eggs in Oviposition Sites.—The methods used for taking samples and estimating egg density in the samples were as previously described (Miura and Takahashi 1972b), except where noted otherwise. Core samples, about 7 cm diameter and 2-5 cm deep, were collected from bunch grass clumps in irrigated pastures. Egg numbers were estimated from the number of larvae that hatched during repeated floodings of each sample in the laboratory.

RESULTS AND DISCUSSION

The number of eggs laid in a given batch varied greatly among individual females (Table 1). The mean number of eggs laid by individually-isolated females was about 125; an average of only 82 eggs were laid by each of the females kept in groups. The maximum number of eggs laid per female was 228 by the isolated females, and 226 by females held in groups.

Table 1.—Mean number of eggs laid by individual *Aedes nigromaculis* females.

Container	No. Tests	Mean	Range[1]	Host
Test tube[2]	41	133	47-228	Rooster
Dixie cup[2]	29	116	55-218	Rabbit
2-quart carton[3]	28	82	23-226	Rooster

[1] Females which failed to lay eggs were excluded.
[2] Females were kept individually isolated.
[3] Females were kept in groups of ten.

Generally, egg production is greatly influenced by many factors such as quantity and quality of blood meal, and presence or absence of mating stimuli (Clements 1963). The number of eggs laid by pasture mosquito females showed a positive correlation with the size of females and also with the amount of blood meal (Miura and Takahashi 1972a). The retention of one or more eggs in the ovary following oviposition also occurred in this species (Miura, unpub. data). Thus, the total eggs produced does not represent a true estimate of the actual reproductive potential of the species.

The reproductive potential of *A. nigromaculis* as determined by the number of oocytes is shown in Table 2. The mean number of oocytes at the follicular developmental stage I was 154, and 156 at stage II. Marked reductions in the number of oocytes were noticed at stages III, IV and V. The maximum number of oocytes recorded was 265; i.e., a single *A. nigromaculis* female can oviposit 265 eggs if conditions are favorable. The maximum number of eggs deposited per female was 228 (Table 1).

While *A. nigromaculis* eggs were being mass-produced via the induced mating method (Miura 1969), the total egg production of the OP S-strain was much higher than that of the OP R-strain (Table 3). To confirm this phenomenon, the mean egg production of field-collected S- and R-strains was

Table 2.–Mean number of *Aedes nigromaculis* oocytes at each follicular developmental stage.

Follicular Developmental Stage[1]	OP S-Strain			OP R-Strain			Resistance Unknown			
	No.	Mean	Range	No.	Mean	Range	No.	Mean	Range	Mean
I	17	152	105-204	9	145	114-176	7	168	138-190	154
II	10	158	105-219	10	132	109-170	13	173	74-221	156
III	9	108	61-192	17	117	72-169	25	154	94-265	133
IV	7	88	50-138	12	107	61-174	4	119	75-144	103
V	18	88	52-206	20	97	42-155	23	140	76-191	110
Mean		118.8			119.6			150.8		131.2

[1] Christophers' classification.

Table 3.–Mean number of eggs laid by OP R-Strain and OP S-Strain of *Aedes nigromaculis* females.[1]

Mosq. Source	R-Strain			S-Strain		
	No. tests	Mean	Range	No. tests	Mean	Range
Field-collected	7	55	22-226	20	92	40-157
Induced mating	76	20	1- 56	50	32	5- 89

[1] Females held in groups.

Table 4.–Some unusually high numbers of *Aedes nigromaculis* eggs[1] recovered from bunch grass clumps collected from the Costa Pasture, Kings County, California.

No. Eggs	Size of Sample	Eggs per 1 ft^2
45,790	456.0 cm^2 (0.49 ft^2)	92,978
2,329	38.5 cm^2 (.042 ft^2)	55,452
2,100	38.4 cm^2 (.042 ft^2)	50,000
16,546	353.4 cm^2 (.382 ft^2)	43,348
1,513	38.5 cm^2 (.042 ft^2)	36,024
1,273	38.5 cm^2 (.042 ft^2)	30,310
976	38.5 cm^2 (.042 ft^2)	23,238
856	38.5 cm^2 (.042 ft^2)	20,381
773	38.5 cm^2 (.042 ft^2)	18,404
992	50.0 cm^2 (.054 ft^2)	18,370
769	38.5 cm^2 (.042 ft^2)	18,310
747	38.5 cm^2 (.042 ft^2)	17,786

[1] Egg numbers assumed same as numbers of larvae that hatched from each sample.

compared (Table 3). The mean egg production of the OP S-strain was greater than that of the R-strain; but the mean number of oocytes in the ovaries of the S-strain was approximately the same as that for the R-strain. Whether or not the mechanism causing resistance is also responsible for reduction of egg production is unknown.

Detection of field oviposition sites and estimation of the number of eggs in those sites is of practical importance for mosquito control agencies. The data reported here are in agreement with the results of our previous study which showed that *A. nigromaculis* females deposited their eggs in bunch grass clumps. Within a given clump, most eggs are deposited at the periphery and near the surface of the ground (Figure 1).

The mean number of eggs estimated from core samples (38.5 cm^2 or 0.042 ft^2) was 247 which is about equal to 256,176,360 per acre. The maximum number of eggs estimated was 45,790 per 459 cm^2 (0.49 ft^2), or 4,050,121,680 eggs per acre (Figure 2). A few samples which produced an unusually large number of eggs are described in Table 4. Thus, this figure agrees with the large numbers of larvae that are frequently found during the field season.

SUMMARY

1. The mean number of eggs laid by individually-isolated *A. nigromaculis* females was 125; the maximum egg output was 228.
2. Based upon oocyte numbers in the ovary, females of this species probably are capable of producing 260 eggs or more. The mean number of oocytes at stages I and II was 155.
3. Actual egg production of the OP S-strain was larger than that of the R-strain; however, number of oocytes per female was almost identical.
4. The maximum number of eggs deposited per 1 ft^2 area of bunch grass clumps was 92,978. The mean was 5,881.

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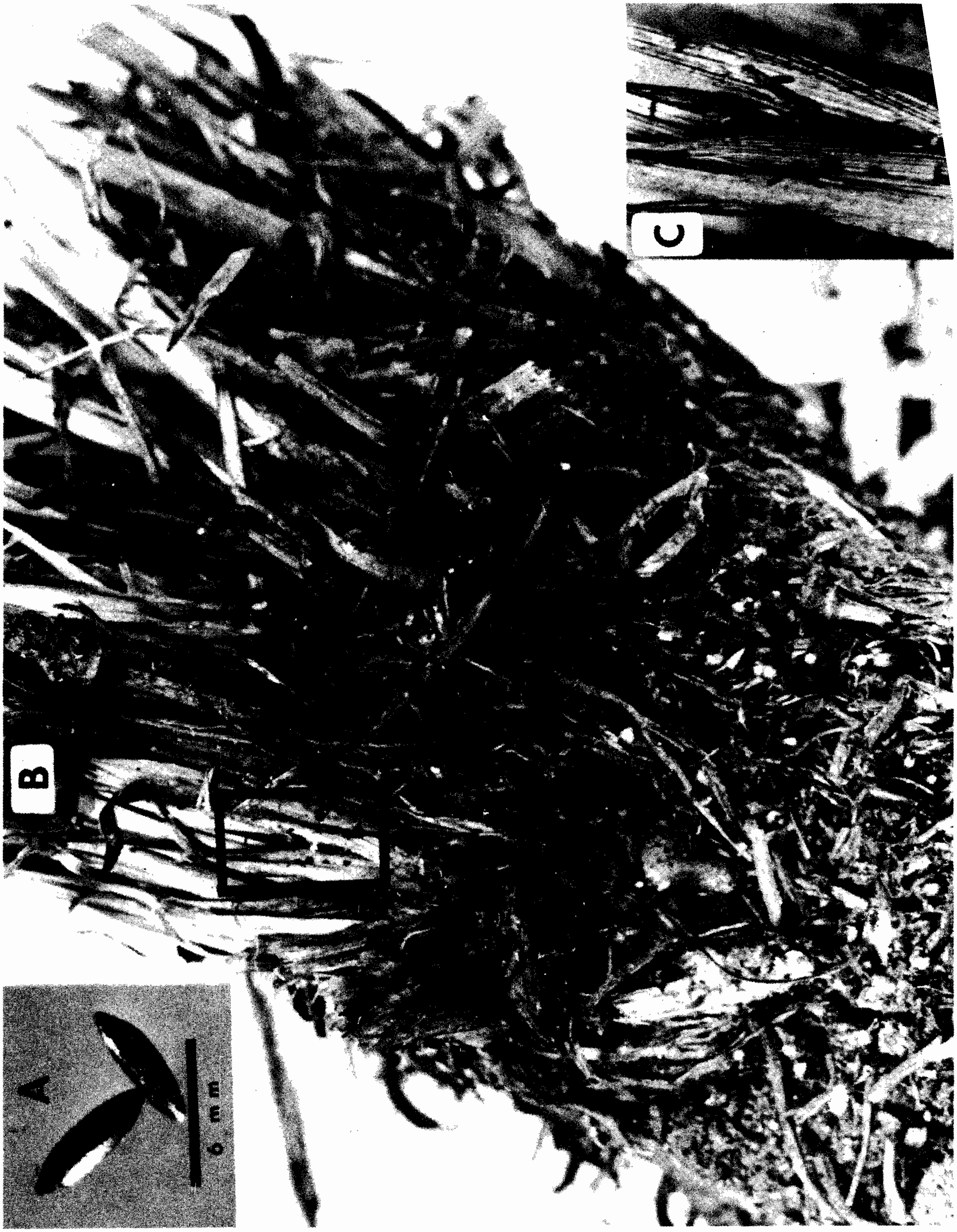

Figure 1.—Natural oviposition sites of *A. nigromaculis*, A, eggs. B, portion of bunch grass clump showing eggs deposited by females (enclosed area). C, close-up of the enclosed area in B.

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Figure 2.—A piece of bunch grass clump. Maximum number of eggs (45,790) was obtained from this clump.

CULEX SALINARIUS COQUILLETT AS A POTENTIAL VECTOR OF *DIROFILARIA IMMITIS* (LEIDY)[1, 2]

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ABSTRACT

Three strains of *Culex salinarius* from Connecticut, Louisiana, and Maryland were maintained under laboratory conditions. Females of the 3 strains were fed on dogs infected with *Dirofilaria immitis*. The Maryland strain fed most readily on the dogs; 46.0% of 1,680 mosquitoes offered dog blood accepted it. Response of the Connecticut strain was 37.2% of 2,402, and of the Louisiana strain 25.1% of 1,409.

All 3 strains supported *D. immitis* larval development although maturation of larvae to the infective stage was observed only in the Connecticut strain. Infective larvae were recovered 11, 18, and 20 days after the infective blood meal. Percentages of Maryland, Louisiana, and Connecticut strain mosquitoes positive for developing larvae of *D. immitis* were 0.57, 1.42, and 3.40, respectively. The intensity of infection (number of developing larvae per infected mosquito) was, respectively, 1.0, 1.33, and 1.05 for the three strains.

INTRODUCTION.–Canine heartworm disease is highly enzootic along the eastern seaboard of the United States, from New Jersey to Texas (Otto 1972). *Culex salinarius* Coquillett reaches its greatest abundance in the Atlantic and Gulf Coast regions (Murphey 1961). Mallack et al. (1971) studied dog heartworm at a hunt club near Upper Marlboro, Maryland. The dogs were American Foxhounds 19 months and older. Of 34 dogs, 28 or 76% were positive for *Dirofilaria immitis* (Leidy) microfilariae. Samples of mosquito populations obtained with CDC-type light traps showed that *Culex* species comprised 50.2% of the mosquitoes collected, and *C. salinarius* predominated.

Ludlam et al. (1970) reviewed the literature and reported that in the United States 25 species of mosquitoes could be considered potential vectors of *D. immitis*. Of these, 5 are in the genus *Culex*. In general, a species has been considered to be a potential vector if females support development of filariae to the third or infective stage. Hu (1931), and Summers (1943), found that *C. salinarius* did not support the development of the filarial parasite to the infective stage. Nevertheless, in Maryland it has been generally known for many years that *C. salinarius* populations are often large in areas where the incidence of canine heartworm disease is high. This does not establish a relationship between the two, but does suggest a need to investigate the possibility that a correlation between the two might exist. To that end, colonies of 3 laboratory strains of *C. salinarius* were established. The objectives of this study were: (1) to allow female mosquitoes of the 3 strains to take potentially infective blood meals from dogs harboring *D. immitis* and (2) to monitor the larval development of *D. immitis* in these mosquitoes to determine if they might serve as an intermediate host.

METHODS AND MATERIALS.–Rearing Mosquitoes--The 3 strains of *C. salinarius* used in this project were: (1) The Louisiana (La.) strain provided by Dr. H. C. Chapman of Lake Charles, Louisiana, where it was colonized in 1966 (Chapman and Barr 1969); (2) The Yale Arbovirus Research Unit (YARU) strain obtained from Dr. R. C. Wallis who colonized this strain at New Haven, Connecticut in 1967 (Wallis and Whitman 1968); (3) The Pocomoke Cypress Swamp (PCS) strain colonized in 1972, from the local source in Maryland, after which it was named, by Col. Bruce Eldridge at the Walter Reed Army Institute of Research and supplied by him (Eldridge et al. 1972).

Rearing and maintenance techniques were those of Wallis and Whitman (1968) with some exceptions. The La. and PCS larval rearing pans were left uncovered. Peeled apple sections were provided daily to the adult stock cages as a supplement to the 10% sucrose solutions. Chickens were the usual source of blood meals for the stock colonies of the 3 strains.

Feeding Mosquitoes on the Dogs--Mosquitoes for experimental feedings on infected dogs were handled in the following manner. Half-gallon ice cream cartons were modified by coating the bottoms with paraffin. The tops were discarded and small-mesh curtain material was stretched taut across the opening and secured with Ambroid® cement. Eighteen mm access holes were then cut into the sides of the cartons. These holes were closed with cotton wads. Unsexed pupae were rinsed and placed in distilled water 12 mm deep in the cartons. After allowing 2 days for emergence of adults, the water was poured off through the mesh top. The usual number of pupae placed in each cylinder was 400 although the number ranged from 196 to 555. Mosquitoes in these cylinders were later presented to the infected dogs for experimental feedings.

Two male mongrel dogs were utilized as the source of infective blood meals. Dog P-5 was provided by Dr. Guillermo Pacheco, Laboratory of Parasitic Diseases, Bethesda, Maryland 20014. This dog had been infected in the laboratory by inoculation of *D. immitis* infective stage larvae. The second dog, dog D, was supplied by Dr. Eugene J. Gerberg, Insect Control and Research, Baltimore, Maryland 21228. This dog was naturally infected in Georgia and was subsequently secured for *D. immitis* research. The exclusive infection of this dog was confirmed by Dr. Paul E. Thompson of the School of Veterinary Medicine, University of Georgia, Athens, Georgia 30601 (Kutz 1971). Both dogs were periodically monitored during the course of the infec-

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tion by examination of stained blood smears. *Dipetalonema reconditum* microfilariae were never observed, and the infections were judged to be solely *D. immitis.*

Immediately before allowing mosquitoes to feed upon a dog, a blood sample was taken from the latter to determine the microfilaremia. A 2-3 cc sample was withdrawn from the cephalic vein into a heparin rinsed syringe. The microfilaremia was determined within 1 hour of the time the sample had been taken. The syringe was agitated and a few drops of blood were expelled from the needle tip which was then wiped to remove any excess. A 20 µl (20 mm^3) disposable micropipette was touched against the needle point and filled to the precalibrated mark. Two drops of normal saline lightly stained with methylene blue were then placed in the center of a glass microslide which had been cleaned with absolute ethanol. The measured quantity of blood was then discharged into the saline and a corner of a 20 x 40 mm coverslip was used to mix the two. This action aided in determination of the microfilaremia by hemolysing the red blood cells and lightly staining the parasites. The coverslip was then allowed to settle over the preparation and the slide was scanned for microfilariae. A second 20 µl aliquot was drawn from the syringe and treated identically to the first. This procedure for determining microfilaremia was repeated at all mosquito feedings. An average of 2 readings is implicit where the term microfilaremia is used in relation to this work.

When the blood sample had been taken, the half-gallon cartons were placed against the shaved side of the dog and the mosquitoes were allowed to feed. A 30 minute feeding period on an unanesthetized dog was the standard technique. Engorged females were then removed with an aspirator and placed in smaller holding cartons. The smaller cartons were 1-pint ice cream cartons which had been modified in much the same manner as the half-gallon ones. Curtain material was stretched across the tops and fixed with Ambroid® cement. Access holes were cut in the sides and plugged with cotton. The bases of the cartons were cut off and replaced with disposable plastic petri dish bottoms. The petri dish bottoms have slightly tapering sides and fit snugly over the open carton ends. These clear bottoms aided in observation of the mosquitoes and could be easily removed for cleaning.

Dissection of Mosquitoes and Detection of Parasites--Mosquitoes were separated into different groups based on the individual strain and age of the group. Mosquitoes in each group were all of the same strain and age. The potentially infected groups were handled in two different ways. Some of the groups were used to monitor the development of *D. immitis* larvae within the mosquito. Some mosquitoes were dissected each day from the day of the blood meal, day zero, through the 18th post-prandial day. On the 18th day all remaining living mosquitoes were dissected.

The following dissection regimen was used. Up to the 4th day after infection midguts and Malpighian tubules were examined. From day 5 through day 7 only the Malpighian tubules were inspected. From day 8 through day 18 the Maliphian tubules and mouthparts were scrutinized.

Mosquitoes were prepared for dissection by first lightly anesthetizing them with chloroform and then removing wings and legs. Dissections were accomplished on a glass microslide. Microforceps were used to grip the last 2 abdominal segments. A curved needle was then placed at the juncture of the thorax and abdomen. The last 2 abdominal segments were then protracted so that the midgut, Malpighian tubules and hindgut were drawn out into a drop of saline. The tubules and midgut were then excised and the rest of the mosquito removed. The head was easily severed at the neck and was examined under a separate coverslip on the same slide. Usually, 5 sets of Malpighian tubules and midguts were placed under 1 coverslip and the 5 corresponding heads were placed under a second coverslip on the same slide.

In an attempt to maximize production of infective stage larvae, other groups of potentially infected mosquitoes were carried until the 18th post-prandial day before any dissections were attempted. When this method was employed, large numbers of surviving mosquitoes were sometimes encountered and dissections continued through the 20th day. Malpighian tubules and midguts were examined.

RESULTS AND DISCUSSION.--Dogs as Hosts for *Culex salinarius*--The feeding behavior of the 3 strains of *C. salinarius* was not a primary concern of this study. However, feeding data were recorded to detect possible differences in acceptability of dogs.

Females of the La. strain ranged in age from 3 to 8 days (Table 1). Different groups of 3-day-old mosquitoes fed irregularly. Percent feeding ranged from 17.7 to 49.5 with an overall average of 25.1. There appeared to be no correlation between the age of the mosquitoes and the numbers that fed on a dog.

Table 1.--Feeding response of *Culex salinarius* Coquillett (Louisiana strain) when presented to an unanesthetized dog for 30 minutes.

Date of blood meal, 1973	Hour of blood feeding	Age of mosq. in days after emergence	Total females offered/taking blood meal	% of females taking blood meal
Mar. 9	2:40 - 3:10 p.m.	3	181/32	17.7
Mar. 27	1:20 - 1:50 p.m.	3	107/46	22.2
May 3	1:10 - 1:40 p.m.	3	99/49	49.5
Mar. 27	1:20 - 1:50 p.m.	4	124/25	20.2
Aug. 8	1:55 - 2:25 p.m.	6	521/132	25.3
Aug. 8	1:55 - 2:25 p.m.	8	277/80	28.9
Totals			1,409/364	25.1

The age of the PCS strain females varied from 3 to 7 days (Table 2). Four-day-old mosquitoes were used on April 26, and 61.7% fed on the dog. This represents the highest percentage of feeding for any group of all 3 strains. There was a great difference in the feeding response of the 2 groups of 3-day-old mosquitoes; only 6.9% of 1 group fed while 53.0% of the second group fed. The 7-day-old mosquitoes of June 18 were fed on the dog between 7:30 and 8:00 p.m., over 4 hours later than the other 7-day-old group. There was more uniformity in feeding response between these 2 groups than there was between the 2 groups of 3-day-old mosquitoes whose feeding times were separated by only 20 minutes. The feeding response was greatest for 4-day-old mosquitoes. Of the 1,680 mosquitoes offered dog blood, 773 (46.0%) fed on a dog.

Of the 3 strains of *C. salinarius*, the YARU strain was used in the greatest numbers (Table 3). Female mosquitoes ranging in age from 1 to 6 days were employed in this series of experiments. The response of 4-day-old mosquitoes averaged 46.3% and the response of 5-day-old individuals averaged 45.6%. Of the 2,402 females offered dog blood, 893 or 37.2% engorged.

The acceptability of the dog as a host by *C. salinarius* was previously reported by Hu (1931), Summers (1943) and Murphey (1961). Many of the reported studies of feeding behavior by various species of *Culex* involved forced feeding, or lack of choice, as was the procedure used in this investigation. Bemrick and Sandholm (1966) and Bemrick and Moorhouse (1968) used dog baited traps, but *C. salinarius* did not naturally occur in the areas where they worked.

Table 2.–Feeding response of *Culex salinarius* Coquillett (Pocomoke Cypress Swamp strain) when presented to an unanesthetized dog for 30 minutes.

Date of blood meal, 1973	Hour of blood feeding	Age of mosq. in days after emergence	Total females offered/taking blood meal	% of females taking blood meal
Apr. 26	1:30 - 2:00 p.m.	3	100/53	53.0
May 3	1:10 - 1:40 p.m.	3	116/8	6.9
Apr. 26	1:30 - 2:00 p.m.	4	133/82	61.7
June 18	7:30 - 8:00 p.m.	7	681/345	50.7
July 11	2:50 - 3:20 p.m.	7	650/284	43.7
Totals			1,680/772	46.0

Table 3.–Feeding response of *Culex salinarius* Coquillett (Yale Arbovirus Research Unit strain) when presented to an unanesthetized dog for 30 minutes.

Date of blood meal, 1973	Hour of blood feeding	Age of mosq. in days after emergence	Total females offered/taking blood meal	% of females taking blood meal
Mar. 9	2:40 - 3:10 p.m.	1	221/19	8.6
Mar. 9	2:40 - 3:10 p.m.	2	217/34	15.7
May 3	1:10 - 1:40 p.m.	3	138/11	8.0
Mar. 9	2:40 - 3:10 p.m.	4	90/31	34.4
Mar. 27	1:20 - 1:50 p.m.	4	198/67	33.8
Aug. 21	5:30 - 6:00 p.m.	4	590/360	61.0
Aug. 22	5:00 - 5:30 p.m.	4	627/239	38.1
Mar. 27	1:20 - 1:50 p.m.	5	131/55	42.0
June 26	1:05 - 1:35 p.m.	5	97/49	50.5
June 26	1:05 - 1:35 p.m.	6	93/28	30.1
Totals			2,402/893	37.2

Ability of *Culex salinarius* to Support *Dirofilaria immitis* Development--Females of the La. strain were fed on an infected dog on 4 occasions (Table 4). The microfilaremia of the dog at the time of feeding ranged from 34-70 mf/20 mm^3 (1,700-3,500 mf/ml). Of the 364 mosquitoes that fed on a dog, 211 were dissected. The remaining 42% died before dissection and were not examined. Dissections commenced at 1 hour post feeding and were continued on a daily basis through the 18th day after the infective blood meal. Most of the mosquitoes, 77.8%, were dissected on days 17 and 18.

One microfilaria was located in the midgut of 1 of 3 mosquitoes dissected 1 hour after feeding. Only dead microfilariae were observed in the midguts on day 1, and no microfilariae were located in ther Malpighian tubules. On day 2, 1 dead microfilaria was located in the midgut of 1 of 3 mosquitoes dissected. From day 3 through 17, no parasites were observed. A total of 4 second-stage larvae were seen in 3 mosquitoes on day 18. Two of the 4 parasites were in the elongate transitional phase. No infective larvae were observed. The percentage of mosquitoes positive for developing stages was 1.42. Percent positive refers to mosquitoes positive for developing stages of filariae in the Malpighian tubules or proboscis. This method of reporting positive and negative results is in conformity with the work of Hu (1931). He stated, "An individual is considered positive when all the filarial larvae found in the mosquito seemed

Table 4.–Susceptibility of laboratory reared *Culex salinarius* Coquillett (Louisiana strain) to infection with *Dirofilaria immitis* (Leidy).

Date of blood meal, 1973	Microfilaremia of dog	Days after blood meal	Number dissected	Negative	Microfilariae in stomach	Microfilariae in Malpighian tubules	2nd stage Malpighian tubules	3rd stage Proboscis
March 9	42/20 mm^3	2	2	2				
		4	2	2				
		14	11	11				
March 27	62/20 mm^3	1 hr.	3	2	1			
		1	3	1	a. 1 (dead)			
					b. 2 (dead)			
		2	3	2	1 (dead)			
		3-15	3/day	39				
		16	1	1				
May 3	70/20 mm^3	18	22	22				
August 8	34/20 mm^3	17	10	10				
		18	115	112			a. 2	
							b. 1	
							c. 1	

Table 5.–Susceptibility of laboratory reared *Culex salinarius* Coquillett (Pocomoke Cypress Swamp strain) to infection with *Dirofilaria immitis* (Leidy).

Date of blood meal, 1973	Microfilaremia of dog	Days after blood meal	Number dissected	Negative	Microfilariae in stomach	Microfilariae in Malpighian tubules	2nd stage Malpighian tubules	3rd stage Proboscis
April 26	108/20 mm^3	13	3	3				
		14-16	6/day	18				
		17	6	5			1	
		18	57	57				
May 3	70/20 mm^3	18	5	5				
June 18	24/20 mm^3	17	134	134				
		18	96	95			1	
July 11	49/20 mm^3	17	199	198			1	

likely to develop completely to the infective stage or had already arrived at that degree of maturity." The intensity of infection was 1.33. Intensity of infection was another term used by Hu as an index of vector potential. This value represented the number of developing parasites per infected mosquito. In its determination Hu considered only cases in which all the filariae were well established and reaching maturity. Negative mosquitoes were not included in determination of intensity of infection.

Because dissections of representatives of the La. strain immediately following the uptake of microfilariae were limited to 3 mosquitoes, Kartman's (1953) host efficiency and infective potential indices were not calculated.

PCS strain mosquitoes were offered blood meals on an infected dog 4 times (Table 5). The microfilaremia of the dog ranged between 24 and 108 mf/20 mm^3 (1,200-5,400 mf/ml). The total number of mosquitoes that fed on the dog was 772 of which 33% died before dissection; 95.9% of the dissections were made on days 17 and 18. Three second-stage larvae were observed, 2 on day 17, and the third on day 18. One of the parasites observed on day 17 was in the elongate transitional phase. The percent of mosquitoes positive was 0.57 and the intensity of infection was 1.

A total of 893 females of the YARU strain took potentially infective blood meals from dogs on 6 days between March 9 and August 22. The results of these findings are presented in Table 6. The microfilaremia of the dog ranged between 26 and 80 mf/20 mm^3 (1,300-4,000 mf/ml). Mortality in these mosquitoes before dissection was 39%. Dissections were begun 1 hour after feeding and were continued daily through day 20, with 39.5% completed between days 17 and 20. Twenty-nine mosquitoes were dissected 1 hour after feeding, and a total of 34 microfilariae were located in their midguts, with 13 of the 34 dead. The day after the blood meal, day 1, 18 mosquitoes were dissected and 1 dead microfilaria was located in the midgut of each of 2 mosquitoes. Three other microfilariae had successfully located in the Malpighian tubules of 2 other mosqui-

Table 6.–Susceptibility of laboratory reared *Culex salinarius* Coquillett (Yale Arbovirus Research Unit strain) to infection with *Dirofilaria immitis* (Leidy).

Date of blood meal, 1973	Microfilaremia of dog	Days after blood meal	Number dissected	Negative	Microfilariae in stomach	Microfilariae in Malpighian tubules	2nd stage Malpighian tubules	3rd stage Proboscis
March 9	42/20 mm^3	2	2	2				
		4	2	2				
		14	38	37			1	
March 27	62/20 mm^3	1 hr.	3	2	1			
		1	3	2	1 (dead)			
		2	3	1	a. 1 (dead) b. 2 (dead)			
		3	3	3				
		6-15	3/day	30				
		16	5	5				
		17	6	6				
		18	3	2				1
		20	26	25				1
May 3	70/20 mm^3	18	2	2				
June 26	26/20 mm^3	18	54	54				
August 21	28/20 mm^3	1 hr.	16	10	a. 4 b. 4 c. 3 d. 3 e. 3 f. 1			
		1	10	8		a. 2 b. 1		
		2-3	10/day	20				
		4	10	9			1	
		5	10	9			1	
		6	5	5				
		7	6	6				
		8-10	5/day	15				
		11	5	4				1
		12-13	10/day	20				
		14	10	9			1	
		15	10	10				
		16	10	9			1	
		17	10	10				
		18	88	83			a. 1 b. 1 c. 1 d. 1	1
August 22	80/20 mm^3	1 hr.	10	4	a. 5 (dead) b. 4 (dead) c. 3 (dead) d. 1 (dead) e. 1 f. 1			
		1	5	4	1 (dead)			
		2-11	5/day	50				
		12-13	10/day	20				
		14	10	9			1	
		15-16	10/day	20				
		17	10	9			1	
		18	37	35			1	1

toes, 2 in 1 mosquito and 1 in another. On day 2, 20 mosquitoes were dissected and 3 dead microfilariae were observed in the midgut of 2 mosquitoes. Dissections on days 3 through 20 revealed 12 second-stage larvae and 5 infective stage larvae. No more than 2 second-stage or third-stage larvae were found per mosquito. Infective stage larvae were found 11, 18, and 20 days after the blood meal. The rapid development to the infective stage by a single parasite occurred during a period of high temperatures in the insectary. During a 6-day period the daily temperatures rose to 86 or 87°F. It is possible that the high temperatures might have caused more rapid development in this instance. The percent positive was 3.40, the intensity of infection, 1.05. The host efficiency was .02912 and the infective potential, .00728. Host efficiency and infective potential are 2 expressions formulated by Kartman (1953). Host efficiency is the ratio between the mean number of developing filariae per mosquito and the mean number of microfilariae ingested per mosquito during the infective blood meal. Infective potential is the ratio of the mean number of infective larvae per mosquito and the mean number of microfilariae ingested per mosquito. As the percentage of ingested larvae undergoing development increases, these ratios approach 1.

CONCLUDING REMARKS.–All 3 strains demonstrated a propensity for feeding on dogs, and some individuals of the YARU strain took a second blood meal from a dog. The YARU strain supported the highest percentage of developing parasites and was the only strain in which the infective stage of the parasite was found. Although no third-stage larvae were recovered from the other 2 strains, advanced phases of the second stage were observed in both. The range of variation in percentage and intensity of infection among the 3 strains was not great. These findings are analogous to those of Hu (1931) who stated that the limited range of variation might be characteristic of host susceptibility peculiar to the different species serving as hosts to *D. immitis*. However, both Hu (1931) and Summers (1943) worked with *C. salinarius* and experienced results unlike those reported here. Summers fed 72 mosquitoes of which 27 (37.5%) became infected. There was no development beyond the second stage and the species was dismissed as unimportant in transmission. Hu inspected only 5 mosquitoes and found 1 second-stage parasite. He believed that if enough time was allowed, the parasites could attain complete development. Although no infective larvae were recovered in either case, there was a higher percentage of infection in both cases. The incongruity between the results of Hu and of Summers and those reported here possibly reflects differences in experimental conditions. Neither Hu nor Summers reported the microfilaremias of the donor dogs. The work of Villavaso and Steelman (1970) emphasized the importance of differing microfilaremias in relation to different levels of infection and mortality in *C. p. quinquefasciatus*.

Species of the genus *Culex* are generally classed as relatively poor carriers of *D. immitis*, although there are reports that *Culex* species may serve as vectors; e.g., Intermill and Frederick (1970) reported that *C. quinquefasciatus* and *C. tritaeniorhynchus* appeared to be important vectors on Okinawa. Kartman (1953) attributed the extremely low density of parasites in *Culex* species to the destruction of the majority of the microfilariae in the midguts during the first 24 hours after ingestion by the mosquitoes. Results of dissections of YARU females during the first 3 days after the infective blood meal tend to confirm Kartman's findings.

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FIELD EVALUATION OF CHEMICAL, RADIOACTIVE AND BLINKING LIGHT SOURCES AS MOSQUITO ATTRACTANTS[1]

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INTRODUCTION.—Since its improvement by Sudia and Chamberlain (1962), the portable battery-operated mosquito light trap has proven to be a highly efficient and reliable tool for collecting adult mosquitoes in the conduct of epidemiological surveys. However, the miniature light trap has been relatively expensive to operate in terms of electrical energy. Utilizing six-volt dry cell batteries, the motor can be driven at a battery cost of about $.10 a night, whereas the light bulb alone consumes ten times that much electrical energy (Barnhart, Pers. Commun.). Attempts to develop a more economical means of power were largely unsuccessful until Johnston et al. (1973) modified existing CDC traps to accept standard "D" size carbon-zinc flashlight batteries or heavier duty alkaline flashlight batteries. This innovation reduced the operating cost of the CDC light trap. As stated previously, the incandescent light consumes a relatively large amount of electrical current regardless of the power source, whereas the motor has a very low current consumption. In fact, four carbon-zinc flashlight batteries are sufficient for only one night's operation when running both the light and motor, except under extreme hot and humid conditions such as occurs in tropical areas. In this instance even one night's operation may not be obtained. However, when the incandescent light is removed, those same "D" cells will power the motor for three consecutive nights. If the standard incandescent bulb is removed and replaced by an alternate attractive light source not requiring battery power, then a considerable cost reduction should result.

This paper describes a field evaluation of alternate light sources as mosquito attractants, conducted during September 1973 on Maryland's Eastern Shore.

MATERIALS AND METHODS.—Three different types of alternate light devices were selected for evaluation. These are shown in Figure 1. Gas-powered tritium lights were purchased from Self-Powered Lighting LTD., Telham, New York under the commercial name BETALIGHTS. Four different colors of Betalights, green, blue, yellow and white were chosen, each measuring 22 x 25 mm, and providing brightness ranging from 390-1300 micro-lamberts. Betalights can provide continuous light for about 7-8 years. (Approximate cost per source is $25.00). Chemical lights were procured from the Naval Weapons Center, China Lake, California under the trade name CHEMLITES. All Chemlites were green in color, and when activated produced bright light for approximately 8-12 hours depending on temperature. However, field tests utilizing the same Chemlite for two nights showed that it was capable of providing enough light on the second night to attract sufficient numbers of mosquitoes. Maximum luminescence per light measured approximately ten foot lamberts/cm. (Approximate cost per Chemlite is $.50.)

Blinking lights were obtained from the LWL, Aberdeen Proving Ground, Maryland. Each of these contained a GE glow lamp 6 AC, and when activated produced two flashes of low intensity light per second for a period of 1-2 years. (Approximate cost of the blinker is $3.00).

CDC miniature light traps modified to accept "D" cell batteries were utilized during the course of the investigation. Traps were purchased from Hausherr's Machine Works, Toms River, New Jersey, and contained new Barber-Colman motors capable of providing up to 2000 hours service.

Pocomoke Cypress Swamp, Maryland was selected as the test area for the study. The alternate light sources were compared to the standard incandescent light trap bulb, and to each other.

CDC traps were fitted with the various light sources and placed randomly in blocking fashion throughout the test area. Within a given block, each trap contained a different light source. Originally there were to have been three blocks, each one containing seven traps. However, due to logistical problems, it was possible to provide only two standard and two blinking traps. Thus, only nineteen traps were established, (7 in Block I, 7 in Block II, and 5 in Block III) each light source having three replications, with the exception of the standard and the blinking which had only two replications each. Traps were located approximately 100 meters apart within blocks.

During the course of the investigation, light traps were activated ½ hour prior to sunset each evening, and catches collected ½ hour following sunrise the next morning. Additionally, a control was utilized from time to time in which only the trap motor was run and no light was used. Trapping was conducted for three continuous weeks during which a total of 373 trap nights was recorded.

The trap index[2] was selected as the tool by which to measure the effectiveness of each light source as a mosquito attractant. For statistical purposes, each of the colored radioactive lights was treated as a separate variant. Accumulated raw data were subjected to an analysis of variance of randomized blocks using a 5% level of error. The standard error of the mean was then used to calculate Duncan's New Multiple Range Test at the 5% level. In order to reduce the variability between nights and weeks, all data were standardized by converting trap index means into natural logarithms.

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[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of Army or the DOD.

[2] Includes both males and females.

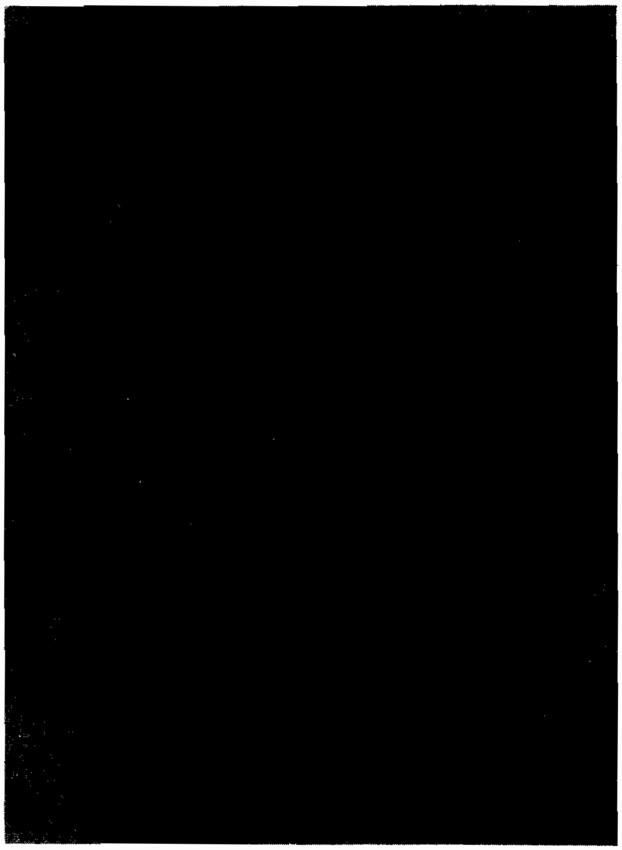

Figure 1.—The three types of light devices utilized during the field evaluation: from top.to bottom: blinker, chemical light, radioactive light.

RESULTS AND DISCUSSION.—Analysis of variance tests performed with data accumulated during the course of the investigation demonstrated the following:

a. There was a significant difference between weeks with respect to numbers of mosquitoes trapped by all light sources. Average trap indices for weeks 1-3 were recorded as 2245, 337 and 69 respectively.
b. No significant difference was noted among the three blocks. That is, the number of mosquitoes trapped in each block did not differ significantly.
c. There were significant differences among some of the light trap sources in terms of performance. (See Table 1.)

The standard chemical sources, Rad-blue, Rad-green and Rad-white, yielded similar results. However, the Rad-yellow and the blinking light sources were less efficaceous.

Although Duncan's New Multiple Range Test demonstrated no significant difference among chemical, standard, blue, green and white, Table 1 shows that the average trap indices vary widely between chemical and white. Rad-green and Rad-white overlap with Rad-yellow. Green and white may be more closely related to yellow and blinking than to chemical, standard and blue. Further experimental evidence may clarify that situation. Of the alternate light sources utilized, chemical is clearly superior to all. Of the radioactive lights, blue compares similarly with chemical and is more attractive than either green, yellow or white.

The blinking light source is far less efficaceous than the other light sources evaluated.

Control traps operated from time to time during the three week period produced no significant mosquito catches. Seventeen mosquitoes were captured utilizing

Table 1.—Comparison of light source attractants using Duncan's new multiple range test at the 5% level of error.

Variants	Chemical	Standard	Blue	Green	White	Yellow	Blink
Means [1]	1366	1102	965	871	602	426	318
Relationship							

[1] Average trap indices

only motors with no light sources. Statistically, the chemical, Rad-blue, Rad-green and Rad-white light sources are suitable substitutes for the standard incandescent bulb. In addition, when used in lieu of the incandescent light, the cost of operating the CDC trap is substantially reduced. For example, during a typical mosquito season of six months, cost per night for the standard (utilizing 6 volt batteries), chemical and radioactive light sources (utilizing carbon-zinc flashlight batteries) is $1.10, $.57 and $14 respectively.

SUMMARY.—A field evaluation of six alternate light sources for use in the CDC trap was conducted to determine their effectiveness as mosquito attractants. All of the alternate sources were compared to the standard incandescent light, and to each other.

There was no significant difference in performance among the chemical, radioactive-blue, radioactive-green, radioactive-white and the standard. The blinking and radioactive-yellow light sources were considerably less efficaceous than the chemical, Rad-blue, Rad-green, Rad-white and standard lights. No significant difference was noted between Rad-yellow and blinking lights.

ACKNOWLEDGMENTS.—The authors express sincere appreciation to Dr. Clyde Barnhart, USA LWL, Aberdeen Proving Ground, Maryland, who was instrumental in the formulation and planning of this study.

We also thank Mr. James Cook, Biostatistics Department, USDA, for his helpful suggestions regarding the utilization and presentation of statistical data.

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COLLECTING OVIGEROUS *CULEX PIPIENS QUINQUEFASCIATUS* SAY NEAR FAVORABLE RESTING SITES WITH LOUVERED TRAPS BAITED WITH INFUSIONS OF ALFALFA PELLETS

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ABSTRACT

Eight grams of alfalfa pellets per gallon of water, aged for three days at summer temperatures, proved to be an effective lure for ovigerous *Culex pipiens quinquefasciatus* Say. Females readily entered several types of traps which were charged with the lure. Screen-wire louvers in any trap used retained a higher proportion of the entering females than other types of trap entrances.

EFFECTIVE SWATH SIDTHS OF ULV AND HIGH VOLUME LABELED DOSAGES OF MALATHION AND CHLORPYRIFOS AGAINST CAGED MOSQUITOES

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ABSTRACT

Twenty-one comparison studies of two ULV converted cold foggers and the military cold fogger were conducted at Skaggs Island, Naval Security Group Activity, Sonoma, California by the Navy Disease Vector Ecology and Control Center, Alameda, California. The machines used were the military cold fogger, a LECO ULV converted cold fogger, and a ULV converted cold fogger constructed at DVECC, Alameda. The insecticides, malathion and chlorpyrifos, were dispersed according to the label to caged *Culex pipiens* (susceptible strain) at distances of 100 to 1500 feet from the cold foggers.

Results from preliminary tests were inconclusive because of variable temperatures and wind velocities. The available data indicated essentially equal results for all three machines with the effective swaths ranging from 500 to 1250 feet (average 800 feet). Malathion had a slightly greater swath width than chlorpyrifos; possibly this was a result of more droplets of malathion being dispersed (4.3 ounces per minute) compared to 1.3 ounces per minute of chlorpyrifos. Further tests will be conducted during 1974.

ULTRA LOW VOLUME TESTS OF SEVERAL INSECTICIDES APPLIED BY GROUND EQUIPMENT FOR THE CONTROL OF ADULT MOSQUITOES

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ABSTRACT

Ultra low volume field tests of several insecticides applied by ground equipment were conducted using caged adult *Aedes taeniorhynchus* and *Culex nigripalpus*. Excellent kills (95-100 percent) of both species were obtained with 0.5 gph of technical fenthion and 20 gph of 1 percent naled in diesel oil at a vehicle speed of 10 mph. In tests of chlorpyrifos, 99 percent kill of *A. taeniorhynchus* was obtained at 0.62 gph at a vehicle speed of 10 mph, but at least 0.75 gph was required for equal kill of *C. nigripalpus*. A solution of 5 percent pyrethrins-25 percent piperonyl butoxide in Klearol at 2.0 gph, vehicle speed of 10 mph, resulted in 96 percent kill of *C. nigripalpus*, but 4.0 gph were required for equal kill of *A. taeniorhynchus*. In tests of Actellic, 99 percent kill of both species was obtained at 1.0 gph at a vehicle speed of 5 mph. Dowco 214 killed 93 percent of *A. taeniorhynchus* at 0.5 gph at a vehicle speed of 5 mph, but 1.0 gph gave only 63 percent kill of *C. nigripalpus*. A solution of 25 percent malathion in peanut oil at 1.0 gph, vehicle speed of 5 mph, killed 95 percent of *A. taeniorhynchus* but only 57 percent of *C. nigripalpus*; 25 percent malathion plus 5 percent naled in peanut oil only increased the kill of *C. nigripalpus* to 64 percent. Resmethrin at 10 percent solution in peanut oil killed 83 percent of *C. nigripalpus*, but was very poor against *A. taeniorhynchus*, giving only 4 percent kill. Therefore, species susceptibility to insecticides is an important consideration in establishing the dosage requirements of various insecticides for effective control of different species of mosquitoes.

EVALUATION OF SEVERAL ULTRA LOW VOLUME GROUND AEROSOLS ON CAGED *CULEX PIPIENS*

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There has been extensive research on the ultra low volume (ULV) ground aerosol method of adult mosquito control in other areas of the country, but little on the primary pest species of the midwest, *Aedes vexans* Meigen and *Culex pipiens* Linnaeus. The initial purpose of this investigation was to evaluate several insecticides applied by ULV to caged females of both species. However, *A. vexans* were not available in sufficient quantities during the study period. This report gives results of tests comparing the effectiveness of several insecticides dispersed as ULV in an open field versus an urban area against *C. pipiens* during the summer of 1973.

METHODS.—Test procedures were patterned after those of Mount and Pierce (1972). Field collected *C. pipiens* pupae were allowed to emerge into plastic cages supplied with cotton balls saturated with a 10% sugar-water solution and held in the laboratory. Twenty 2 - 5 day old adult females were later aspirated from the cages and transferred to 6.5 x 15 cm cylindrical 14-mesh galvanized screen wire cages. The cages were placed in styrofoam coolers supplied with ice, taken to the field and exposed to insecticides dispersed by a Leco® ULV Model HD cold aerosol generator moving at 10 mph. After transfer back to the laboratory, the mosquitoes were slightly anesthetized with CO_2 and placed into pint paper cups covered with gauze. The mosquitoes were in the screen cages for a maximum of one (1) hour before and after exposure. A cotton ball moistened with a 10% sugar water solution was placed on each cup. The mosquitoes were held for 24 hours in constant light at approximately 70% RH 75°F. after which mortality counts were made.

Tests were conducted in Lyons, Illinois between 8 and 10 a.m. with temperatures ranging from 60°-82°F. and wind velocities from 2-7 mph.

The Leco ULV was operated at an air pressure of 3.5 psi and since the dosage rate varies with temperature, the driver monitored this function to insure proper flow rate.

Area I--In one series of tests the mosquitoes were exposed in an open park by hanging the cages 6 feet above the ground on stakes 150 and 300 feet downwind, in two rows perpendicular to the line of travel of the generator. Four chemicals were tested at various dosage rates with a minimum of 4 replications per rate. Control cages receiving no treatment and a standard treatment of 4.3 fl oz/min of malathion were included in each trial. Tests with greater than 10% mortality in the control cages and/or more than 80% mortality of the standard were not recorded. The chemicals were as follows:

Cythion + heavy aromatic naphtha (HAN) (1:1 ratio)
Pyrocide® (pyrethrins 5% + piperonyl butoxide 25%)
Cythion + HAN + Lethane® 348 [2-(2-butoxyethoxy) ethyl thiocyanate] (1:1:1 ratio)

Area II--In a second test replicated 4 times, 23 cages of mosquitoes were exposed to single applications of 4.3 fl oz/min of Cythion at 10 mph in an urban situation. An average city block in Lyons, Illinois, with approximately 20 year old homes, a tree lined street, and groomed lawns and shrubbery was chosen. This block was 400 feet deep and 20 cages were placed in shrubbery and open yards throughout the area. Locations in the shrubbery were inside the plant canopy and were chosen to simulate mosquito resting places. The open yard locations were chosen as representative of backyard recreational areas.

Three cages were placed in an open parkway perpendicular to the line of treatment and parallel to the test cages at distances of 150, 300 and 400 feet. These locations were similar to the open field trials in Area I. Control cages receiving no treatment were also set up.

RESULTS AND DISCUSSION.- Area I--The LD_{90}'s of the chemicals tested were estimated from an eye-fitted line drawn on probit graph paper with a minimum of 4 points per line and 320 insects per point. The mortalities at 150 and 300 feet were averaged (Mount and Pierce 1972).

Cythion at 3.7 fl oz/min or 0.046 lb A/A yielded an LD_{90}, based on a 300 ft swath width. Cythion plus HAN in a 1:1 solution produced an LD_{90} at 7.5 fl oz/min (0.047 lb A/A Malathion), about twice that of Cythion alone. A solution of Cythion, HAN and Lethane 384 in a 1:1:1 ratio produced an LD_{90} at 13.1 fl oz/min (0.054 lb A/A of Malathion). These results demonstrated that toxicity of ULV malathion applications to mosquitoes was directly related to the actual amount of Malathion applied per acre, which is contrary to the findings of Thompson (1973). Mount and Pierce (1972) reported that Lethane does not enhance the toxicity of Malathion applied ULV and these results support that observation.

Pyrocide had an LD_{90} at 11.1 fl oz/min. Excluding the weight of the synergist (piperonyl butoxide) as did Mount and Pierce (1972), the LD_{90} rate per acre based on a 300 ft swath was 0.005 lb actual pyrethrins.

Table 1 is a summary of the results and also includes cost figures based on our district's 1973 purchases. From these data it is apparent that for ULV control of *Culex pipiens* in the midwest, pyrethrins give better control on a pound per acre basis but Cythion is superior when compared by flow rate and cost.

Area II--The tests conducted with caged mosquitoes in a typical urban setting revealed that open field evaluation of ULV sprays of Cythion can be correlated to actual urban use. Cages placed around the homes within 150 ft of the line of discharge had 90 to 100% mortality, whether located in the open or in the shrubbery. One cage placed 18 inches inside the canopy of a lilac bush was an exception and averaged 65% mortality.

Table 1.—Results of various chemicals applied by ground ULV to Caged female *Culex pipiens.*

	LD90		
Chemical	Flow Rate oz/min	lb. A/A 300 ft swath	Cost/acre (cents)
Cythion	3.7	0.046	$.034
Cythion + HAN (1:1)	7.5	0.047[1]	-
Cythion + HAN + Lethane 384 (1:1:1)	13.1	0.054[1]	-
Pyrocide	11.1	0.005[2]	$.321

[1] A/A of malathion
[2] A/A of pyrethrins

The caged mosquitoes located in the open parkway had mortalities equal to those observed in the open field test of Area I with an average mortality of 93%. Mortality in the test cages in the open in this zone averaged 87% and those in the shrubs averaged 57%.

The cages at the end of the block (400 ft) averaged 86% mortality in the open parkway and 71% in the open front yard.

These results demonstrate the effectiveness of Cythion ULV applications for mosquito control in urban situations, and chemicals accepted for the control of mosquitoes, when properly applied by the ULV method, should give excellent control of flying mosquitoes and fair to good control of resting mosquitoes.

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EMERGENCY MOSQUITO ADULTICIDING OPERATIONS CONDUCTED IN THE PALO VERDE VALLEY, EASTERN RIVERSIDE COUNTY, CALIFORNIA

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ABSTRACT

Pooled light trap collections of *Culex tarsalis* mosquitoes from the Palo Verde Valley, Eastern Riverside County, California were found to be over 50 percent postive for encephalitis virus. Because of the possible threat of mosquito-borne disease the State Department of Health, Vector Control Section, recommended immediate control measures be instituted to reduce the adult mosquito population. The Coachella Valley Mosquito Abatement District was asked by Riverside County for assistance in conducting the control program. Adulticiding operations over a 42 square mile area surrounding the City of Blythe were begun on 1 September, utilizing two non-thermal aerosol generators (Micro-Gen type) on loan from the U.S. Navy. The insecticide dispensed from these generators was naled (Dibrom) diluted 1 to 5 with cottonseed oil. Pre- and post-treatment light trap counts were tabulated. Pre-treatment counts averaged 246 per trap night. When control operations were terminated on 20 September or 19 days later, the count was reduced to an average of three mosquitoes per trap night.

ULV CHEMICAL CONTROL OF MOSQUITOES, *CULICOIDES* AND TABANIDS IN COASTAL NORTH CAROLINA[1]

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INTRODUCTION.—The coastal area of North Carolina is experiencing rapid growth in recreation and tourist facilities. There is increasing interest in improving the control of insects affecting man (Gerhardt et al. 1973). The major mosquito pests are: *Aedes sollicitans* (Walker), *Aedes taeniorhynchus* (Wiedemann), and *Anopheles bradleyi* King. Other species on occasions assume local importance. The principal sand flies attacking man are *Culicoides furens* (Poey), *C. hollensis* (Melander and Brues), and *C. melleus* (Coquillet). Although more than 40 species of Tabanidae are commonly collected in the coastal area, the principal pests of man are usually the "black deerfly" *Chrysops fuliginosus* Wiedemann, the "yellow deerfly" *Chrysops atlanticus* Pechuman and the "greenhead fly" *Tabanus nigrovittatus* Macquart. In-depth investigations on the biology and control of these coastal mosquitoes and biting flies in North Carolina have been undertaken only recently with reports now beginning to be issued (Dukes et al. 1974a, b; LaSalle and Knight 1973).

Control of these coastal pests will involve judicious integration of a variety of methods into management programs tailored to the local conditions. It is reasonable to assume that temporary adult insect control will often be a part of such management programs. In fact, many localities have used thermal fogging machines intermittently for many years. Recently, ground application by ULV (ultra low volume) non-thermal aerosol machines has come into use, mostly for mosquito control, in many areas other than North Carolina (e. g. Fultz et al. 1972). Data on the effectiveness of this method with the species and conditions in coastal North Carolina have not been available, although ULV machines are being introduced. Therefore, we conducted tests in 1973 and a preliminary summary of our results is presented herein.

MATERIALS AND METHODS.—A truck mounted LECO® Model ULV-HD machine (Lowndes Manufacturing Co., Inc., Valdosta, Georgia) was used. The chemicals used were; 95% malathion (Cythion® ULV, American Cyanamid Co.) and 5% pyrethrin synergized with 15% piperonyl butoxide (Pyrocide® Fogging Formula 7067, MGK Co.). Application rates were mostly those commonly recommended for mosquito control with the machine moving at 10 mph.

Tests were conducted with caged tabanids and with the natural populations of mosquitoes and *Culicoides* sand flies. Each cage test included 3 replicates at several distances from the ULV machine, but only the data from cages at 100 ft (and suspended 5 ft above the ground) are used in this report. The number of flies per cage was usually about 50, but varied from 30 to 90.

Untreated cages were in a nearby, uncontaminated area and handled in the same manner as the treated ones. Flies were removed from the cages in the field 30 minutes after treatment and held in subdued light in screen-topped jars with a supply of sugar-water solution in the lab (about 24°C.) to determine posttreatment mortalities.

Mortalities were determined by counts made after holding in the jars for 15 min, 1 hr and 3 hrs. Mortalities in the untreated controls were less than 4% in most cases. In 3 of the 14 tests, Abbott's formula was used to correct for higher mortalities in the controls.

In the tests on mosquitoes and *Culicoides*, a small community (Atlantic, N. C.) was treated using the existing road and alley system (Figure 1). The area under treatment was about 250 acres. Vehicle speed was 10 mph. All applications were made beginning at dusk (about 8:00 p.m.). The degree of mosquito control was determined by comparing the numbers collected by a light trap during the night before treatment and the night after treatment and by comparing 5-min netting collections (from around a person) made about 15 min before treatment to those made 1 hr after treatment. *Culicoides* control was evaluated from the light trap collection and not from the netting collections which did not yield *Culicoides*.

RESULTS AND DISCUSSION.—A summary of the results of the cage tests is given in Table 1. The 5% pyrethrin (synergized with 15% piperonyl butoxide) gave the fastest knockdown and kill of the tabanid flies. Sometimes the flies recovered. At the dosage rates used, 100% mortality was not achieved. It should be noted that this was a 5% formulation so the amount of pyrethrin was very small. Malathion (95%) gave very little immediate knockdown and mortality, however, after 3 hours over 90% mortality of *C. atlanticus* was achieved at the higher dosages. Further tests are in progress, but these preliminary results indicate that considerably higher dosages will be required to kill tabanids than are commonly used against mosquitoes. The results suggest that synergized pyrethrin has the desirable characteristic of quick action against tabanids.

The results of 29 nights of community treatment are shown in Table 2. Treatment by 95% malathion gave an overall 29% mosquito reduction based on the light trap data and 67% reduction based on the netting data. Synergized 5% pyrethrin gave an overall 38% mosquito reduction based on the light trap data and 92% control based on the netting data. Essentially, the light traps measured longevity of control (i. e. overnight) while the netting measured immediate (1 hr posttreatment) effectiveness of the treatment. Thus, the pyrethrin treatments gave a greater degree of immediate reduction and both chemicals gave about the same

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[1] This research was supported by the North Carolina State University Agricultural Experiment Station and by NOAA, Office of Sea Grant, U. S. Department of Commerce, under Grant No. 2-35178. The U. S. Government is authorized to produce and distribute reprints for governmental purpose not withstanding any copyright notation that may appear hereon.

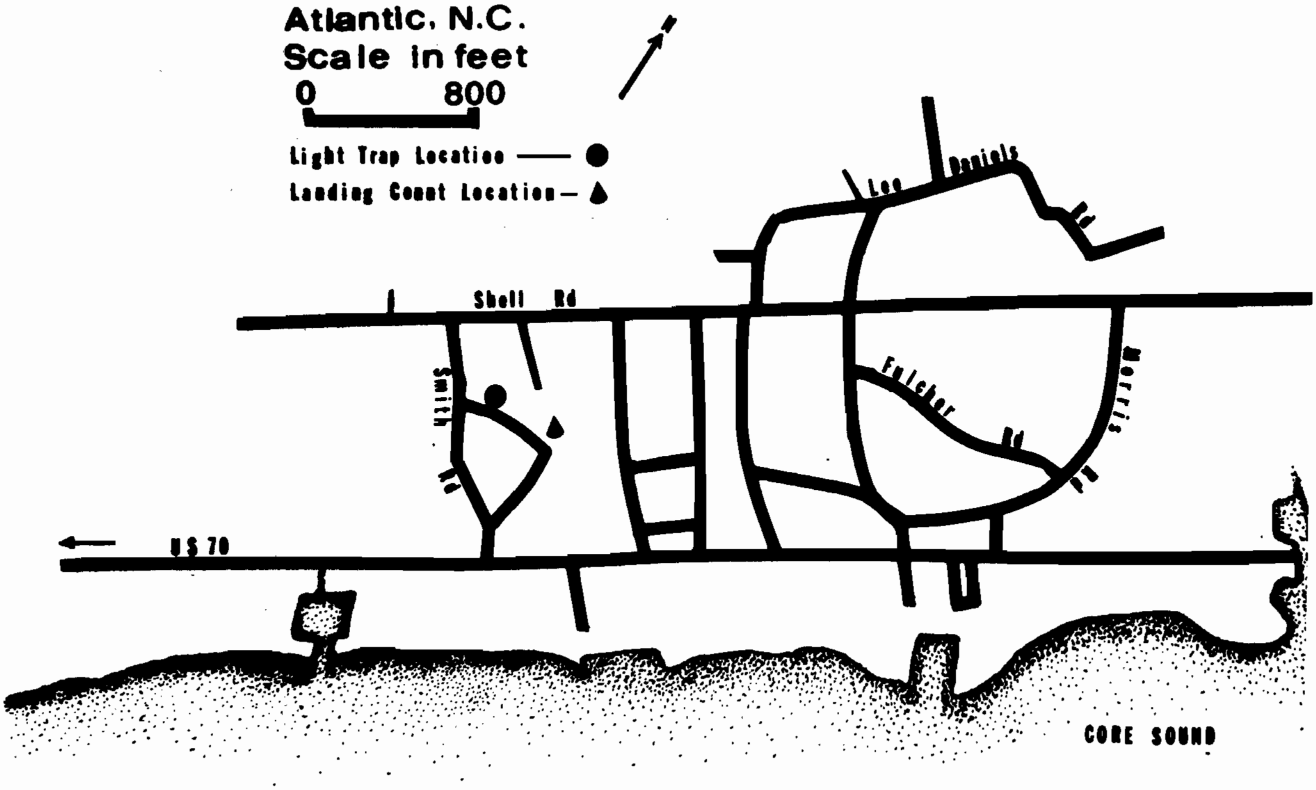

Figure 1.–Diagram showing roads used in whole community treatment by ULV ground equipment. Atlantic, Carteret County, North Carolina.

Table 1.–Mortalities of caged tabanid flies at various times following ULV applications of 5% pyrethrin synergized with 15% piperonyl butoxide and 95% malathion.

Chemical	oz/min	No. Tests	Percent Mortality 15 min	1 hr	3 hr
			Tabanus nigrovittatus		
pyrethrin	3.5	1	72	82	78
malathion	4.3	1	5	0	28
			Chrysops fuliginosus		
pyrethrin	4.0	1	33	52	28
malathion	4.3	2	0	29	54
			Chrysops atlanticus		
pyrethrin	3.5	3	58	54	52
	4.0	1	54	58	32
	7.0	1	61	74	61
malathion	3.5	1	2	26	66
	4.3	2	5	19	91
	7.0	1	2	69	99

level of reduction for the remainder of the night. The species of mosquitoes collected were *A. taeniorhynchus*, *A. sollicitans* and *An. bradleyi*, in that overall order of relative abundance but the proportions varied among the tests.

Table 2.–Control (%) of mosquitoes and *Culicoides* sand flies by whole community treatment with ULV application (10 mph) of 5% pyrethrin synergized with 15% piperonyl butoxide and 95% malathion.

Chemical	oz/min	Tests	Mosquitoes Trap	Mosquitoes Net	Sand flies Trap
pyrethrin	3.5	6	48	77	64
	4.0	12	34	99	27
malathion	3.0	3	43	58	0
	4.3	8	24	92	47

Culicoides reduction (overnight) was slightly greater (34%) overall with the malathion treatments than with the synergized pyrethrin treatments (25% overall). *C. furens* was the only species present in large numbers during these tests. Variations in reduction were large among the tests and further testing and development of better sampling methods are in progress.

These community tests indicate that ULV ground applications of malathion and synergized pyrethrin, at the rates used, can be expected to give a satisfactory level of immediate posttreatment control of these common coastal species of mosquitoes. The degree of *Culicoides* reduction is less certain although considerable relief may be expected.

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FIELD TESTS OF INSECTICIDES APPLIED BY ULTRA LOW VOLUME GROUND EQUIPMENT FOR THE CONTROL OF ADULT STABLE FLIES, *STOMOXYS CALCITRANS* (L.)

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ABSTRACT

Field tests of malathion, resmethrin (SBP-1382), actellic, Dowco 214, Baytex, Dursban, Pyrethrins (5%), and naled (Dibrom) applied as non-thermal aerosols with ultra low volume ground equipment were conducted against caged adult stable flies, *Stomoxys calcitran* (L.)

Resmethrin and Actellic were highly effective (95% +) against stable flies at dosage rates of .25 and 1 gal./hour, respectively at a vehicle speed of 5 mph. Baytex, malathion, Dursban, and Pyrethrins (5%) also gave high mortality (92% +) of flies at dosage rates of .5, 1, 1, and 4 gals./hour respectively at 10 mph. Dowco 214 was sublethal at both rates of .5 and 1 gal./hour and 5 mph.

Tests of 1% Dibrom 14 by volume in diesel oil dispersed at the rate of 20 gals./hour and 10 mph killed 98% of the flies. This dosage rate was .01 lb./acre A.I. based on a swath of 300 feet. This is one fourth the dosage of Dibrom 14 and one-half the volume recommended for control of adult stable flies in Florida by thermal aerosol.

LABORATORY SUSCEPTIBILITY TESTS OF SOME FLORIDA STRAINS OF *AEDES TAENIORHYNCHUS* (WIED.) AND *CULEX NIGRIPALPUS* THEOB. TO MALATHION AND NALED, 1972-1973

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ABSTRACT

First generation larvae from field collected adults of *Aedes taeniorhynchus* (Wied.) from two offshore islands on the southwest coast, the Tampa area, the Florida Keys, and Brevard and Martin counties on the east coast of Florida were found to be from 2-30 times more resistant to malathion at the LC_{90} level than the susceptible laboratory colony. F_1 adults from some of these areas were up to 35 times less susceptible than those of the laboratory colony. indicating a decrease in susceptibility to malathion; however, one area (near Tampa) and several others reported during previous years were as susceptible as the laboratory colony. No resistance to naled was found in either the larval or adult stages. F_1 larvae and adults of *Culex nigripalpus* Theob., collected from five counties in the state were shown to be as susceptible to malathion and naled as those of the laboratory colonies.

FIELD EVALUATION OF BLACKFLY CONTROL – GROUND APPLICATIONS[1]

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INTRODUCTION. Adequate protective measures against blackflies and other biting diptera at Camp Drum, New York have been needed for many years, but the need now has become more urgent with the reorganization of the U.S. Army and its emphasis on better trained and equipped Reserve and National Guard Forces. The annoyance and irritation caused by blackflies is adverse to morale, training and recruitment, especially during the spring and summer training months.

In May 1973, a preliminary study was initiated to evaluate ultra low volume (ULV) ground applications of nonpersistent insecticides for the control of adult blackflies. In the past five years, numerous field studies have shown excellent results and success with such application for the control of adult mosquitoes as well as other pestiferous insects. Mount et al. (1968) showed that the dispersal of ultra low volumes of undiluted insecticides as nonthermal aerosols using Malathion applied at 0.68 gallon per hour (gph) was as effective against caged adult mosquitoes as diluted thermal aerosols dispersed at 40 gph. Lower costs and less environmental contamination offered by this type of application (Taylor and Schoof 1971) make this technique more desirable and ideal for selection. This reasoning coupled with the recent innovations of commercial ULV dispersal equipment has created considerable interest in this concept for insect control. The following tests were made during the spring and summer of 1973 at Camp Drum, to determine: (1) the efficacy of various aerosols as adulticides; (2) the species composition of blackflies attacking man during the study; (3) the most favorable time of day for ULV spraying; and (4) the re-entry time of blackflies after spraying.

MATERIALS AND METHODS. Seven test sites including one check area were used, each approximately a mile long and 300 feet wide. The following insecticide formulations were applied: (1) 33.8% Malathion plus 14.5% Lethane®; (2) Pyrocide® 7067 (5% Pyrethrum plus 25% Piperonyl Butoxide); (3) Pyrocide X-2749 (5% Pyrethrum, 25% Piperonyl Butoxide plus 5% Repellent 11); (4) 85% Dibrom-14®; (5) 9.3% Dibrom-14 and (6) 6.5% Baygon®. The test area is described as a decidious forest with a combination of dense and semi-dense vegetation consisting mostly of single canopy with some small open areas measuring approximately 100 square yards. A Rotary-Tube Sprayer described and designed by Barnhart and Sheldon (1973) was used for dispersing all of the compounds. The principle of the rotary-tube sprayer is somewhat analagous to the "flit gun", in which air is passed at a high velocity over the tip of a liquid-filled tube. In the rotary-tube sprayer, the tube of liquid is rotated so that its tip moves rapidly through the air. The effect is atomization in both cases. This principle was first developed by Barnhart (1962) at Fort Belvoir, Virginia.

The Rotary-Tube Sprayer has two polypropylene plastic tubes, 1/8 in OD, mounted on a metal bowl set on edge, which is belt-driven. The rotary-tubes are located in the outlet air stream of a 17,000 cu ft/min vanaxial fan, also belt-driven. The air stream serves to move the atomized liquid from the vicinity of the sprayer. A six horsepower gasoline engine powers both fan and rotary-tubes. The speed of rotation of the tubes can be varied between 3,000 to 6,000 rpm. An automotive type tachometer registers the speed.

Liquid to be sprayed is pumped to the rotating bowl by means of an electric fuel pump. The sprayer bowl has a constricted rim. Centrifugal force prevents overflowing and no packing gland or rotary seal is needed where the liquid enters the bowl. The liquid leaves the bowl through two oppositely mounted 1/8 in OD polypropylene tubes, each of such length that the distance from the center of the bowl to the tip of the whip is 11 in. The Rotary-Tube ULV Sprayer was calibrated to deliver three gallons per hour of the material tested with an RPM setting of 5,600.

In order to determine the efficacy of the aerosols tested, prior to each test live flies were collected in hand sweep nets, and also collected in the passenger cab area of the evaluators' truck by the use of an aspirator. Ten flies each were placed in clean, No. 30 mesh nets, positioned behind and/or on top of vegetation approximately three feet above ground at fixed distances downwind from the sprayer. To ensure that the netted flies were not influenced by heat or other adverse factors prior to testing, they were held for at least 30 minutes 200 yards upwind of the sprayer and observed for mortality. Immediately after exposure, the flies were taken out of the bags and placed in clean white enamel pans (12 x 24 x 2) and observed for mortality. During each test, pre-counts of blackflies were made at locations approximately 150 feet into the test site. This was accomplished by sweeping three times above the investigators head every 15 seconds, taking the highest count. After each sweep, flies were killed by crushing in the nets to prevent recapture. After spraying, re-entry time was noted by counting the number of minutes elapsing before the flies were seen to return. After determining re-entry time, a "posttreatment count" was conducted in the manner described above. At least two replicates were used for each compound tested. The passes were made at a rate of three gallons per hour with a vehicle speed of 5 mph. The dosage in lb/acre was calculated on the basis of a swath width of 300 feet (182 acres per hour). This method of recording dosages probably does not give a true measure of insecticidal deposit per acre; however, it does enable investigators to compare one test to another. Droplet size was determined by handwaving

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[1] The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the DOD. Research sponsored by the U.S. Army Medical Research and Development Command, Washington, D. C., 20314, under Contract/Grant No. DA 3A 061102B71P01.

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teflon-coated slides at 25 feet from the sprayer according to the method of Yeomans (1949). A minimum of 200 droplets was sampled for each compound tested. Any droplet with a spread factor greater than 1.48 received no correction factor. All tests were conducted in the afternoon during the period 1600-1800 hrs. Temperature and humidity were determined at the test site by thermometers and sling psychrometers. Windspeeds were measured with a Dwyer® wind meter.

To determine what time of day would be most advantageous for spraying, a seven day field monitoring test was conducted at one of the sites. Each day was divided into four time periods as follows: sunrise (0500-0800); noon (1130-1230); afternoon (1600-1630) and sunset (1900-2100). Temperature, humidity, general weather conditions and blackfly activity were noted. Blackfly activity was determined by use of the handsweep-net over and around the investigator's head. During each time period, specimens were collected, mounted and later identified.

RESULTS AND DISCUSSION. Results of the ground ULV spraying are shown in Table 1. Although kill was shown for all compounds except Malathion plus Lethane up to distances of 225 feet, the natural populations at all sites were not reduced for any period longer than 3-6 minutes. All compounds were effective but there was a difference in time required to obtain 100% kill. Dibrom-14 in both concentrations was the most effective for quick kill (3 min.), whereas, Malathion/Lethane took the longest time to produce 100% mortality (25 min.). There was no significant difference between the pyrethrum compounds. Table 2 shows the results of the compounds tested against natural population of blackflies. The average temperature was 75°, humidity 65%, windspeed 6 mph.

Table 1.—Kill of netted adult *Simulium venustum* Say obtained with ULV rotary-tube sprayer.

Compound	Discharge gph	No. Tests	Distance (ft) & Kill (%)			
			50	125	225	300
malathion (38.8%)/ lethane (14.5%)	3	4	100	70	0	0
dibrom 14 (85%)	3	2	100	100	100	0
dibrom 14 (9.3%)	3	3	100	100	100	0
pyrocide 5%	3	3	100	100	70	0
pyrocide x-2749	3	2	100	100	70	0
baygon (6.5%)	3	2	100	100	50	0

Two species of blackflies, *Simulium venustum* Say and *Prosimulium hirtipes* complex were responsible for attacks on man in the test area and were the most persistent and abundant species collected. Figure 1 shows activity at different times of the day. Adult flight activity was influenced by the following: temperature, light intensity, windspeed, rain and other meterological factors. There appeared to be an upper and lower temperature gradient which strongly in-

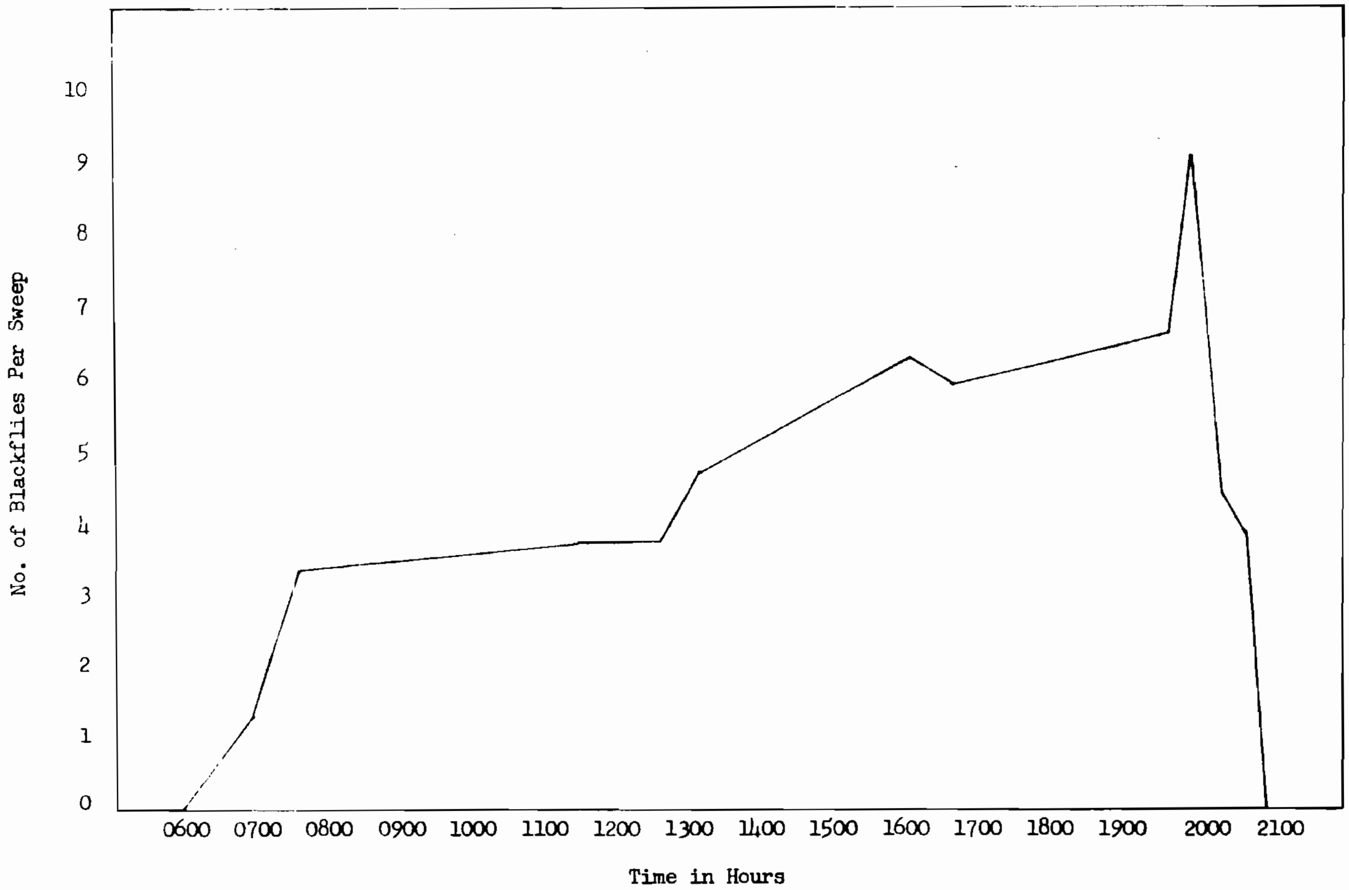

Figure 1.—Average daily distribution of blackflies during a seven day period (29 May - 4 June).

Table 2.—Data from spraying with ultra low volume aerosols at Camp Drum, N. Y.

Compound	Dosage lb per acre	Re-entry Time Minutes	Avg. Flies per Sweep Pre-spray	Avg. Flies per Sweep Post-spray
malathion (38.8%)/ lethane (14.5%)	.062/.018	3	6	7
dibrom 14 (85%)	.230	6	5	7
dibrom 14 (9.3%)	.022	6	7	7
pyrocide (5%)	.007	5	5	6
pyrocide x-2749	.007	6	6	6
baygon (6.5%)	.011	3	5	6

fluenced blackfly activity. Blackflies became active at temperatures above 50°. Windspeeds greater than 7 mph decreased blackfly activity to nil. Observations indicate that only a small percentage of the blackflies in any area are flying at a given time. Most are resting in dense vegetation or other protected sites. When an individual or a vehicle moved into an infested area, at first there was no great sign of blackfly activity; however, within 2-3 minutes the flies appear to sense the host and actively seek him out.

The Rotary-Tube Sprayer used for these tests presented a variable in the test data because of inconsistent performance. The shroud enclosure trapped a considerable amount of material which leaked onto the engine, creating inhalation hazards, and allowed significant loss of material to occur, and the production of larger droplets. Mount (1970) concluded that for effective ground aerosol applications, a mass median diameter droplet size of 5 to 10 µ is optimum to impinge on target insects. Lofgren et al. (1973) conducted tests with soybean oil dispersed by a truck-mounted Leco Model HD® aerosol generator and found that aerosol droplets of 2-16 µ impinged on all body sections of caged mosquitoes, while under field application conditions, droplets of 1-8 µ diameter impinged on outdoor non-caged specimens. In both cases impingement of droplets greater than 16 µ was not obtained. The results of our tests show that most of the aerosol particles produced by the Rotary-Tube Sprayer were not that small and plentiful enough to give adequate coverage, and yet impinged readily on the body surface of the blackflies. Observations on the amount of material trapped in the shroud substantiate these findings even when consideration is given to the inability of the hand-waved slide collection technique to collect droplets smaller than 5 µ (Rathburn 1970). Perhaps the rotary-tube whips should be located outside the shroud to alleviate this problem. The sprayer used failed to give effective penetration into dense vegetation and to adequately clear the vehicle. As a result, there was excessive wetting by spray material on the side of the vehicle after each application. Droplet ranges and mass median diameters of the compounds tested are shown by Table 3.

CONCLUSIONS.—Ground ULV applications of non-persistent insecticides produced kills of trapped adult blackflies at distances up to 225 feet downwind. Dibrom at two concentrations tested was the most effective compound, and required the least amount of time to give 100% kill (three min.). Control of the natural adult population was not achieved: (1) dispersal of the insecticide beyond 225 feet could not be accomplished; (2) re-entry and infiltration from the outer perimeter not sprayed was great; and (3) a significant amount of the material released did not reach the target insect; (4) the particles were too large to penetrate vegetation and to impinge on the blackflies.

Additional research should be done to evaluate the effect of environmental and meterological factors such as temperatures, windspeed, etc. which strongly influence blackfly activity and are directly correlated with the effectiveness of control measures.

Table 3.—Mass median diameter and range distribution of ultra low volume ground aerosols tested.

Compound	Range (µ)	Average (µ) Dia.	mmd (µ)	Discharge gph
malathion (38.8%)/ lethane (14.5%)	8-55	19	33	3
dibrom 14 (85%)	10-38	14	24	3
dibrom 14 (9.3%)	19-38	22	28	3
pyrocide (5%)	8-57	18	27	3
pyrocide x-2749	3-76	27	37	3
baygon (6.5%)	8-36	19	28	3

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CULEX TARSALIS:
RESISTANCE TO ORGANOPHOSPHORUS INSECTICIDES IN RETROSPECT

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Reports of control failures and laboratory studies of field populations have firmly established the presence of organophosphorus (OP) insecticide resistant populations of *Culex tarsalis* Coq. in California. The array of chemicals to which this species has developed resistance is amazing. In many areas resistance extends to all licensed mosquito larvicides, namely malathion, parathion, methyl parathion, fenthion, EPN, chlorpyrifos, and Abate (Womeldorf et al. 1972).

A comprehensive investigation into the mechanisms and genetics of OP resistance in *C. tarsalis* has just been completed in our laboratory (Apperson 1974). The results of this study enable us to provide an explanation for the rapid development of extensive OP resistance in *C. tarsalis* in recent years.

Malathion resistance was first reported for a population of *C. tarsalis* from the San Joaquin Valley of California (Gjullin and Isaak 1957). By 1965 resistance had developed in many other areas of California (Womeldorf et al. 1972). Malathion is a unique insecticide in that it is detoxified almost exclusively by one enzyme, a carboxyesterase. This has been substantiated through biochemical studies (Bigley and Plapp 1962, Matsumura and Brown 1963a, b) and by genetic studies (Matsumura and Brown 1963b) of malathion-resistant *C. tarsalis*. Consequently, cross-resistance in malathion-resistant populations extends only to malaoxon (Darow and Plapp 1960) and other closely related analogs (Dauterman and Matsumura 1962, Plapp et al. 1965).

The consequences of use of other substitute OP larvicides were manifested in 1969 when Georghiou et al. found extensive OP multi-resistance in the formerly malathion-resistant population of *C. tarsalis* from the Central Valley. A similar situation was reported by Apperson and Georghiou (1974) in the Coachella Valley in 1971. Larvicides, such as parathion and fenthion, are different from malathion in that they possess common structural sites which are vulnerable to enzymatic attack. Thus, the use of any one compound should produce cross-resistance to the other, although the levels of resistance may vary according to the selecting agent. Since additional mechanisms potentially can be involved in resistance to these chemicals, their use could produce a population of mosquitoes that contain a multiplicity of resistance factors.

Our studies confirm that this is what has occurred with *C. tarsalis*. When methyl parathion selection pressure was applied to a multi-resistant field strain of *C. tarsalis* in the laboratory (Apperson and Georghiou 1974), resistance was increased to the greatest extent against those compounds most similar in structure to the selecting agent. Use of synergists suggested the involvement of several enzymes in resistance. Biochemical studies revealed that at least two separate resistance mechanisms were involved. Subsequently, in studies of the inheritance of resistance, it was found that the F_1 hybrid exhibited a high degree of dominance. OP resistance was also found to derive from a multiplicity of genes. Clearly then, the rapid development, high levels and broad spectrum of OP resistance manifested by *Culex tarsalis* is due to the integration and consolidation of a multiplicity of defenses into field populations. Through interaction, the totality of these defense mechanisms becomes sufficiently non-specific to enable the population to withstand a diversity of toxicants.

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INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES IN CALIFORNIA: STATUS OF ORGANOPHOSPHORUS RESISTANCE IN LARVAL *AEDES NIGROMACULIS* AND *CULEX TARSALIS* THROUGH 1973

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Resistance to chemical pesticides has complicated mosquito control in California since failure of DDT against *Aedes nigromaculis* was reported by Smith (1949) and against *Culex tarsalis* by Gjullin and Peters (1952). Widespread failures of the organochlorine insecticides followed and the organophosphorus compounds were substituted as they became available. Inevitably, resistance followed and one organophosphorus larvicide was substituted after another.

Generally, an agency with extensive *A. nigromaculis* breeding areas, typically those in the Great Central Valley, uses the same chemical throughout its jurisdiction. Thus, *C. tarsalis* larvae, which frequently occur in excess irrigation water following a brood of *A. nigromaculis*, are exposed to the same pattern of insecticide use as the pasture mosquito.

In 1963 the (now) Vector Control Section of the California Department of Health, in cooperation with mosquito control agencies, instituted a surveillance program designed to define the extent and severity of resistance as it applied to operational mosquito control in California. Results were first reported by Brown et al. (1963) and have since been reported periodically (Gillies 1964, Womeldorf et al. 1966, 1968, 1971, 1972). As resistance developed, high levels of tolerance for several species of California mosquitoes were listed for each agency by species and year of the first laboratory confirmation of a population mean lethal concentration (LC_{50}) exceeding 0.005 parts per million (ppm) parathion, methyl parathion, and fenthion, or 0.1 ppm malathion. Slopes of the log concentration-probit lines (1c-p) were not reported.

To demonstrate the severity of the resistance problem, this paper reports the highest LC_{50} records for each mosquito control agency during the past decade for *A. nigromaculis* and *C. tarsalis* larvae, the two species most affected by resistance. Malathion, parathion, methyl parathion, fenthion and chlorpyrifos are included to represent the organophosphorus larvicides.

Test results were obtained as described by Gillies and Womeldorf (1968). All data were processed through the computerized probit analysis program developed in conjunction with the surveillance program. Test results were considered invalid if there were fewer than three data points, lack of mortality above or below 50%, mixed species, pupation or statistically significant heterogeneity.

Tables 1 and 2 list the data for each agency by chemical, year of test, slope of the line, and the LC_{50} and its 95% confidence limits. The inclusion criteria caused some test data to be left out, so that not all chemicals or agencies are listed although tests were performed in some cases. Chemicals are listed in their approximate sequence of introduction and use. Mosquito control agencies are listed from north to south, except for some isolated agencies in southern California.

INTERPRETATION.–Levels of less than 0.005 ppm at the LC_{50}, accompanied by an LC_{90}/LC_{50} ratio of less than two, are generally descriptive of susceptible populations which can be controlled by parathion, methyl parathion or fenthion when applied at 0.1 lb/acre. As the LC_{90}/LC_{50} ratio increases, incipient resistance is suspected and limited field failures may occur when this depressed slope is combined with an LC_{50} of approximately 0.005 ppm. An LC_{50} of 0.01 ppm or above signifies moderate resistance. Control in the field decreases rapidly above this point and a new chemical is generally substituted. Continued pressure with a replacement organophosphorus material may push the LC_{50} even higher, but when the LC_{50} reaches 0.1 ppm, it is only of academic importance, as complete loss of control with that chemical occurred long ago.

A homogenous susceptible population is, then, characterized by a low LC_{50} accompanied by a steep slope of the 1c-p line. That is, mortality occurs within a very narrow range of concentrations and the LC_{90} is generally less than twice the LC_{50}. Gillies et al. (1968) demonstrated the change in the slope of the lc-p line as resistance progresses. In the beginning, the proportion of more tolerant individuals in a population increases and is demonstrated by an increase in the LC_{90}/LC_{50} ratio. This initial change in slope may or may not be accompanied by a marked change in the LC_{50}, but is the first indication of incipient resistance and occurs well in advance of noticeable control failures in the field. As resistance increases, both the LC_{50} and the LC_{90}/LC_{50} ratio increases until the less tolerant individuals in the population are eliminated and the LC_{90}/LC_{50} ratio decreases to again approach two and the population is again fairly homogenous, but now for resistance. In the highly resistant field populations of *A. nigromaculis* that we have tested, developing homogeneity for resistance is evident, but the LC_{90}/LC_{50} ratio has not again become less than two. However, in *C. tarsalis*, LC_{50} levels above 0.01 ppm parathion, methyl parathion or fenthion are not accompanied by a marked increase in the LC_{90}/LC_{50} ratio, indicating that the populations are fairly homogenous for the increased tolerance.

CURRENT STATUS.–*Aedes nigromaculis* resistance currently extends to all organophosphorus larvicides in many areas of the state (Table 1, Figure 1). The first laboratory verification of resistance in this species occurred in

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TABLE 1.–Highest laboratory demonstrated level of organophosphorus tolerance in larvae of *Aedes nigromaculis* through 1973.

AGENCY	MALATHION					PARATHION					METHYL PARATHION					FENTHION					CHLORPYRIFOS				
	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits
Pine Grove	1965	2.22	3.78	.18	.15 - .21	1965	5.92	1 65	.0045	.0041- .0050	1964	3.36	2.41	.0012	.0010 -.0015	1964	8.33	1.42	.0013	.0012 -.0014	1972	6.07	1.63	.00082	.00074-.00090
Shasta						1971	1.26	10.6	.055	.035 - .097	1971	.92	24.72	.011	.0066 -.016	1971	1.20	11.70	.0058	.0041 -.0079	1971*	3.74	2.20	.00077	.00061-.00098
Tehama County	1969	5.63	1.69	041	.033 - .050	1969	1.37	8.62	.067	.051 - .089	1972	1.33	9.20	.0075	.0049 -.012	1970	3.64	2 25	.0018	.0014 -.0023	1969	1.29	10.03	.0025	.0019 -.0038
Los Molinos	1972	2.61	2.85	.055	.046 .067	1970	2.87	2.80	.0030	0025- 0037	1972	4.36	1 97	.0014	.0012 -.0016	1972	3.81	2.17	.00099	.00081-.0012	1972	6.91	1.53	.00072	.00063-.00082
Corning						1965	2.02	4.31	.0046	.0038- .0058	1967	2.51	3.24	.00071	.00033-.00096	1966	6.40	1.59	.00079	.00067-.00093					
Butte County	1969*	4.63	1.89	.063	.059 - .069	1969*	2.34	3.53	.23	.18 - .28	1969*	1.28	10.21	.035	026 049	1972*	2.17	3 90	.0023	.0018 -.0029	1971*	2.33	3.55	.00029	.00021-.00039
Colusa	1966	6.00	1.64	.021	.019 - .024	1966	2.56	3.17	.028	.023 - .034	1971	1.50	7.15	.10	.074 -.15	1971	1.49	7.25	.0090	.0069 -.012					
Sutter-Yuba	1969*	3.73	2.21	.039	.033 - .045	1970*	3.14	2.56	.48	.40 - .57	1969*	1.29	10.03	.20	.15 -.27	1969*	1.67	5.85	.035	.027 -.045	1969*	1.66	5.92	.031	.019 -.12
Sacramento-Yolo	1969	4.37	1.97	.017	.014 - .021	1972	2.12	4.02	.012	.0096- .015	1969	2.22	3 78	.0011	.00077-.0014	1971	2.00	4.37	.0032	.0024 -.0045	1971	2.82	2.85	.0040	.0029 -.0075
Solano County	1970	5.40	1.73	052	.045 - .060	1970	1.98	4.44	.030	024 - .038	1970	2.63	3.07	.0054	.0045 -.0066	1972	8.25	1.43	.0012	0010 -.0013	1970	3.80	2.18	.00092	.00082-.0010
Diablo Valley						1971	3.74	2.20	.51	.40 - .65	1971	1.76	5.35	.17	.13 -.24	1971	2.02	4.31	.024	.016 -.066					
No. San Joaquin	1963	1.38	8.48	.17	.096 - .28	1964	4 83	1.84	.0023	.0021- .0025	1971	3.75	2.20	.0014	.0011 -.0018	1964	9.79	1.35	.0030	.0028 -.0032	1972	6.18	1.61	.00075	.00069-.00083
San Joaquin	1972*	11.13	1.30	.031	.028 - .034	1964	2.38	3.46	.0056	.0046- .0074	1972*	2.57	3.15	.0038	.0029 -.0051	1971*	4.21	2.02	.042	.033 -.052	1971*	2.50	3.26	.0040	.0032 -.0052
Eastside	1972	1.84	4.97	.10	.081 - .13	1971	2.56	3.17	.014	.013 - .018	1971	1.76	5.35	.0022	.00074-.0035	1972	1.67	5.85	.014	.0065 -.022	1967	4.03	2.09	.00063	.00050-.00076
Turlock	1963	4.02	2.09	.061	.042 - .074	1968	1.70	5.67	.10	.072 - .16	1971*	2.36	3.49	.25	.18 -.40	1971*	2.10	4.08	.081	.059 -.11	1971*	2.36	3.49	.054	.033 -.066
Merced County	1967	1.53	6.88	.12	.099 - .15	1972	1.24	11.01	46	.29 -1.11	1972	1.55	6.71	.15	.11 -.22	1972	1.63	6.11	.062	.045 -.098	1972	1.13	13.62	.014	.0091 -.056
Madera County	1972	4.65	1.89	069	.059 - .081	1972	1.99	4.41	.0088	.0065- .014	1972	1.73	5.51	.0020	.00071-.0030	1973	2.44	3.35	.15	.12 -.18	1972	3.51	2.32	.00064	.00048-.00078
Fresno Westside	1972	3.72	2.21	21	.19 - 24	1972	1.69	5.73	30	.21 - .56	1972	1.96	4.51	.37	.31 - 43	1972	3.16	2.27	.21	.19 -.24	1972	3.20	2.51	.034	.030 -.039
Fresno	1963	3.21	2 50	.027	.020 - .035	1963	1 44	7.76	.049	.029 - .15															
Consolidated	1965	2.54	3.20	.87	.71 -1 1	1970	2 79	2 88	.020	.015 - .026	1972	3.16	2.54	.019	.015 -.022	1970	2.49	3.27	.0042	.0035 -.0049	1970	2.81	2.86	.0058	.0045 -.0077
Delta	1964	2.26	3 69	19	16 - 24	1963	3.07	2.62	.17	.13 - .22	1972	3.65	2.25	.17	.14 -.19	1972	4.32	1.98	.010	0078 -.012	1969	1.73	5.51	.0017	.0012 -.0037
Kings	1971	2.09	4 10	.23	.11 - .34	1970	2.53	3.21	.37	.27 - .51	1970	2.27	3.67	.64	.50 -.85	1970	3.35	2.41	.025	.022 -.029	1972	3.12	2.58	.0073	.0063 -.0090
Tulare	1965	2.05	4.22	.39	.27 - .73	1971	2.72	2.96	.68	.58 - .85	1971	1.25	10.80	.50	.35 -.72	1970	2.97	2.70	.053	.042 -.079	1971	3.59	2.28	.013	.011 -.015
Delano	1972	3.43	2 37	.14	.12 - .16	1972	2.24	3.73	.11	.085 - .13	1972	1.66	5.92	.17	.12 -.25	1972	5.68	1.68	.0087	.0077 -.0099					
Kern	1964	1.63	6.11	.50	.31 - .94	1964	2.26	3.69	.17	.13 - .22	1965	2.71	2.97	.080	.059 -.15	1965	5.91	1.65	.011	.0094 -.014	1973	2.61	3.10	.0040	.0032 -.0050

* Mosquito control agency data

FOUR-SITE METHOD FOR MOSQUITO REPELLENT FIELD TRIALS

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INTRODUCTION.—As part of a Letterman Army Institute of Research (LAIR) program to find a mosquito repellent better than the repellent most frequently used, N,N-diethyl-m-toluamide (DEET), new repellents and repellent formulations have been evaluated by comparison to DEET in laboratory screening tests using a four-site testing method. To determine the efficacy of these compounds under field conditions and to evaluate laboratory screening techniques, a series of laboratory trial results were compared to field test results. This paper is a report on the four-site method used in the field trials.

MATERIALS AND TEST DESIGN.—The compounds which were evaluated in the field trials are as follows:

cyclohexamethylene-carbamide (carbamide), 99% pure, (Stepanor 1967 and Dremova 1970)

n-butane-hexamethylene-sulfonamide (sulfonamide), 99% pure, (Maslii 1966; Zhogolev 1970; and Prevomaiskii 1967)

3,6,9-trioxapentadecan-1-ol (SRI-6), 96% pure, (Skinner 1974)

N,N-diethyl-m-toluamide (DEET), 95% pure, (Eastman Chemicals, Practical Grade)

The standard LAIR testing procedure is a four-site design (Brodel 1974) using two sites on each forearm, with defined areas of 7 x 10 cm each. This design permits testing three compounds simultaneously in addition to a control repellent. This capability is of importance when performing field trials because of day to day changes in climate, test subject, and mosquito populations. Moreover, the data can be analyzed by a paired comparison of a test formula to DEET (Ostle, 1963).

In the laboratory, repellent solutions are applied to two 7 x 10 cm test sites on each forearm, and are spread with a clean glass rod (Brodel, 1974). The arm is then inserted into a plastic sleeve measuring 20 cm x 70 cm (Khan, 1968). This sleeve has two 5 x 8 cm holes, exposing the repellent application sites to the mosquitoes.

Testing takes place for three minutes in two 1.25 cubic foot cages each containing approximately 250 avid female *Aedes aegypti* mosquitoes. The volunteers[3] are tested each hour in alternate cages. Failure of a site is determined by two bites during a testing period or two consecutive bites over two periods (Brodel 1974).

For field trial testing, the LAIR method (Brodel, 1974) was modified to include the total circumference on each forearm. The field trials were accomplished in the following manner:

1. Adhesive backed foam strips about ¾″ wide were wrapped around the wrist, upper forearm, and mid-forearm. The foam strips served as boundaries and as protective devices against abrasion for the given sites. The foam strips outlined two sites on each forearm of every volunteer; four sites per individual.
2. One of the four repellents, DEET, SRI-6, carbamide, or sulfonamide, was applied in ethanol solution to each site at a constant concentration per unit of forearm area. The volume applied varied according to the surface area of the site on the various individuals. To determine the surface area, the circumference of the arm was measured at points 3 cm, 12 cm, and 21 cm from the wrist. The surface area of each test site was calculated as the area of a frustum of a cone: $A = s(R_1 + R_2) \times \pi$ (Selby, 1968) where A = area, s = 9 cm, and R_1, R_2 are the radii at the upper and lower boundaries of a site.
3. The total circumference of the area marked off by the foam strips was covered with the repellent solution for that site. The repellent was applied so that no repellent appeared on a given site more than once in any group of four individuals. Each subject had his own DEET control.

   All results were analyzed using a paired comparison with DEET and having statistical significance at the 95% level as determined by the Student's t-distribution. Six and 7 August were replicates and the data for the two days were combined for analysis.
4. The twelve individuals used in the test were divided into three equal groups each containing four volunteers and the application times for the volunteers were staggered to obtain differentiation between repellent protection. Testing occurred from one-half hour before to one-half after sundown, except on 10 July 1973. A concentration of 0.48 mg/cm^2 was used in repellent tests except as stated elsewhere. This concentration was designed to last approximately from an initial morning application to testing at sunset. The application times are as follows:

	25 Jun 73	6, 7 Aug 73	8 Aug 73
Group I	0800	0700	1000
Group II	1000	0930	1000
Group III	1200	1200	1000

5. During the actual field trials the volunteers were issued the following items: tropical fatigues and boots, plastic gloves for the hands, head nets, canvas square to sit on,

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[3] Informed consent was received from each volunteer prior to participation in the tests.

pen and notebook for recording their own results, and pen size flashlight for verifying bites after sunset. Only the actual repellent sites were exposed to mosquitoes.

RESULTS AND DISCUSSION.–On 10 July 1973 the first of two preliminary experiments was conducted. The first experiment was designed to compare the four-site field method using the total forearm circumference to the four-site technique designed at LAIR (Brodel, 1974). Two of four individuals had field test sites on the right forearm and two had the field test sites on the left forearm. The other forearm of the individuals had the standard laboratory test sites. Repellents were applied at 0900 hours. For this test two stock repellent solutions, carbamide and DEET, were applied at 0.48 mg/cm^2 and testing was done by the usual LAIR laboratory method (Brodel, 1974).

The results show the field test values were exactly two hours longer in protection on each individual than in the lab method. This situation is probably an experimental artifact resulting from the small population at risk. The DEET field test method gave a longer dry protection time than the DEET four-site method, while with the carbamide the four-site method gave a longer time than the field test method. This difference was not statistically significant probably because carbamide at 0.48 mg/cm^2 gives protection between 20-25 hours such that abrasion is a substantial factor. In the field method, the total circumference of the arm was covered by repellent while a smaller rectangular site on the ventral forearm is used in the laboratory design. Abrasion would be more likely to occur when using the field design.

On 25 July 1973, twelve volunteers were divided into three groups of four volunteers with each group having its repellents applied at LAIR at different times as described above. After busing to Hamilton Air Force Base, about twenty miles north of San Francisco where the climate is much warmer, the men did twenty minutes of calisthenics, followed by a 1.5 mile run. Then the test subjects played softball for one hour, creating an abrasion factor. At sundown, upon returning to LAIR, test sites were exposed in the laboratory for one minute in one of two test cages. The total number of bites received in one minute was recorded.

It was found that the 0800 group (Table 1) shows carbamide as having significantly fewer bites than DEET. Under similar circumstances without abrasive factors, one would expect to find that sulfonamide offers protection equivalent to that of carbamide. The sulfonamide data have a greater standard deviation attributed to abrasion. In a small population group, the abrasion factor disturbs the normal distribution and becomes the dominant influence in statistical analysis. As a result of this, the softball game was excluded from planned activities in field trials.

The 1000 hours group showed no compound significantly better than DEET due to the absence of bites in one individual which prevented any statistical differentiation between the repellent compounds (Table 1). Moreover, abrasion in at least one other site gives a large standard deviation for the group data. Carbamide and sulfonamide showed significantly better results than DEET in the 1200 group where abrasion factors did not predominate.

On 5 August 1973, a group of 12 volunteers went to Camp Lejeune, North Carolina. On 6 and 7 August the applications were made and volunteers engaged in moderate activity such as jogging and marching. During the test the individuals were distributed randomly around the testing area. Each volunteer sat on a canvas square and held his arms away from his body. One volunteer was chosen randomly each night from the group as a treated control while one individual acted as an untreated control. Mosquitoes collected from the treated control showed what species bit each repellent site. Mosquitoes collected from the untreated control were a measure of species population and density of the mosquitoes. Mosquito population screening during all tests showed that *Aedes taeniorhynchus* was the most prevalent species along with *Aedes sollicitans* and *Anopheles crucians* which were present in small numbers. Mosquito density was estimated at 200 man bites/hour. The *Aedes taeniorhynchus* whole body count from the untreated control was 193, while only 10 were collected from the treated test site used as a control on 6 August. Repellency results were analyzed by paired comparison of the number of bites received on a test repellent site to the control DEET site.

On 8 August the test area was changed. All applications were made at a dose rate of 0.31 mg/cm^2 at 1000 hours. Otherwise the testing procedures followed that of the two previous days.

The purpose of the field trial was to relate laboratory results to field results by comparing the test repellents to the standard Army repellent, DEET. Results on 6 and 7 August (Table 2) are reported in bites per one hour of exposure to mosquitoes at Camp Lejeune. In the 0700 group, carbamide and sulfonamide show significantly better protection time than DEET. The two other repellent site groups, 0940 and 1200 groups, did not receive enough bites to differentiate between repellents. The three application times in the field trials allowed one to detect differences in repellents regardless of high or low mosquito density and/or avidity. For instance, the repellents could not be differen-

Table 1.–Preliminary field test, Hamilton Air Force Base, California.

	Repellent Efficacy (Bites/Minute/Test Site)[a]			
	SRI-6	Carbamide	DEET	Sulfonamide
0800	5	3[b]	4	3
	3	3	6	0
	10	0	2	0
	1	0	2	0
1000	1	0	0	0
	0	0	1	0
	4	0	14	0
	4	0	8	0
1200	2	0[b]	2	1[b]
	1	0	3	0
	0	1	2	0
	1	0	2	1

[a] Test 25 July 73 on 12 volunteers at a dose rate of 0.48 mg/cm^2. Each number represents an individual test site.

[b] Significantly different than DEET at a 95% level.

Table 2.--Mean bites per test site in one hour exposure in field trial[a].

	0700		0930	
	$\bar{X}$	S_d	$\bar{X}$	S_d
DEET	2.63	2.13	1.00	1.60
SRI-6	3.13	3.68	0.38	0.52
Sulfonamide	0.13[b]	0.35	0.00	0.00
Carbamide	0.00[b]	0.00	0.00	0.00

[a] Test 6 and 7 August 73 on 12 volunteers at a dose rate of 0.48 mg/cm^2.

[b] Significantly different from DEET at 95% level.

Table 3.–Ranking of repellents: Lab and Field.

	LAIR	Camp Lejeune	
	$\bar{X}$ hrs protection to date	Total Bites/hr 6, 7 Aug (0700)	8 Aug
Carbamide	17.4 ± 5.1[a]	0[a]	3[a]
Sulfonamide	14.3 ± 5.6[a]	2[a]	8[a]
SRI-6	8.5 ± 4.9	15	50
DEET	6.6 ± 1.7	21	66
Application rates	0.31 mg/cm^2	0.48 mg/cm^2	0.31 mg/cm^2
Number of volunteers	8-28	12	12

[a] Significantly different from DEET at 95% level.

tiated if all volunteers had had their repellents applied at 0930.

Results from 8 August 1973 (Table 3) show that the carbamide and sulfonamide offer significantly better protection than DEET in decreasing the number of bites. By comparing repellents to DEET, we can obtain information on how the repellents' protective properties under given tests relate to DEET. In laboratory testing (Table 3) we found carbamide and sulfonamide proved to give significantly better protection than DEET, likewise in the field results. Thus, laboratory methods are indicative of the field results under conditions tested and also, carbamide and sulfonamide are superior to DEET under the test procedures used.

Under the test conditions described, SRI-6 was not found to be significantly better than DEET. SRI-6 has poor wash-off resistance (Spencer, 1974) and sweating, a form of wash-off, would detract from this compound's repellent properties in field trials. As a result of poor wash resistance, the SRI-6 compound was not expected to be better than DEET in the field.

SUMMARY.--To determine if our laboratory results would predict a repellent's relative protection time under action field conditions, a field study was undertaken to compare three repellents to the standard military repellent, DEET. Preliminary testing was done to determine the feasibility of our field methods which was found to be satisfactory. Actual field testing produced the same relative comparison to DEET as was determined in our laboratory test (Table 3).

Carbamide and sulfonamide give significantly longer protection time than DEET in the laboratory and significantly less bites than DEET under field conditions, while SRI-6 offered protection which was not significantly different from DEET. For the mosquito species and field conditions encountered, the laboratory screening procedure appears to have predicted the field results.

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CANADA COMMITTEE ON BITING FLIES

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The "Canada Committee On Biting Flies" was formed in 1973 and consists of 18 members representing provincial and federal departments of agriculture, universities, and other federal departments. It is responsible for analyzing the problem of biting flies in Canada and recommending activities which should contribute to the solution of the problem. Reports are made annually to the parent Canada Agricultural Services Coordinating Committee which is composed of provincial Deputy Ministers, Deans of Agriculture and Veterinary Medicine, Canada Department of Agriculture and other agricultural elements.

In 1972, two gatherings were held in Canada which led to the formation of this Committee, one a Symposium on Biting Fly Control and Environmental Quality in Edmonton, the other a Work-Planning Meeting on Biting Flies and their Control in Saskatoon. These have provided current information on the status of research on biting flies and recommended approaches to be taken in setting up priorities among the major problems.

One meeting of the Committee was held in December; a second will occur in March. Plans include: (1) a Newsletter once or twice each year giving information on regional problems and recommendations, and similar items; (2) an Inventory relating to biting fly work in Canada listing current research, personnel, and significant reports and publications; (3) preparation of Retrospective and Current Awareness bibliographies pertaining to the Canadian situation; (4) feasibility studies of a Biting Fly Research Institute; (5) recommendations to the parent Committee of those research priorities that can be designated at this time.

I expect this Committee to be a focal point of biting fly research in Canada and welcome this opportunity to speak to U.S. workers with similar interests in the A.M.C.A. We have always recognized the similarity of pest problems in our two countries and earnestly desire to strengthen this collaboration by any exchanges of information that would further this relationship.

COMPUTER USE IN MOSQUITO ABATEMENT DISTRICT MANAGEMENT

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Cascade County Mosquito Abatement District was formally established in August of 1968, after 25 years of sporadic larval control and fruitless attempts at adult control in the Great Falls area. The District includes the entire land area of Cascade County, creating a mosquito control program encompassing nearly 1.8 million acres, of which 30 thousand acres are potential mosquito breeding sites.

The population of Cascade County is approximately 80,000 people, 75 percent centralized in Great Falls, and the rest widely distributed. A great majority of the County is agricultural land with relatively low taxable value. Small population and low taxable value severely restrict the budget resources available. An operating budget of approximately $150,000 was set for the District. The exaggerated ratio of small budget to relatively large area needing service requires very strict management to accomplish the best control possible per dollar spent.

As the Federal Environmental Pesticide Control Act of 1972 takes full effect, greater emphasis is being placed on maintaining complete and accurate records of all pesticide usage. Federal, state and local environmental organizations are continually increasing pressure on applicators to use extreme care to insure safe and accurate chemical treatments, applied only where absolutely necessary. This increasing awareness of potential pesticide problems has demanded improved methods of management and record keeping.

Our record-keeping program was evaluated to determine what would be necessary changes to meet the increased demand for accurate information. Among the several types of information required from field personnel were the following: (1) locations of sources and places where pesticides are applied must be recorded by township, range, section and station; (2) the number of sampling dips, number of larvae present, and the stages of development; (3) chemical information, including the chemical name, concentration, amount of mixture applied, and acreage treated; (4) the date, management area or zone, type of equipment, employee's name, and a complete breakdown of time usage. Most of this information was already being recorded by field personnel, and it was apparent that the main problem was not lack of data, but lack of means for rapid and accurate evaluation.

A major problem in evaluation is the correlation of pre-application data to relevant post-application information. A method was devised which incorporated various measureable limiting factors. These included temperature, larval development period, chemical persistance, and their effects on each other. In correlating these factors with sampling and application dates, many calculations and comparisons are necessary. Time required for analysis by standard methods is too long to be feasible as a management tool, but personnel time input can be minimized by analysis of the data by computer.

Accordingly, we arranged to use an IBM System 3 machine available through the county government. There was no one available at reasonable cost to write the necessary programs, but at the suggestion of IBM personnel, this was resolved through formal IBM training and on-the-job experience to develop the skills needed to write programs. This gave us the management tool needed for rapid and accurate program evaluation on a regular basis. The administrative use of periodic evaluations has contributed to efficiency of the total program, as well as economy of single projects.

Information from every station visited is transferred daily from field reports onto punch cards and fed into the computer for analysis. The data are then processed to determine which are pertinent for each situation. Computer computations determine the density of larvae per square foot for each sample. Pre-application sample data are then correlated with respective post-application data, to indicate the efficacy achieved by each control operation. This factor is termed a "control indicator", and is useful in determining the efficiency of respective operations.

Records of chemical applications can be studied by computers. Chemical usage is accumulated and compared for all levels by stations, townships, drainage areas, and applicators. The figures are then correlated with acreage data to compare application rates with recommended rates and effective dosages. Frequent computer analysis has provided a means of continually evaluating the effectiveness of each chemical in use, and of comparing it with other chemicals applied as to persistence and efficacy under various environmental conditions. These data provide a valid basis for determining the particular pesticide for each specific purpose, and also show when a change in chemicals or application rates is necessary to provide maximum results with maximum safety and minimum expenditures. The effects of chemicals upon individuals can be used as a supplementary safety guide, by frequent sampling of cholinesterase levels in the blood of persons applying greater than average amounts of organic phosphate compounds. The computer speeds up the handling of the data. It is also useful in preventive maintenance or field breakdowns, and increasing the safety factor by anticipating when badly worn application equipment should be overhauled or replaced.

Computerized records or labor input and costs, correlated with chemical usage, permits determination of unit costs of control accomplished. The computer also provides total expenditures on each project. This convenient access to accurate figures contributes to operation efficiency.

The records give management information useful in making decisions on crew reorganization for better coverage of problem areas, determining the right time for major changes in program priorities and reassignment of duties to insure achieving maximum control.

Data that would take weeks to evaluate by older procedures can be processed in minutes. Analysis of an entire year's data for all pertinent factors can be done in roughly two hours, with improved accuracy. A primary advantage of computers is prompt and rapid evaluation of field data to provide current information on the current status of each control activity.

Future use of computers for better management evidently will be limited only by the imagination of the user.

PORTRAIT OF AN OPERATOR

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Mosquito Control Operator, Inspector Operator, Abatement Operator, Zone Operator, Zoneman, Fieldman, Sprayman, or whatever you call the basic operational person, is the person who does the essential field work. He is a responsible, reliable and well trained person. If he does not now possess these characteristics, he soon will have to and it will be up to the District Manager to see that he does.

By 1975, persons who use pesticides for mosquito control in California will have to be certified by the California State Department of Health as competent in the proper safe, effective and economical use of pesticides. They will have to know specific entomological facts about mosquitoes, disease potential of mosquitoes, biological and ecological facts about prevalent species; comprehensive mosquito control (biological, physical and chemical measures), evaluation techniques and safe and legal operations. The worker will have to apply this knowledge to field operations to be an effective operator.

DUTIES AND RESPONSIBILITIES.–With the certification and the increasing sophistication of the tools he uses in his work, there will be an expansion of the duties and responsibilities operators now have.

A list of the duties and responsibilities of a control operator in the Orange County Mosquito Abatement District is as follows:

1. He is responsible for the effective control of mosquitoes and other vectors within his zone.
2. He uses judgment in organizing and carrying out his control program, and schedules inspections and operations to effectively cover his assigned zone.
3. He enters public and private property to carry out his responsibilities.
4. He evaluates mosquito producing conditions.
5. He is the District's direct representative to the public.
6. He interviews each property owner reporting mosquito annoyance, evaluates the problem, determines its cause, and sees that abatement procedures are carried out to prevent a continuing nuisance to that resident.
7. He prepares, signs and gives to each resident reporting mosquito annoyance a "Report to Service Requestor," advising the resident of the cause of the annoyance and the corrective action.
8. He prepares, signs and gives to other residents whose premises he has entered and inspected for possible mosquito breeding sources a "Report to Householder," advising that he has entered the property, why he has entered the property, the results of his inspection and any corrective action that should be taken.
9. He teaches and seeks cooperation of, and provides advice and assistance to, the resident or property owner responsible for a mosquito breeding source on how the public nuisance can be controlled or abated.
10. He issues a written notice, "Mosquito Source Inspection Report" to the responsible party when necessary, requesting abatement of the public nuisance and advising him of the property owner's responsibility under the Mosquito Abatement Act.
11. He chooses the insecticide and method of application for a particular situation.
12. He uses safe practices and procedures when applying insecticides, to protect the public and the environment.
13. He observes potential problems developing in the field.
14. He prepares and maintains maps, records, and documentation of all breeding sources and abatement actions.
15. He prepares an Annual Report summarizing the mosquito control activities within his zone and presents recommendations for improvements in methods and equipment.
16. He directs seasonal control operators assigned to his zone.

The Orange County Mosquito Abatement District will soon be expanding its responsibility to cover other phases of vector control within Orange County. This means ten operators, plus four additional operators to be hired, will be accepting new responsibilities in the control of flies, rats and other vectors. They will have to be trained in these new areas and adapt to new and changing conditions.

These operators are stable persons with an average of eight years of service with the District, and average age of 34. All are high school graduates, with an average of one year of college experience. The average salary is $12,182. and the ten operators have an average of 1.8 children.

These men have chosen this special type of work as a life-time career and they prefer outdoor work and contact with people. They are interested in nature and in protection of the environment. They have a desire to continue learning new techniques for the control of mosquitoes and other vectors. They are interested in the affairs of the California Mosquito Control Association and they participate in its activities and professional meeting whenever possible. They identify with their job, use personal initiative and have pride in their work. They are individuals with the highest standards of honesty, reliability and morality.

AERIAL PHOTOGRAPHIC SURVEILLANCE OF MOSQUITO BREEDING SOURCES IN AN URBAN ENVIRONMENT

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Neglected and abandoned swimming pools are an important mosquito source in urban Orange County. These sources cause a great number of service requests in the cooler parts of the year when the pool heater and filtering systems are disconnected. A Control Operator often spends many hours locating sources when responding to a complaint and he cannot always be sure he has located the source. Aerial photographic surveillance has improved our swimming pool source detection and prevented a number of complaints.

The use of aerial photographic surveillance of breeding sources in Orange County began in March of 1973. It was an unexpected result of a flight taken to photograph large mosquito sources to facilitate mapping and documentation.

While passing over the City of Orange, a variation in color was noted between several private swimming pools in a typical residential area. Most were clear blue and well taken care of; a few, however, were dark green in color. Circling the area at 2,000 feet, several photographs were taken before proceeding to the original destination.

Careful examination of this series of photographs was instrumental in the detection of a total of 30 swimming pools, nine of which appeared to be neglected and seven of which were in fact breeding mosquitoes. Statistically, 25% of the swimming pools photographed were breeding mosquitoes, and 77% of the pools which appeared to be neglected were infested with mosquito larvae.

Aerial observation as a means to discover and precisely locate actively breeding or potential mosquito sources in Southern California has been used by the Southeast Mosquito Abatement District, in cooperation with Los Angeles area law enforcement agencies who used aircraft in their routine patrol activities. Arrangements were made for District personnel to ride as observers and note new sources.

The Board of Trustees of the Orange County Mosquito Abatement District at its April 1973 Board Meeting approved the use of aerial photographic surveillance on a limited basis, as a result of the photographs and followup inspections of the previous month. After verifying that insurance coverage was adequate, methods and procedures for making routine surveillance flights in Orange County were developed.

In the interest of economy, precise plans and criteria were established for subsequent photographic missions, thus, minimizing the waste of expensive flying time. The procedure now used is as follows:

1. The foreman and operators determine which areas are to be photographed, based on general knowledge of the presence of swimming pools in the area, the number of service requests, the age, general condition, and past record of pool problems in the area.
2. The District Vector Ecologist (who acts as photographer) and the District Pilot (an observer if required) go over the proposed flight plan and the area to be photographed. A preliminary plan of the flight is based on a standard Thomas Brothers Road Atlas. The scale is 1″ = 2,800′, and each sheet represents a nine square mile area, which has proved to be an ideal size for this phase of planning. This map is encased in plastic, allowing notation and outlining the areas to be photographed with felt tip pen.

   The final flight plan is plotted on a map which is scaled 1″ = 800′ and which is representative of one square mile. The flight path is drawn in and during the flight the number of the photograph is entered directly on the map over the area photographed, and simultaneously entered on a log sheet, thereby reducing the possibility of error. (Orientation errors are sometimes possible due to the large number of urban areas in Orange County which have a similar appearance in a photograph, making it difficult to locate them precisely.) A record is made on the log sheet of the flight number, date, crew, aircraft number, duration of the flight, and the camera and film types.
3. The flight is carried out in accordance with the flight plan, photographing areas in question and recording the data.
4. The photographs are developed within forty eight hours. The Area Foreman and Zone Operator analyze the photographs and determine which sources appear to be neglected. These sources are then located precisely on a map which has a scale of 1″ = 400′, each sheet of which represents a ¼ mile square. This map shows individual property plots, which facilitates more precise location of the pools. When the pools have been inspected and appropriate treatment carried out, the findings are recorded on the map, which with other records become a permanent log of our findings and the actions taken. We then use the standard routine of periodic inspection and treatment until the swimming pool is back in use.

The equipment used is a hand held, Minolta SRT-101 F 1.7, 35 mm camera equipped with a 55 mm lens. We prefer Kodak Kodachrome film for transparencies, but Kodak Ektachrome-X can be used.

Two types of high-wing aircraft have proved to be satisfactory. The first is the Model 150 Cessna, a two-place aircraft. The second is the Model 172 Cessna, which will accommodate four passengers. Both airplanes offer good visability and the front seat side windows may be opened in flight. They may be operated safely as slowly as 70 to 80 miles per hour indicated airspeed.

The photographs are the oblique type, shot at an angle of between 35° to 45° from the vertical plane, with the aircraft in a level flight attitude.

TABLE 2.–Highest laboratory demonstrated level of organophosphorus tolerance in larvae of *Culex tarsalis* through 1973.

AGENCY	MALATHION					PARATHION					METHYL PARATHION					FENTHION					CHLORPYRIFOS				
	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits	Year	B	LC_{90}/LC_{50} ratio	LC_{50}	95% limits
El Dorado						1969	5.62	1.69	0026	.0019 -.0036	1969	3.86	2.15	.0032	0022- 0045	1966	13.39	1 25	0022	.0020- 0025	1969	5.05	1.79	.00022	.00016-.00031
Pine Grove	1965	4.44	1.95	.020	.018- .023											1972	6 76	1 55	.0027	.0024- 0031					
Shasta	1967	1.20	11.70	.087	.054- .14	1972	9.55	1.36	.0021	.0019 -.0024	1972	4.71	1.87	.0031	0027-.0037	1972	6 67	1.56	0031	.0027-.0035	1971*	3.44	2.36	.00052	.00044-.00062
Tehama County	1968	1.30	9.85	.053	.032- .083	1964	4.75	1.86	.0031	.0027 -.0035	1971	6.00	1.64	.0041	0036-.0047	1971	4.48	1 93	0056	.0047-.0073	1972	7.19	1.51	.00053	.00048-.00058
Los Molinos	1963	3.18	2.53	.057	.045- .071	1972	3.68	2.23	.0017	.00069-.0022	1970	6.08	1.63	.0032	.0027-.0036	1970	7.69	1.47	0014	.0013-.0016					
Corning	1967	1.45	7.65	.092	.061- .14	1963	4.81	1.85	.0024	.0020 -.0028	1970	9.89	1.35	.0032	.0029-.0036	1967	6.83	1.54	.0022	.0022-.0025	1970	5.48	1.71	.00023	.00020-.00027
Butte County	1966*	1.46	7.55	.10	.069- .25	1972*	3.18	2.53	.0045	.0040 -.0052	1966*	7.58	1.48	.0023	.0020-.0026	1971*	2.84	2.83	0079	.0068-.0091	1968	7.18	1.51	.00028	.00026-.00030
Colusa	1971	3.64	2.25	.045	.035- .058	1968	4.14	2.04	.0015	.0011 -.0018						1970	5.57	1.70	.0023	.0020-.0027	1970	4.84	1.84	.00027	.00020-.00036
Sutter-Yuba	1966*	1.64	6.05	.41	.26 -1.20	1966*	7.95	1.45	.0024	.0021 -.0028	1966	7.20	1.51	.0029	0026-.0033	1970	4 72	1.87	.0043	.0030-.0060	1971*	3.89	2.14	.00090	.00070-.0012
Sacramento-Yolo	1966	1.56	6.63	.11	.081- .19	1966	7.42	1.49	.0032	.0027 -.0040	1963	7.61	1.47	.0042	.0039-.0045	1968	6.74	1.55	.0031	.0027-.0035					
Solano County	1965	1.16	12.73	.11	.066- .17	1965	6.43	1.58	.0023	.0025 -.0033						1969	11.16	1.30	.0027	.0023-.0031					
Diablo Valley						1970	5.30	1.75	.0013	.0011 -.0015	1971	4.70	1.87	.0042	.0035-.0051	1971	5.24	1.76	.0031	.0027-.0036					
No. San Joaquin	1967	1.38	8.48	.068	.051- .091	1969	8.66	1.41	.0014	.0012 -.0016						1972	4.48	1.93	.0044	.0038-.0054	1967	7.32	1.50	.00043	.00038-.00049
San Joaquin	1967*	2.23	3.76	.071	.052- .096	1971*	6.68	1.56	.0070	.0061 -.0081	1971*	5.20	1.76	.0079	0067-.0099	1972*	4.71	1.87	.0058	.0047-.0072	1971*	2.15	3.95	.0011	.00064-.0016
East Side						1966	12.37	1.27	.0017	.0015 -.0019	1965	5.73	1 67	.0026	.0024-.0029	1972	2.03	4.28	.0033	.0024-.0045					
Turlock						1966	5.43	1.72	.0021	.0017 -.0025	1969	6.46	1.58	.0044	.0041-.0049	1970*	2.28	3.65	.016	.013 -.022	1969	5.04	1.80	.00035	.00031-.00039
Merced County	1966	1.15	13.01	.47	.26 -1.8	1966	12.11	1.28	.0038	.0035 -.0043						1970	3.34	2.42	.0048	.0042-.0054	1969	4.23	2.01	.00051	.00045-.00057
Madera County	1969	1.22	11.47	.25	.15 -1.30	1970	4.11	2.05	.011	.0092 -.014	1969	2.64	3.06	.0059	.0048-.0072	1970	2.66	3.03	.013	.0099-.017	1970	1.38	8.48	.019	.0093 -.14
Fresno Westside	1972	2.01	4.34	.23	.14 - .30	1969	5.89	1.65	.022	.018 -.028	1969	9.57	1.36	.018	.016 -.021	1969	6.28	1.60	.023	.021 -.027	1969	3.61	2.27	.0089	.0074 -.011
Fresno	1963	1.02	18.05	.095	.049- .15	1969	2.19	3.85	.0053	.0045 -.0063	1969	4.07	2.07	.0085	.0063-.010	1969	4.33	1.98	.015	.013 -.017	1969	1.75	5.40	.0017	.0012 -.0029
Consolidated	1969	3.14	2.56	.24	.19 - .36	1970	3.31	2.44	.023	.018 -.028	1970	4.03	2.08	.0046	.0037-.0058	1970	7.16	1.51	.053	.047 -.060					
Delta	1964	1.59	6.40	.080	.056- .12	1964	4.26	2.00	.0030	.0025 -.0036	1964	7.36	1.49	.0045	.0039-.0051	1964	6.69	1.55	.0049	.0043-.0056					
Kings	1972	1.38	8.48	.75	.55 -1.06	1972	2.74	2.94	.054	.045 -.065	1972	2.04	4.25	.050	.039 -.068	1972	3.25	2.48	.040	.034 -.046	1972	2.82	2.85	.027	.023 -.032
Tulare	1966	2.22	3.78	.25	.18 - .40	1971	2 77	2.90	.0099	.0052 -.013	1971	2.02	4.31	.023	.017 -.029	1969	4.02	2.09	.017	.012 -.023	1971	1.47	7.45	.011	.0069 -.015
Delano	1965	2.15	3.95	.058	.044- .077	1964	4.09	2.06	.0031	.0026 -.0037	1964	9.01	1.39	.0039	.0037-.0041	1969	3.20	2.51	.0049	.0037-.0059	1969	1.67	5.85	.0011	.00063-.0018
Kern	1964	1.74	5.45	.065	.046- .088	1964	4.69	1.88	.0074	.0066 -.0083	1964	10.31	1.33	.0066	.0058-.0073	1964	16.21	1.20	.0063	.0057-.0070					
West Side	1969	1.15	13.01	2.1	1.3 -4.8	1965	6.08	1.63	.0032	.0028 -.0036	1966	3.29	2.45	.0024	.0020-.0027	1970	6.20	1.61	.0062	.0045-.0085	1966	9.70	1.36	.00049	.00045-.00053
Goleta Valley	1966	8.76	1.40	.054	.048- .060	1966	7.34	1.50	.0024	.0023 -.0027	1966	5.70	1.68	.0019	.0018-.0021	1969	4.35	1.97	.0044	.0037-.0054	1966	6.66	1.56	.00063	.00057-.00069
Carpinteria	1966	3.82	2.17	.030	.027- .034											1966	5.11	1.78	.0072	.0060-.011					
Southeast	1967	3.05	2.63	.069	.059- .080											1969*	3.72	2.21	.042	.036 -.053	1970	3.39	2.39	.00083	.00069-.00099
Long Beach																1972	2.37	3.47	.012	.0097-.015	1972	1.63	6.11	.0019	.0014 -.0026
Orange County	1973*	3.21	2.50	.060	.047- .080											1973*	3.03	2.65	.0078	.0062-.012					
Riverside City	1972	2.61	3.10	.033	.028- .040	1972	2.93	2.74	.0026	.0021 -.0032	1972	3.61	2.27	.0034	.0029-.0039										
Northwest	1971	4.98	1 81	.016	.014- .018	1971	6.73	1.55	.0016	.0015 -.0018	1971	5.41	1.73	.0015	.0013-.0016	1971	7.95	1.45	.0026	.0023-.0029	1971	3.67	2.24	.00024	.00022-.00028
San Diego County	1972	2.36	3.49	.12	.099- 15	1972	3.87	2.15	.0038	.0033 -.0047						1972	3.20	2.51	.0070	.0061-.0082					
Coachella Valley	1972	4.45	1.94	.073	.062- .086	1970	3.06	2.62	.016	.013 -.020	1971	2.21	3.80	.0075	.0060-.0098	1972	1.95	4.54	.0086	.0058-.011	1971	2.06	4.19	.026	.019 -.046
Antelope Valley	1968	3.10	2.59	.024	.016- .032											1971	7.06	1.51	.0040	.0033-.0049					
Inyo County	1969	10.20	1.34	.026	.024- .029											1972	4.18	2.03	.0059	.0047-.0081	1969	9.79	1.35	.00045	.00041-.00048

* Mosquito control agency data

1958, when parathion resistance was reported from the Kings Mosquito Abatement District (Lewallen and Brawley 1958). Parathion resistance has now been confirmed for nearly all agencies that must deal with *A. nigromaculis*. Larvae from more than half the agencies tested are either moderately or highly resistant to parathion's replacement, methyl parathion, and its use has been discontinued.

Half of the agencies tested have moderate resistance to the next substituted material, fenthion with an LC_{50} greater than 0.01 ppm. These are generally the same agencies that have populations highly resistant to parathion and methyl parathion. There is a difference in slope separating populations in the north from those in the southern portion of the Central Valley. This pattern was first noted by Womeldorf et al. (1971) and has since become more pronounced as more areas have become involved. Districts from Merced County north have resistant populations characterized by an increased LC_{90}/LC_{50} ratio, similar to that for parathion, while those from the south have low ratios even though the LC_{50}'s are of the same magnitude as the northern populations. Since the information in Table 1 is based on a single high level test result, the difference in slope does not show clearly.

Chlorpyrifos has not been widely used for area wide control. Moderate but operationally-hampering resistance is mainly limited to the general areas which were the focal points for initial resistance to parathion. These areas are the Sutter-Yuba Mosquito Abatement District in the Sacramento Valley, the Turlock Mosquito Abatement District in the northern San Joaquin Valley, and the Kings and Tulare Mosquito Abatement Districts in the southern San Joaquin Valley. These agencies and their immediate neighbors have also experienced severe resistance to the organophosphorus adulticides and have led in the use of the carbamate adulticide, propoxur.

Malathion has been used intensively in agricultural areas by only a few agencies and mosquito larvae from these agencies are generally characterized by high levels of tolerance to this material. However, other agencies are affected by cross-tolerance.

Culex tarsalis larvae were first reported resistant to malathion in the Fresno Mosquito Abatement District in 1956 (Gjullin and Isaak 1957). Since then, malathion resistance has become general throughout most of the state as evidenced by field failures and confirmed by LC_{50}'s greater than 0.1 ppm and an increased ration of LC_{90}/LC_{50} (Table 2).

Prior to 1969 resistance was generally restricted to malathion, with some scattered instances of high levels of tolerance to fenthion. Then, quite suddenly, in mid-1969 severe organophosphorus resistance was verified in larval populations of *C. tarsalis* from several contiguous agencies in the southern San Joaquin Valley, extending from the Fresno Mosquito Abatement District in the north to the Kern Mosquito Abatement District in the south (Figure 2). All currently available organophosphorus larvicides were included (Georghiou et al. 1969; Schaefer and Wilder 1970; Womeldorf et al. 1971, 1972). The resistance has since spread northward and currently includes most agencies south of the Madera County Mosquito Abatement District in the Central Valley. Resistance of the same magnitude appears to have developed independently in the Coachella Valley Mosquito Abatement District in southern California.

With the exception of malathion, LC_{90}/LC_{50} ratios are generally less than those for *A. nigromaculis*, indicating more homogenous populations. The LC_{50}'s are generally lower, not exceeding 0.05 ppm. Nevertheless, these levels are high enough to cause control failures in the field.

ACKNOWLEDGMENTS.—Personnel at all of the mosquito control agencies listed aided in collecting and testing larvae. We are especially appreciative that technical staff of agencies who themselves performed the laboratory work, as noted in the tables, provided us with their data.

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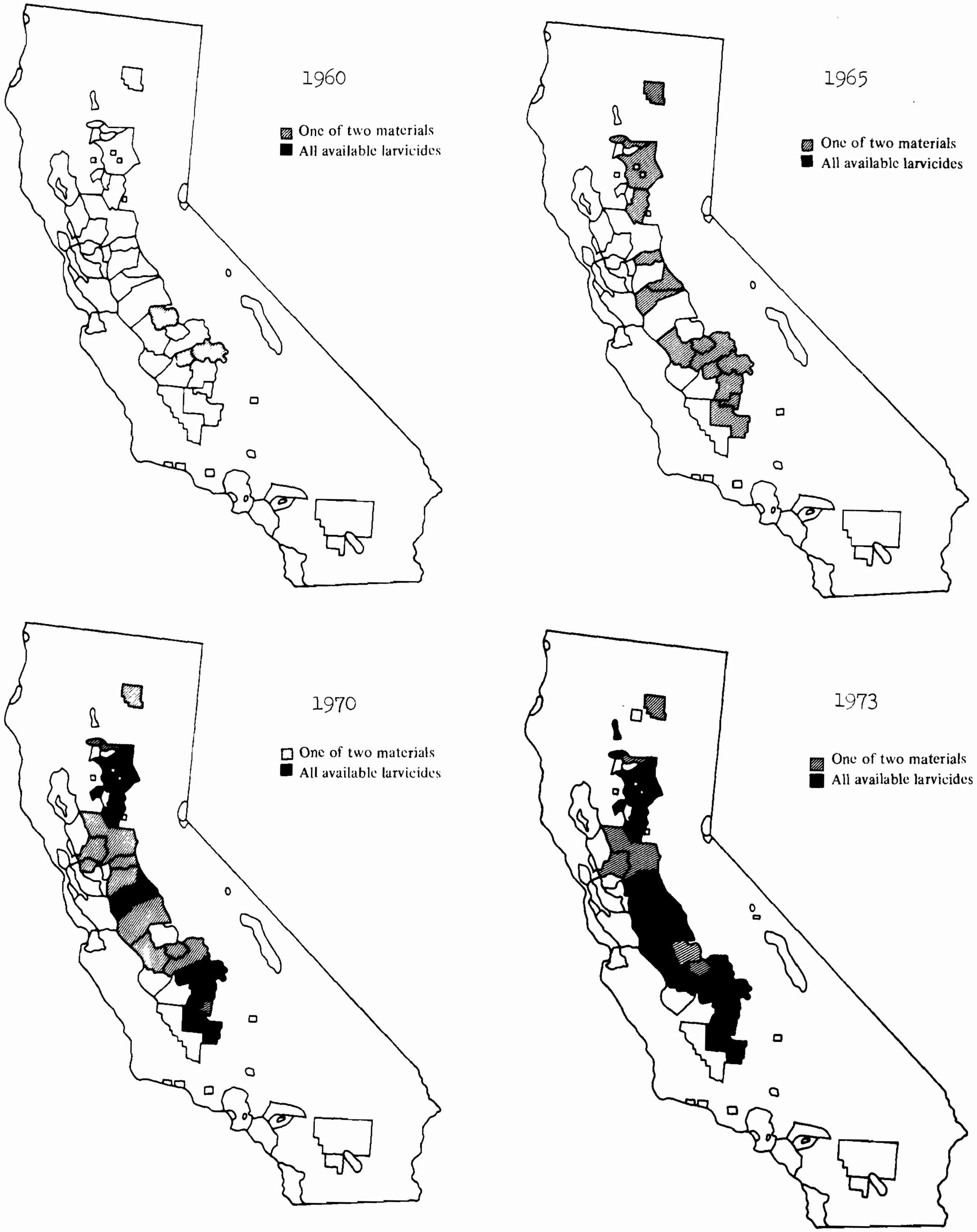

Figure 1.– Documented organophosphorus resistance in *Aedes nigromaculis*, California, 1960-1973.

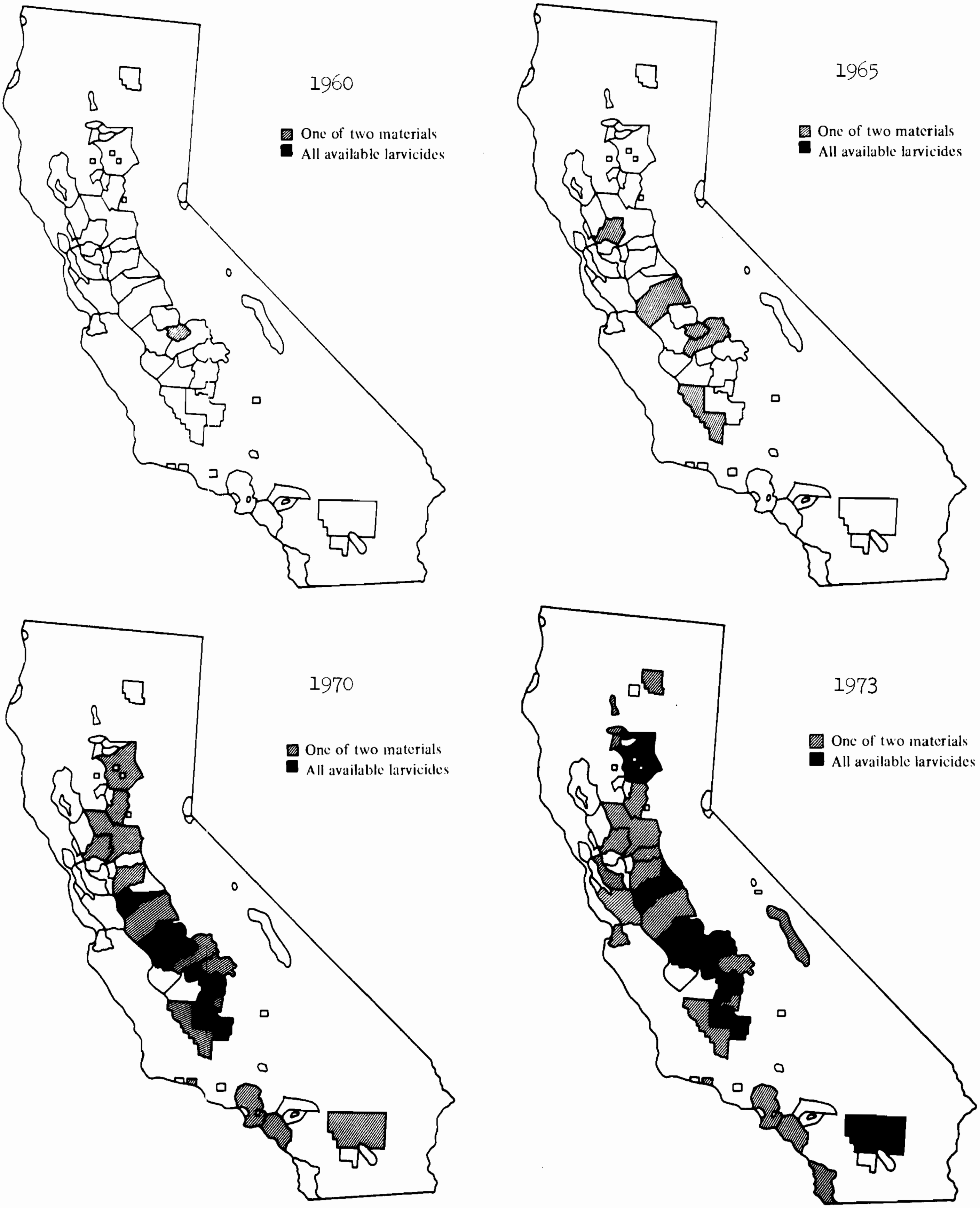

Figure 2.—Documented organophosphorus resistance in *Culex tarsalis*, California, 1960-1973.

FAILURE OF TIME EXPOSURE TECHNIQUE TO MEASURE PROPOXUR RESISTANCE IN ADULT *AEDES NIGROMACULIS*

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INTRODUCTION.–Control of adult *Aedes nigromaculis* has not been possible with organophosphorus compounds in areas of California where resistance has become extremely severe. The carbamate propoxur is now used to suppress the adults, but sporadic failures of this compound have been reported by several mosquito control agencies. The need for a practical laboratory method to monitor developing propoxur resistance in California's adult mosquitoes has been increasingly apparent. The lethal dosage method (i.e., exposing insects to a range of concentrations for a constant time), did not clearly differentiate between susceptible and operationally resistant adult *A. nigromaculis* (Zboray and White, 1973). There were indications that the lethal time method (i.e., exposing insects to a standard concentration for a range of time periods) might clarify the difference between susceptible and non-susceptible strains. This technique has been used by the World Health Organization (Anonymous, 1970).

In 1972, preliminary experiments with this technique (Zboray and White, 1973) established that 4.2 microgram/cm^2 propoxur was a useful standard concentration, and that the range of equal log time exposure intervals should be, for S(usceptible) populations, between 10 and 160 minutes; and for R(esistant) populations, between 20 and 320 minutes. In 1973, these standard concentration and exposure time intervals were used to investigate the possibility of separating median lethal times (LT_{50}) between susceptible and operationally-resistant adult *A. nigromaculis.*

MATERIALS AND METHODS.–Larvae were collected from the field and reared to adults in the laboratory as described by Zboray and White (1973). The same strains of *A. nigromaculis* were utilized for these experiments as for the 1972 experiments establishing the mortality range. The susceptible strain came from a pasture in Tehama County outside the Tehama County Mosquito Abatement District jurisdiction. The resistant strain, from a pasture in the Turlock Mosquito Abatement District, has repeatedly survived aerial application of .05 lb/acre of propoxur.

All tests were aimed at establishing lethal time regression lines by utilizing three or more equal log time exposures per test to the chemical propoxur. All tests were run in duplicate. The testing procedure was essentially as described by Georghiou and Metcalf (1961) with the modification of Darwazeh (1972). Fifteen to twenty adult mosquitoes were aspirated from the rearing cage into the vial. The sexes were not separated and the females not blood-fed. Insecticide-treated filter paper (Whatman No.2) was then placed into the test vial which was then covered with a nylon screen. After the appropriate time-exposure periods, the mosquitoes were transferred into an eight-ounce paper cup and held for 24 hours. Mortality was determined thereafter. The holding cage contained a cotton wick saturated with 5% sugar water providing food and moisture.

Probit regression lines were fitted to the data using the computer program of the Data Processing Center of the California Department of Health. The program includes a statistical test of the homogeneity or regularity of each set of data in terms of how well it fits a probit line. Only tests for which an acceptable fit was obtained were included in the analysis.

RESULTS AND DISCUSSION.–The following observations were based upon the 95% confidence limits of the LT_{50} values of the probit lines (Table 1).

1. The 1973 S mosquitoes tested, except for one collection, showed significantly higher tolerance than the 1972 mosquitoes.
2. All the 1973 R tests, except one, showed a tolerance level comparable to that of the one valid test obtained in 1972.

Table 1.–Probit line values of bioassays of propoxur against adult *Aedes nigromaculis* with concentration of 4.2 micrograms/cm^2 and variable exposure time in minutes – California, 1972 and 1973.

(S = OP susceptible; R = OP resistant)

1972	Strain	Slope	LT_{50}	95% Confidence Limits	LT_{90}	95% Confidence Limits
6/21	S	7.02	21.0	18.6 - 23.4	31.8	28.2 - 38.4
9/13	S	3.02	28.8	24.6 - 33.0	76.3	62.4 - 105.
9/20	S	3.81	24.	17.4 - 31.8	51.6	7.8 - 97.80
1973						
6/4	S	8.46	40.	36. - 43.	56.	41. - 66.
10/1	S	9.49	39.	33. - 46.	53.	45. - 82.
10/1	S	4.62	35.	27. - 46.	67.	51. - 120.
7/27	S	5.71	16.	11. - 20.	26.	21. - 43.
1972						
9/20	R	2.82	53.4	41.4 - 64.8	151.2	117.6 - 229.2
1973						
7/30	R	7.50	40.	35. - 47	60.0	51. - 86.
5/3	R	2.78	71.	50. - 95.	200.0	140. - 410.
6/12	R	3.08	81.	61 - 110.	210.	150. - 380.
9/1	R	4.41	100.	84. - 130.	200.	160. - 320.

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[1] Vector Control Section.

[2] Program Analysis and Statistics Section.

3. The confidence limits of two of the five R tests overlapped the confidence limits of two of the seven S tests.

CONCLUSIONS.—From the information, it was obvious that the tests did not clearly separate the R and S strains. One possibility is that the resistance of the R strain to propoxur was not high enough to be differentiated or conversely that the S strain was not susceptible enough. However, actual control failures occurred several times at the pasture where the R strain was collected, and the S strain was never knowingly treated, so for all practical purposes it must be assumed that the strains were actually resistant and susceptible, respectively, to propoxur.

The conclusion, therefore, must be that the sensitivity of the method was not suitable to define propoxur resistance or susceptibility in *A. nigromaculis*. It is speculated that two major factors may be responsible for the inconsistent results: variable availability of the propoxur; and vapor pressure.

The LT method could yield consistent results if the insects were forced to pick up the poison and the same amount of the poison was always available to the insects in every test. However, in our tests, filter paper was treated with a propoxur-acetone solution, which was evenly distributed on the filter paper and the acetone allowed to evaporate before the mosquitoes were exposed to it. There is a distinct possibility that some of the propoxur crystals were hidden in the pores of the filter paper and unattainable to the mosquito. The availability of the poison, therefore, may have greatly varied from test to test.

Mitchell et al. (1972), working with *Culex pipiens fatigans*, noted inconsistencies in propoxur tests and concluded that airborne propoxur may have contributed to this. In our tests, it is also speculated that mortality may have been partly due to vapor. The tolerance of *A. nigromaculis* to propoxur vapor is not known.

The time exposure technique should not be ruled out as a means of differentiating propoxur resistant and susceptible *A. nigromaculis*. It does not, however, appear that further work with the method as employed here would be productive. Rather, a re-assessment of the techniques would be in order with an eye toward modifying them to overcome the problems we encountered.

SUMMARY.—The measurement of the resistance of *A. nigromaculis* to propoxur with the time exposure method did not render the desired separation of the susceptible and resistant strains. It is suspected that the airborne effect of propoxur, and the variable availability of the propoxur on the filter paper due to entrapment of propoxur crystals in the pores may have distorted the results. The techniques should be modified to avoid these difficulties.

ACKNOWLEDGMENT.—The assistance, cooperation and encouragement of Melvin L. Oldham, Tehama County Mosquito Abatement District; Stephen M. Silveira and Marc R. Pittman, Turlock Mosquito Abatement District; and Don J. Womeldorf, Vector Control Section, California Department of Health; are gratefully acknowledged.

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SUSCEPTIBILITY LEVELS IN LARVAL AND ADULT *AEDES NIGROMACULIS* (LUDLOW) OF THE CENTRAL VALLEY

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INTRODUCTION.–In the past, mosquito control in California has dealt primarily with controlling larval populations. However, due to the ever increasing problem with larval resistance and cross-tolerance of *Aedes nigromaculis* (Ludlow) to the organophosphorus compounds, control of adult mosquitoes has become necessary within many mosquito control agencies, particularly those within the Central Valley. Mosquito resistance in these agencies has been well documented (Brown et al. 1963; Womeldorf et al. 1968, 1971, 1972). However, little laboratory work has been done to determine the susceptibility levels of the adult populations.

Georghiou et al. (1966) selected larvae of *Culex pipiens fatigans* Wiedemann with propoxur and other closely related carbamate insecticides. Following 35 generations of selection, larval tolerance increased 25-fold while adult tolerance increased 3-fold. Selection of the adult stage for 25 generations resulted in a 5-fold increase in tolerance and only a 2½-fold increase for the respective larvae. The authors considered these results to be an encouraging indication as to the usefulness of this class of compounds for mosquito control.

Wilder and Schaefer (1966) determined susceptibility levels of parathion, fenthion and chlorpyrifos to both larval and adult *A. nigromaculis*. The magnitude of adult resistance was found to be considerable and was consistent with field control problems with the same insecticides.

Womeldorf et al. (1971) discussed the relationship between larval resistance and adult field failures and concluded that when fenthion was applied by aircraft at 0.1 lb/acre, adulticidal failures could be anticipated when there was appreciable 24-hour larval survival at a laboratory concentration of 0.01 ppm.

This paper deals with susceptibility levels of larval and adult *A. nigromaculis* to fenthion, naled and propoxur. Mosquitoes tested, representative of a cross section of populations from the Central Valley of California, ranged from susceptible to highly resistant to the organophosphorus chemicals.

METHODS AND MATERIALS.–All mosquitoes were field collected in the larval stage and transported to the laboratory for treatment. Larvae were tested as described by Gillies and Womeldorf (1968).

Additional larvae from the same collections were reared to adults and were tested against propoxur and naled three days following emergence. The adults were tested as described by Georghiou and Metcalf (1961) with the modification of Darwazeh (1972).

All test data were processed through a computerized probit analysis program. The larval response to fenthion was selected for comparison as representative of response to the organophosphorus compounds.

RESULTS AND DISCUSSION.–Larval test results are shown as LC_{50} and LC_{90} for each population tested and are ordered by larval LC_{50} to fenthion. The most susceptible populations, those from uncontrolled areas, are shown at the top of the graph (Figure 1). The distance between the LC_{50} and LC_{90} indicate the slope of the log concentration-probit line.

Larval LC_{50}'s ranged from 0.0007 to 0.034 ppm, an increase of 49-fold. Limited testing of adults (results not shown) indicated comparable resistance to fenthion although results were quite variable.

Naled was tested against adults only (Figure 2) and demonstrated considerable cross-tolerance to the organophosphorus compounds as represented by larval tests against fenthion. Again, there is some variability, but the populations most highly resistant to fenthion are those most tolerant to naled. This is substantiated by the failure of naled in the field when applied at 0.1 lb/acre by aircraft against highly organophosphorus resistant *A. nigromaculis* adults in the Tulare Mosquito Abatement District (Ramke et al. 1969) and by the lack of mortality of caged adults of this species when compared against *Culex pipiens* following application of naled from a nonthermal aerosol generator (Gillies et al. 1972). This material has not been widely used in the field.

Both larvae and adults were tested against propoxur (Figures 3 and 4). Although there is some variation, there is no apparent trend toward increased tolerance when tested against highly fenthion resistant populations. Field failures have been reported from areas with highly resistant *A. nigromaculis* (Womeldorf et al. 1971). Propoxur has been used operationally for several seasons for adult control in areas with high levels of organophosphorus resistance. However, laboratory results do not demonstrate any marked change in the susceptibility of the populations tested.

SUMMARY.–According to the data compiled, there appears to be a definite cross-tolerance to naled in adult *A. nigromaculis*. Propoxur, a carbamate compound, appears little affected by cross-tolerance as evidenced by laboratory tests against both adults and larvae by this compound.

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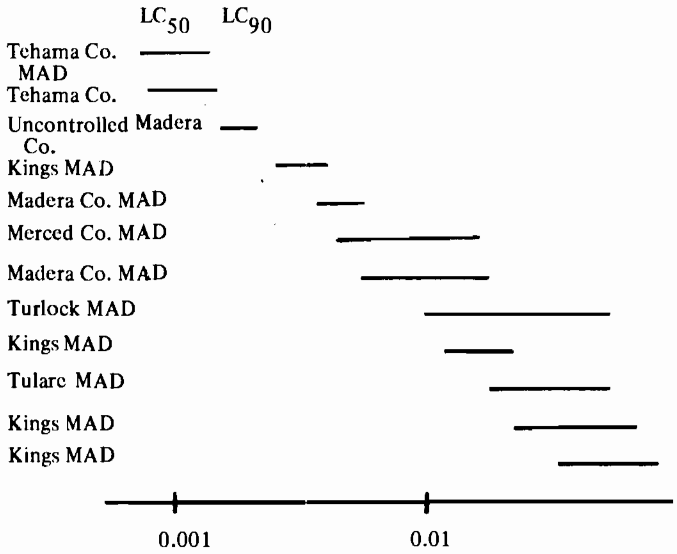

Figure 1.—Response of *A. nigromaculis* larvae, fourth instar, to fenthion. Plotted by LC_{50} and LC_{90} in ppm.

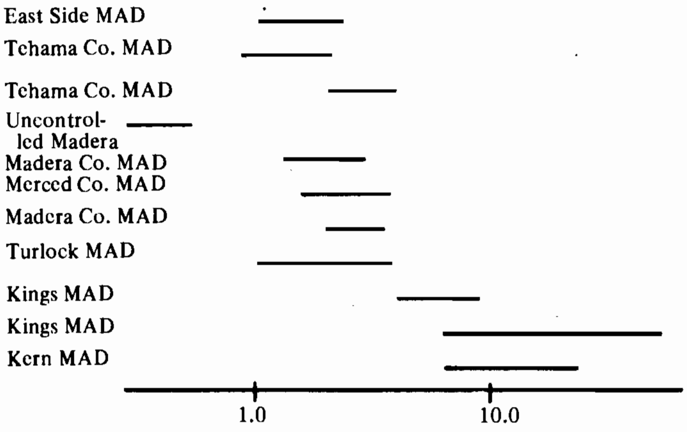

Figure 2.—Response of *A. nigromaculis* adults to naled. Plotted by LD_{50} and LD_{90} micrograms/cm^2. Ordered by of larvae to fenthion.

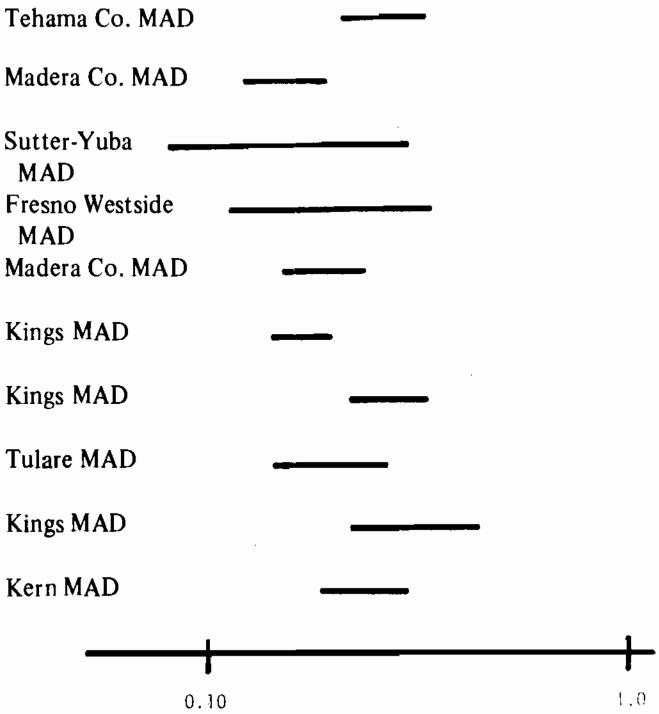

Figure 3.—Response of *A. nigromaculis* larvae, fourth instar, to propoxur. Plotted by LC_{50} and LC_{90} in ppm.

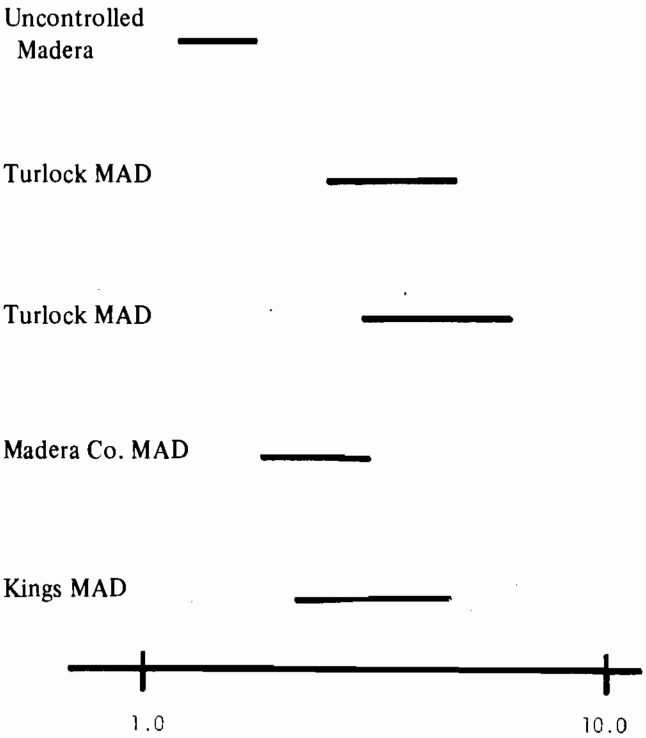

Figure 4.—Response of *A. nigromaculis* adults to propoxur. Plotted by LD_{50} and LD_{90} in micrograms/cm^2. Ordered by response of larvae to fenthion.

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ASSESSMENT OF POTENTIALITY OF *CULEX TARSALIS* FOR DEVELOPMENT OF RESISTANCE TO CARBAMATE INSECTICIDES AND INSECT GROWTH REGULATORS

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The results reported briefly here are part of a continuing project on the potentiality for development and characteristics of resistance to new control chemicals by mosquitoes. This is a report on the selection of *Culex tarsalis* by the carbamate propoxur and the insect growth regulator (juvenile hormone mimic) Altosid® (ZR 515).

Selection by propoxur was undertaken in view of the demonstrated effectiveness of this carbamate against organophosphorus-resistant field populations of the species. Altosid was used as a representative of the novel group of synthetic compounds which mimic the action of the natural insect juvenile hormone.

The population employed in both studies was derived from larvae obtained from the Coachella Valley in late 1971 and early 1972. The population was reared under standard conditions and bioassayed by methods previously described (Georghiou et al. 1966, Apperson and Georghiou 1974).

SELECTION BY PROPOXUR.—Selection by propoxur consisted of exposing groups of 50 4th-instar larvae in 100 ml of water in glass beakers containing the required concentration of the chemical. Adult selection was by exposure of newly emerged mosquitoes to treated glass fiber filter paper in shell vials (Georghiou and Gidden 1965). Two series of selections were performed. Series I involved larval pressure on 9 generations. Five of these were selected in the adult stage as well. The average pressure in series I was at the 75% level of mortality. Series II was initiated by outcrossing part of the F_4 generation of series I to a new collection obtained from the same locality in the Coachella Valley as the original. Seven generations of this series were selected in the larval stage. Two of these were also selected in the adult stage. The average selection pressure in series II was at the 48% level of mortality. The application of higher pressure was considered inadvisable since previous experience had indicated that colonies severely stressed by carbamates suffer a substantial decline in fecundity and fertility (Georghiou 1965).

Dose-mortality data obtained on larvae and adults of each generation indicate no significant change in susceptibility to propoxur. The larval LC_{50} level was 0.14 ppm in the parental generation, 0.19 ppm in the F_9 of series I and 0.21 ppm in the F_{10} of series II.

It is not possible to offer a positive explanation for the recalcitrance of *C. tarsalis* to develop resistance to propoxur, or to postulate that the species lacks entirely the potentiality for development of such resistance. It is of interest, however, to note that tests with piperonyl butoxide, an inhibitor of multifunction oxidase (MFO) enzymes, have produced negligible synergism in *C. tarsalis* (Apperson 1974) and thus that metabolism of carbamates by MFO action may not be a potential source for resistance in this species, in contrast to the situation in *Culex fatigans* (Shrivastava et al. 1970).

The failure of the twice colonized population of *C. tarsalis* from the Coachella Valley to manifest a potentiality for development of resistance to propoxur must be considered as encouraging from the standpoint of practical usefulness of carbamates against this species in the immediate future.

SELECTION BY ALTOSID.—Selection by this compound required the formulation of a new method of treatment and bioassay which will be described in a separate paper. The selection method employed involved daily application of Altosid to water containing 4th instar larvae, thereby obviating the problem of chemical instability of the compound. It was thus ensured that every individual contributing to the new generation had indeed been exposed to the required dose during the critical period of sensitivity. Up to the present time selection has involved 14 generations at an average pressure of 70.8%.

Figure 1 indicates the gradual increase in the selective concentration of Altosid employed from an initial 0.1 ppb to 5 ppb., i.e. an increase of 50-fold. Complete dose-mortality regression lines for the F_{12} selected generation, the pa-

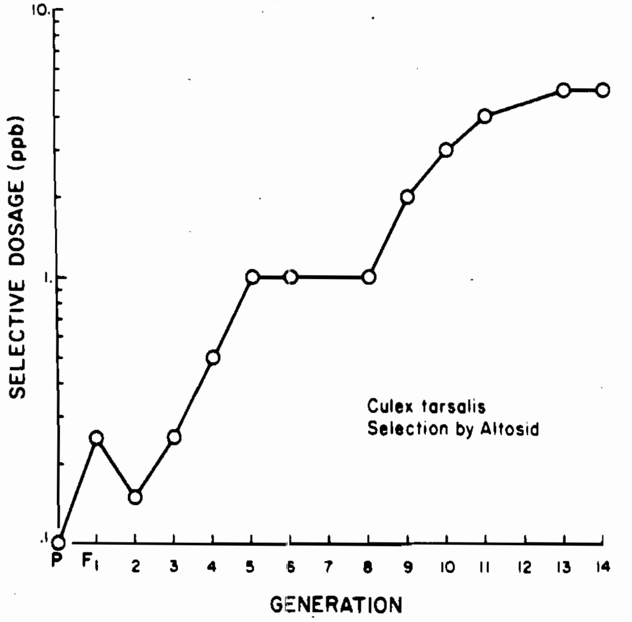

Figure 1.–The gradual increase in concentration of Altosid applied in the course of selection of *Culex tarsalis.*

rental (Coachella) and a susceptible laboratory strain (Bakersfield) were also determined. These revealed that at the LC_{50} level, the selected strain was 8.9-fold less susceptible to Altosid than the parental strain, and 85.9-fold less susceptible than the Bakersfield strain.

We are currently continuing the selection of this strain by Altosid and also investigating the specific mechanisms responsible for the tolerance which was developed. Special emphasis is placed on two likely mechanisms of resistance, namely increased ability to metabolize the hormone mimic or to cope with higher concentrations of it and still complete a normal development.

The development of increased tolerance to Altosid in *C. tarsalis* as reported here corroborates the finding of cross resistance to this compound and to other juvenile hormone mimics in certain insecticide-resistant strains of the house fly reported earlier by Cerf and Georghiou (1972- 1974).

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EXTENSIVE APPLICATION OF ALTOSID™[1] AS A MOSQUITO LARVICIDE

Lawrence L. Lewallen[2] and Dennis A. Ramke[3]

During 1973, Altosid™, a compound that acts as an insect growth regulator, was registered for experimental control of mosquito larvae in California. This product is of interest for use where extreme levels of insecticide resistance in mosquitoes have developed to other available insecticides. The most effective formulation for larviciding incorporates the microencapsulation process and is known as SR-10. It contains 0.8579 lbs/gal of active ingredient. It has been registered for use on floodwater type mosquitoes only.

Extensive testing of SR-10 was carried out on *Aedes nigromaculis* larvae in the Alpaugh area from July 30 to October 31, 1973. A zone two miles in depth was established around the center of town. Sources treated were irrigated pastures and alfalfa fields.

Pre-treatment adult mosquito counts were made using the pants-leg count technique. Despite routine applications of conventional mosquito larvicides to sources in this area, pants-leg counts averaged around 8/leg prior to Altosid™ application.

In the two-mile zone surrounding the town, Altosid™ was applied by hand sprayer and by aircraft at 4 fl. oz. of concentrate per acre. The finished spray was formed by adding water to the microencapulated concentrate. The third instar to early fourth instar larvae were treated. Operations were carried out in the same manner as with conventional insecticides.

Within two weeks after initiating the spray test, pants-leg counts dropped to 0 and remained that way for the duration of the test. By contrast, an untreated irrigated pasture (10 miles distant) had counts of 50/leg on several occasions during the test period.

Post-treatment observations on larvae were made by taking samples into the laboratory, and also by verification in the field that abnormal pupae had developed, which resulted in no adult emergence.

During the test period, no complaints from Alpaugh were received at the District. Complaints were registered before and after the test was terminated.

Thirty gallons of concentrate were applied over 368 acres of irrigated pasture and 586 acres of alfalfa, amounting to a total of 954 acres. 104 acres were treated by hand equipment and 850 acres by aircraft.

The lack of adult mosquitoes in the test site indicates that Altosid™ successfully controlled the irrigated pasture mosquito at a cost of approximately $2.00 per acre for material. Since the test was made in an area of extreme insecticide resistance, it would appear likely that Altosid™ could be used successfully to reestablish chemical control in this area.

The authors wish to acknowledge the assistance rendered by Larn C. James, Jr., Operator Zone 9, in carrying out inspections and treatment of the test area.

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[1] The results reported here do not necessarily constitute endorsement of this product by the California Department of Health or the Tulare Mosquito Abatement District.

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SYNTHETIC MOSQUITO REPELLENTS VIII: 2-THIO-4-THIAZOLIDINEONES AND 2, 4-THIAZOLIDINEDIONES

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ABSTRACT

2-Thio-4-thiazolidineone and 2,4-thiazolidinedione derivatives with substituents in the 3- and 5-positions were synthesized and evaluated on human skin for repellency against female *Aedes aegypti* mosquitoes. In the 2-thio-4-thiazolidineone series, compounds with boiling points approximately 120°C/0.5 mm Hg, and in the 2,4-thiazolidinedione series, compounds with boiling point ranges of 95-115°C/0.5 mm Hg exhibited the longest duration of repellency. The best compound approaches N,N-diethyl-m-toluamide (DEET) in the duration of topical repellency.

IMPACT OF MALATHION ON *A. STEPHENSI MYSORENSIS* IN SOUTHERN IRAN

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ABSTRACT

Iranian malaria eradication has reached an advanced stage. About 2/3 of the country with a population of about 19.5 million is in the pre-maintenance, early, or late consolidation phase. The development of resistance by *A. stephensi mysorensis*, the main malaria vector in southern Iran, first to DDT in 1957 and then to Dieldrin in 1959, practically stopped the eradication of malaria in subsequent years.

In order to solve this problem, in 1958 the field research units of the Institute of Public Health Research, University of Teheran, started trials with new insecticides. In 1959 Malathion spraying was performed in Jiroft and in Shabankareh (Kazerun) areas and repeated in 1960 and 1963. Village-scale trials with Malathion showed that the residual effect of this insecticide is 6 weeks on non-organic surfaces and 12 to 16 weeks on organic surfaces. Subsequently the Malaria Eradication Organization and Institute of Public Health Research decided to carry out a large-scale trial in the Bandar Abbas region, which covers 435 villages with a total population of about 138,570.

The first round of Malathion spraying, at the rate of 2 g/m^2, was performed in October 1964, which coincided with the end of the seasonal activity of *A. stephensi*. The effect of this spraying was remarkable and, until the next round of spraying, which was about five months later, no *A. stephensi* were recorded in the selected villages, although they were the most favorable ones in the area. From October 1964 to March 1966 the area was sprayed with Malathion. The results of an investigation of the five times of Malathion spraying, indoor density, larval density, night-bite collection, age-grouping dissection and window trap collection, indicate that a Malathion spraying program of 3 times per year in areas where *A. stephensi* is active throughout the year is able to keep down the density of *A. stephensi*. Bioassay tests on mud walls showed that after 3 weeks the mortality rate decreased to zero. The same tests performed on wood surfaces showed that for 4 months after each spraying at one-hour exposure, the mortality rate was 98%, according to the results obtained from the large-scale Malathion trial in the Bandar Abbas area.

In October 1967, the southern part of the country with a population of about 4 million was sprayed with Malathion 2-3 times per year, according to the seasonal activity of *A. stephensi* and the vulnerability of the area. An entomological and epidemiological investigation showed that in areas where *A. stephensi* is the sole vector, such as the plain of Khuzestan, the littoral shore of Bandar Abbas and the coastal areas of Fars Province, the interruption of malaria transmission was possible. In hilly areas where *A. stephensi* is mixed with other vectors such as *A. fluviatilis*, *A. dthali* and *A. superpictus*, in spite of intensified anti-malaria measures, 2 times Malathion spraying, 2 rounds DDT, treatment of positive cases, introduction of Gambusia fish in active and potential breeding places, etc., due to the exophagic and exophilic tendency of local vectors as well as outdoor sleeping habits of the local population during the malaria transmission season, population movement and abundance of temporary dwellings (tents, huts, etc.), the transmission of malaria has not been successfully interrupted.

Susceptibility tests carried out during the past 10 years in areas that have been treated 17 times showed that *A. stephensi* is susceptible to Malathion. It should be mentioned that, since 1967, each year about 700 tons of Malathion have been used for malaria control in the southern part of Iran. There have been no reports of harmful effects of the insecticide, either among spraymen or inhabitants.

ORGANOPHOSPHATE PESTICIDES IN AMBIENT AIR IN AND AROUND MOSQUITO CONTROL BUILDINGS

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ABSTRACT

The air in and around a pesticide storage shed and mosquito control buildings was sampled and relative concentrations of organophosphate pesticides in general and malathion in particular were correlated with the proximity to storage areas and mixing areas. A unique and inexpensive sampling technique was employed.

Table 1.–Aerial Photographic Surveillance.

	Location and Number of Flight			
	City of Orange Number 1	Villa Park – Orange Los Alamitos, No. 2	Central Garden Grove Number 3	1973 TOTAL
Date of Flight	2-8-73	4-19-73	12-3-73	
Flight Time	1.0 hrs.	2.0 hrs.	1.1 hrs.	4.1 hrs.
Approximate area covered by photograph	0.25 sq. mi.	9.0 sq. mi.	1.0 sq. mi.	
No. photographs taken	3	27	33	63
Swimming pools detected				
Total number	30	502	152	684
Number concrete	25	408	95	528
Number plastic	5	94	57	156
Appearing neglected				
Total Number	9	87	39	135
Number concrete	4	31	9	44
Number plastic	5	56	30	91
Breeding Mosquitoes				
Total Number	7	35	11	53
Number concrete	3	11	1	15
Number plastic	4	24	10	38

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Experience has shown that 1,500 feet above ground level is a good altitude for this type of photography, after experimenting with several other altitudes at higher and lower levels. We also found that cross-light is most satisfactory, and most photographs are made toward the east and west. North may be photographed with less satisfactory results, and toward the south almost always yields unsatisfactory results due to the presence of intense midday sunlight. We schedule a flight when the sun will be at its highest point overhead, thus lessening shadow distortion. Our experience has shown that visability of less than five miles is unsatisfactory to achieve the desired photographic quality for this type of surveillance. Since some degree of haze is generally present in Southern California, we intend to experiment with several types of light filters to reduce the effects of haze and smog.

In December of 1973, an operational flight was made to photograph one square mile in the central Garden Grove area. 33 photographs were made, showing 152 swimming pools. However, the normal number of photographs for satisfactory results is 16 per square mile.

After analysis, it appeared that 39 of the pools were neglected, and possible mosquito breeding sources. Inspection showed that 11 or 7.2% of the total were breeding mosquitoes.

Two other operational flights were made in 1973 with the results summarized in Table I. Statistically, Table I indicates that of 684 swimming pools detected in the photographs, 135 or 20% gave the appearance of being neglected, while ground inspection confirmed the presence of mosquito breeding in 53 or 8% of the total. Fifteen of the pools found breeding were of the concrete type and 58 were of plastic construction, indicating a much higher frequency of neglect associated with plastic pools than with concrete swimming pools.

This type of surveillance program offers several advantages, the most obvious being the preventive aspect. We are able to find and control more sources before they become a serious problem, and at the same time carry out our program of public education to the property owners. The next advantage is the time involved in locating sources. We can effectively photograph two square miles in one hour of flight time. A two square mile area in a typical urban area will contain an average of 2,000 properties. A two-man ground team can inspect only 10 to 12 properties in one hour.

We rent airplanes at $12.00 per hour for the two place model and $15.00 per hour for the four place. We pay an additional $226.00 annually for liability insurance, and our

Workman's Compensation Policy increased at the rate $3.32 per $100.00 of coverage. This additional coverage adequately protects the District.

A permanent position of Staff Pilot is not required in the District, because Control Operator Beams is a licensed Commercial Pilot.

With the possibility of the District assuming other Vector Control responsibilities, we are exploring other possible uses of aerial photographic surveillance. The detection of large scale rat harborages, fly larval sources, and animal census operations might be accomplished using similar techniques. We have gained a considerable amount of valuable information from just a few flights, and believe that the project has added depth to our mapping and documentation projects.

USE OF PERMAFOAM FILLED TIRES IN CONCRETE LINED FLOOD CONTROL CHANNELS

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The desire of mosquito abatement districts to attain and maintain low costs of operations and at the same time attain a high level of efficiency was the criterion used in developing a program to effectivley control mosquitoes and midges in the 430 miles of concrete lined flood control channels in the Southeast Mosquito Abatement District. The District at one time attempted to control the problem by the use of two and three man crews working from the roadside along the channels with a crew in the bottom of the channel. Costs of such a program were high for salaries alone. We began to look for alternatives, and found that some of the channels were built with ramps to allow equipment to drive into the channel for maintenance and repair. A vehicle was equiped with a rear mounted spray boom so that one man could operate the unit on the floor of the channel, for spraying operations.

The equipment worked well, except that the vehicle had two or three flat tires daily. The public likes to use these channels as trash dumps; bottles, nails, broken concrete, and bits and pieces of scrap iron, etc. are numerous, and sharp items such as these puncture tires much more easily in an aqueous environment. With all the debris we had to drive over, a set of tires would only last for 2 to 3 weeks, with consequent high cost for repair and replacement, and loss of time.

Various types of steel cord tires and sealant so-called puncture proof tires were tried. All of were found to be unsatisfactory under the conditions in the channels.

Industrial type tires were found to meet the requirements. Inherent defects of such a tire are an inability to properly balance them, reduced road speed (25 mph or less), and heavy weight of the tire which causes wear and tear on bearings, shocks and springs. Even with these defects, the saving in cost and time for the operations was substantial. However, the search for something better was continued.

In the early months of 1972 we read an article describing Goodyear's Permafoam filled tires with cushioned ride, improved handling, reduced driver fatigue, decreased vehicle maintenance and improved flotation on soft ground, and a sustained highway speed of 35 mph and up to 50 mph intermittently. In a conference with a company representative, it appeared that the Permafoam filled tires were suitable. Rims for Goodyear tires inflated with Permafoam are standard rims furnished by the customer, which must be sent to the factory for processing. Drop center rims restrict the amount of inflation needed and the specific pressure needed must be determined in relation to the application. Rim and tire assemblies are shipped ready to mount. Cost of a set of 4 tires including shipping, rims and taxes, for size 7:00-15, is $707.59 as of September 1973.

The District has used one set of Permafoam tires in our operations for 1½ seasons and has found them to meet all of our requirements. These tires are retreadable and should last approximately 5 to 7 years. We have received a second set of tires which will be put into service on a second unit used in the channel spray program.

The comparatively high first cost of the Permafoam tire has been more than offset in the savings realized for salaries, repair and replacement, and most importantly the savings in down-time.

REPORT OF INVESTIGATION OF POTENTIAL MOSQUITO BREEDING IN UNDERGROUND UTILITY ENCLOSURES

Charles M. Myers[1], Edward W. Colson[2] and John W. Bunnell[3]

INTRODUCTION.–In 1972, concern was expressed by several mosquito abatement districts regarding potential mosquito production in subsurface transformer enclosures. In response to this concern, the California Department of Health contacted the two largest utilities known to be involved: Pacific Gas & Electric Co. (PG&E) and Southern California Edison Co. (SCE). The remaining utilities involved in underground electrical distribution systems were informed of the potential problem through the Western Underground Committee in late 1972. PG&E amd SCE agreed in 1972 to study the problem with the Department of Health. Early in 1973, interim recommendations to help the potential problem in the increasing numbers of new enclosures being installed were made to all of the utilities by the Department of Health, and an initial study plan was formulated.

METHODS.–Three areas were chosen for the study, and inspection times were designated to correspond to the period of anticipated mosquito breeding in each area. The initial inspections were carried out by utility company staff. Information on the existence, condition and sources of water, the presence and amount of silt, and the relationship of the enclosure to level grade was gathered.

Following the initial inspections, further information on the presence of mosquitoes was acquired.

RESULTS.–Southern California Edison Company conducted a survey of 3,789 of its more than 10,000 enclosures in response to the contact from the Department of Health. The survey was taken in April through August. Of these enclosures, 219 (5.8%) contained standing water. Twenty-seven of these enclosures were inspected for mosquitoes and 15 (56%) had water and 7 (26%) had mosquitoes at that time.

In the two PG&E inspection areas, more detailed data were gathered for 395 enclosures. Water was found in 114 (19%) of all enclosures sampled. This varied from a high of 58% in the Vacaville-Fairfield area to a low of 6.2% in the Fresno-Madera area. Of 57 randomly selected enclosures with water, mosquitoes were found in 7 (12.3%).

Many enclosures may receive water from more than one source. The following data indicate the probable sources and numbers of enclosures affected:

Groundwater	335
Lawn sprinklers	303
Enclosure cover below grade	151
Rain gutter down spouts	39
Other (e.g., irrigation systems)	9

Although the data indicate groundwater and lawn sprinklers were the most probable sources of water, information on rainfall and water tables will be obtained to verify the data.

No significant correlation could be made between the water depth and mosquito presence due to the small sample size of mosquito-positive enclosures. The average water depth in the Vacaville-Fairfield area was 25 inches compared to 14 inches in the Fresno-Madera study area.

Water was recorded as being fresh or stagnant by utility crews. Approximately 75% of the enclosures contained water designated as being stagnant. In all of the enclosures with mosquitoes the water was stagnant. No correlation could be found between age of the enclosures installed from 1969 through 1973 and those containing stagnant water.

Silt, a likely contributor to stagnant water, was recorded in 42% of all the enclosures. At the time of the original survey, silt levels were not recorded for enclosures containing water. Subsequent limited observations indicate silt is always present in those enclosures with water. The elimination of silt from enclosures should lessen the probability of water retention, thus lowering the potential for mosquito breeding. This premise will be analyzed this year.

Although the utilities attempt to locate the enclosure top caps at or above grade during construction, final landscaping often causes the top caps to be below grade. Thirty-eight percent of the top caps were below grade in this survey. Of the 100 enclosures in the Vacaville study with water, 66% had top caps below grade.

CONCLUSIONS.–Mosquitoes occur in some subsurface transformer enclosures. Initial observations indicate the top caps being below grade and silting are contributing causes to enclosure flooding and mosquito production. The utilities recognize this potential problem, and since future enclosure installations will number in the thousands, remedial steps will be taken to minimize present and future mosquito presence in subsurface enclosures.

Recommendations to alleviate this potential problem will be submitted to the Department of Health by the utilities. Studies by the Department of Health and the utilities will continue to monitor the impact of the implementation of these recommendations.

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A TECHNIQUE FOR SEXING PUPAE OF *HIPPELATES PUSIO* DIPTERA: CHOROPIDAE

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ABSTRACT

Approximately 24 hours before emergence the pupae of *Hippelates pusio* were separated from the culture media by winnowing. The cleaned pupae were placed on a light background and separated on the basis of sexual dimorphism. Characteristics between sexes reared under optimum growing conditions were distinct size differences and a slight color difference just prior to emergence. The male pupae appeared slightly deeper brownish-black than females as viewed in reflected light.

Pupae measuring 2.1 through 2.4 mm in length averaged ca 88% males per 1,000 while those measuring from 2.45 through 2.7 mm were 82% females.

TRANSIENT MOSQUITO PROBLEMS OF IRRIGATED PASTURES

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One of the serious problems in California is the control of mosquitoes in irrigated pastures. In California there are approximately 1,000,000 acres (404,690 hectares) of irrigated pastures of which 80% are capable of producing mosquito control problems. These fields are irrigated from 15 to 20 times a year and each irrigation may produce large numbers of mosquitoes. Three mosquito species are associated with pastures: *Aedes nigromaculis* (Ludlow), *A. melanimon* Dyar, and *Culex tarsalis* Coquillet. A high degree of insecticide resistance has developed in these populations. In many mosquito abatement agencies irrigated pastures are the main target of their control effort.

A study was conducted in cooperation with the Delta Vector Control District in Tulare County, California, of pasture land use practices as they relate to mosquito control procedures. The primary objective was to determine how irrigated pastures changed from year to year, their duration and rotation with other crops, and to evaluate this management or any other applicable abatement procedure.

MATERIALS AND METHODS.—The Delta District maintains records of mosquito sources and these records are cross referenced to land use. Maps are prepared from vertical aerial photos using a scale of 1:7920. These maps divide the District into units one mile square. Each of these sections shows roads, farm fields, and various other features. The location and acreage of field crops is accurately determined on each map. A new set of maps is prepared each year and all changes in crops and acreages are recorded. In California this is referred to as the "Section Survey System" of recording inspections and treatments.

A single township (T17, R23, Sections 1-36) was selected for this study. The period of study was limited to ten years of data (1948 through 1957). Section maps showing agricultural land use were examined in sequence and the history of each pasture was recorded by section and year. Since some of the fields did not retain their original size and shape during the entire period of study, it was necessary to compensate for these changes by assigning unit size numbers to each portion of the original pasture that was retained as pasture for the study period. In a few cases this divided the land used as pasture into two or three separate fields. However, most fields did not follow this extreme pattern of change but retained most of their original size and shape. Crop acreage was determined with a planimeter. Land use preceding and following each pasture crop was also recorded.

The area selected for this study was comparable, in terms of the mosquito control problems and pasture age, to other areas in the District with the same types of soil.

RESULTS AND DISCUSSION.—Within the study township (36 sections), irrigated pastures were planted in 26 sections at least sometime during the ten-year period. There was an average of 2,388 acres of pasture in Township 17 during this period. Table 1 shows the total acreage by year, the number of fields, and the average acreage per field unit. There was a small decline in the number of fields and an increase in average field size and in total acreage.

Table 2 shows the number of fields for each period of longest existence. Note that 84% of the fields were in existence for five years or less. Only 16 fields were continuously in pasture during the ten-year period.

Table 1.—Irrigated pastures during ten years of study in a 36-square-mile area.

Year	Total Acreage	Number of Pastures	Average Acreage per Pasture
1948	1972	122	16
1949	1990	129	15
1950	2327	144	16
1951	2087	105	20
1952	2752	126	22
1953	2642	124	21
1955	2487	112	22
1956	2664	117	23
1957	2568	107[1]	24

[1] Fifteen fewer fields in ten years.

Table 2.—Number of irrigated pastures by longest period of existence.[1]

Years	Pastures
1	78
2	64
3	63
4	43
5	23
6	16
7	9
8	9
9	6
10	16
Total	324

[1] 84% of the fields were in existence five years or less.

Table 3.–Life of pastures in years.[1]

Duration in Years	Number of Pastures
1	60
2	43
3	46
4	29
5	18
6	12
7	7
8	6
9	6
10	16

[1] 80% of these pastures existed five years or less.

Table 4.–Irrigated pastures and number of times rotated from 1948 to 1957.

Rotation		Pastures
0		243[1]
1		70
2		11
3		0
	Total	324

[1] A majority of these fields existed for five years or less.

The pastures that were not rotated during the study period to other crops and back to pasture again are shown in Table 3. A high proportion (196) of the pastures (80%) existed for five years or less.

Table 4 illustrates the total number of irrigated pastures that were under study during the ten-year period. Of the 324 fields studied, 243 were never rotated and most of these endured for five years or less, 70 were rotated with another crop once, 11 twice and none three times. This also means that only 81 pastures were rotated one or more times during the period of study.

Further analysis of the section maps revealed that 122 fields were in pasture at the start of the study period (1948) and, except for the 16 fields that were continuously in pasture for ten years, they were all completely replaced by another crop before the end of the study period (1957). Fifty-one of the 122 fields that were initially in pasture in 1948 were only in pasture for a single period of time. Initially 107 fields were in another crop or were uncultivated before they were planted to pasture sometime after 1948 and these continued in pasture until the end of the study period (1957). Of the 107 fields, 54 were never rotated with another crop.

CONCLUSION.–Most irrigated pastures in the township studied were temporary. They were usually planted on land that had been previously cultivated for another crop. Untilled land was not placed in pasture when first cultivated. Of the 324 pastures that occurred in the study township during the ten-year period, 308 were associated with alternate crops.

During the ten-year period covered by this study, there were approximately 200 opportunities to influence the owner or operator of a farm to prepare the land for proper irrigation and drainage to reduce or eliminate mosquito production. This occurred each time a field was rotated with another crop or when it was initially prepared as a pasture.

The frequency of rotation and the short duration of most irrigated pastures shows that this crop is greatly influenced by farming enterprise values and that in the township under study, as well as in comparable areas, irrigated pastures are of a transient nature. This information should be taken into consideration both in planning water and land management programs to minimize mosquito production or when using the legal approach to mosquito source abatement.

CONTINUING EFFECTIVENESS OF SHORE LINE MODIFICATIONS CONSTRUCTED FOR MOSQUITO ABATEMENT

Don M. Rees[1], Glen C. Collett[2], and Robert N. Winget[3]

In 1966 ten small units were constructed on the grassy shore line of a fresh water impoundment in a marsh bordering the Great Salt Lake. The construction of these units was financed by a research grant[4]. The purpose was to determine the effects of this kind of shore line modification on the production of mosquitoes and other biota.

The description of the units and the initial results obtained were reported in Mosquito News by Rees and Winget in 1968 and 1969. Inspections of the units for mosquito larvae has continued during the mosquito season on a weekly basis. Observations have also been made of the effects on other biota. These inspections were made by the personnel of the Salt Lake City Mosquito Abatement District and the results are summarized in this report.

The grassy shore line that was modified is 90-100 feet from the open water zone to the upland vegetation zone. Salt grass (*Distichlis stricta* (Torr) Rybd.) is the dominant vegetation species and is interspersed with other moist soil and emergent marsh plants. *Aedes dorsalis* Meigen is the most abundant mosquito species produced and the larvae are largely confined to the grassy margins. Large numbers of *A. dorsalis* larvae generally appear after each successive flooding. If the water remains for extended periods after flooding, considerable numbers of larvae of *Culex tarsalis* Coq. and *Culiseta inornata* Will. may appear.

The ten modified units are approximately 90 feet wide by 90-100 feet long. All the vegetation was removed and the soil excavated in a unit to a depth of 3, 4, 6, 8, 9, or 12 inches below the adjacent soil levels. The ten modified units were interspaced by undisturbed shore line units of approximately equal size.

In previous published results it was stated that no mosquito larvae were found in any of the modified shore line units but larvae of *A. dorsalis*, *Culex tarsalis* and *Culiseta inornata* continued to appear in the undisturbed control units when conditions were favorable. The vegetation in the control units remained about the same with a slight increase in alkali bulrush, *Scirpus paludosus* A. Nels., and Cattails, *Typha latifolia* L. In the modified units submergent plants began to appear in 1967, primarily sago pondweed, *Potamogeton pectinatus* L.; algae, *Cladophora* spp.; and floating duckweed, *Lemna minor* L. It was also reported that sediment was being deposited on the bottom of the modified units at the rate of 0.5 to 1.5 inches in a four month period, in 1967 and 1968. Both the modified and unmodified units were reported to be used extensively by waterfowl and other marsh birds.

The inspection and observations made on the ten modified and adjacent control units during the five-year period, 1969-73, have produced continuing results similar to those previously reported. The modified and unmodified units in general show very little change since modification. It is significant that no mosquito larvae have appeared in nine of the modified units since their construction. In the control units mosquito larvae of the three species present have continued to appear in abundance when conditions were favorable.

In one modified unit excavated three inches or less in depth, some grass and associated vegetation have appeared. In this unit mosquito larvae have been found at times in this vegetation when conditions were favorable. In the other nine units the kinds of submergent vegetation previously reported and widgeongrass *Ruppia martima* L., which later appeared with a few other species, fluctuate in amount from year to year. No emergent plants are present in these units. The rather rapid deposition of sediment reported in the excavated units has filled the units with soil to a level equal to the bottom of the adjacent open water in the impoundment. The rate of deposition in the units is now approximately the same as in other similar open water in the impoundment.

In the unmodified units the vegetation has remained fairly stable in kinds and amounts except for an obvious increase in the amount of alkali bulrush and dock, *Rumex crispus* L., in some of these units.

CONCLUSIONS

1. The removal of salt grass and the soil beneath to a depth of four inches or more on the impoundments with gradual sloping grassy shore lines has eliminated mosquito larvae in this modified area.
2. This mosquito control method has maintained its effectiveness for seven years and little physical change has occurred on the units to date.
3. The cost of modifying shore lines by this method is expensive but results and duration of effectiveness justify the expense in certain areas, when compared with the expense of applying other acceptable control measures over such a long period of time.

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INSECT DEVELOPMENT INHIBITORS: EFFECTS OF ALTOSID®, TH6040 AND H24108 AGAINST MOSQUITOES (DIPTERA: CULICIDAE)

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ABSTRACT

Aircraft trials with Altosid® (isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) demonstrate the operational feasibility of controlling *Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar larvae at rates of .020 and .025 lb AI/acre. Residues of Altosid on pasture grasses declined quickly and steadily after treatment at the latter rates. TH6040 [1-(4-chlorophenyl)-3-(2, 6 difluorobenzoyl-urea] inhibits molting of mosquito larvae, causes mortality in the pupal stage and interferes with adult emergence. TH6040 offers promising activity against *A. nigromaculis, A. melanimon* and *Culex tarsalis* Coquillett in field trials. H24108 [3-butyn-2 yl N-(p-chlorophenyl) carbamate] is another type of chemical structure that causes pupal mortality following treatment of mosquito larvae.

INTRODUCTION.—In previous reports (Schaefer and Wilder 1972, 1973), the practical potential of ZR-515, now named Altosid® (isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate), for controlling *Aedes nigromaculis* (Ludlow) and *Aedes melanimon* Dyar was shown. In June of 1973 the Environmental Protection Agency issued an "Experimental Sales Permit" to Zoecon Corporation for the first marketing of Altosid; thus, the first official approval of a juvenile hormone-type compound as an insecticide was made. Further research on Altosid was conducted during 1973 to determine the lowest effective dose rate under operational conditions and to determine residue levels on pasture grasses which result from such treatments. Other compounds representing different types of chemical structures, and which have biological activity similar to that of juvenile hormone-type compounds, were studied during the past year. Hercules 24108 [3-butyn-2 yl N-(p-chlorophenyl) carbamate] is very interesting as it is a carbamate. It does not produce larval intoxication (except at high doses), but mosquitoes treated as larvae die during the pupal stage.

Thompson-Hayward 6040 [1-(4-chlorophenyl)-3-(2,6 difluorobenzoyl)-urea] (Wellina et al. 1973) does not cause direct larval intoxication, but mortality occurs during a molt, in the pupal stage at lower concentrations, and in the partial emergence of abnormal adults at still lower concentrations. TH6040 has been shown to inhibit chitin synthesis (Post and Vincent 1973), and the first report of its effects on mosquitoes has recently been published (Jakob 1973). Laboratory and field evaluations of H24108 and TH6040 against several species of mosquitoes were conducted during 1973 and are reported below. Simultaneous evaluations were made in many field tests on the effects of treatment on target and nontarget organisms as in previous studies (Miura and Takahashi 1973).

Each field test has been assigned a Field Test Number, and these are referred to as F. T. Nos. in the text. Since it is impractical to include all the details recorded for each test; e.g., temperature, wind, skycover, water depth, etc., this information will be kept on file and is available to interested persons.

MATERIALS AND METHODS.—The methods for laboratory and field evaluations of developmental inhibitors were the same as previously described (Schaefer and Wilder 1972, 1973), except where noted otherwise.

The SR-10 formulation of Altosid was used in all of the field tests; it is a liquid suspension of polymer-Altosid particles with an average particle diameter of one micron. The technical Altosid is encapsulated in an amide polymeric matrix and the formulation is 10% AI by weight or .8579 lb gal EC formulation was used, but a 25% WP formulation became available and was used for all subsequent tests. For field testing of H24108, a 50% WP formulation was used.

Samples of pasture grasses were collected at random from pastures that were treated by aircraft with Altosid at dosages of .020 and .025 lbs AI/acre. Grass samples were collected immediately before and after treatment and at 1, 3, 5, 7, and 9 days following treatment. The samples were held at -20°C or below until analysis. Duplicate 100 gm aliquots from each sampling time were extracted and analyzed by the methods of Miller et al. (1974).

In one field test with Altosid (F. T. No. 73-20), 600 ml water samples were collected immediately before and after treatment and at 24 hours following treatment; these were collected, processed and analyzed as described earlier (Schaefer and Dupras 1973).

RESULTS AND DISCUSSION.—Altosid--A summary of 1973 aircraft trials with Altosid SR-10 is presented in Table 1. The data demonstrate operational feasibility at doses of .025 and .020 lb AI/acre. Doses of .016 and .014 were too low. In one test (F. T. No. 73-20) a dose of .025 lb AI/acre gave very good results even though the vegetative canopy was fairly high and dense and the wind was high during application. The very limited residual of Altosid in water is in agreement with 1972 studies (Schaefer and Dupras 1973).

Residue levels of Altosid on pasture grasses treated with operationally feasible doses are shown in Table 2. It is apparent that Altosid concentration declines quickly and steadily after treatment. It is also likely that residues would have been even less if the samples had been collected earlier in the season when temperatures were higher.

TH6040--Laboratory data on the biological activity of TH6040 against *Culex* species are shown in Table 3. Pupae are insensitive except at relatively high concentrations. Medium to late 4th-stage larvae of the southern house mosquito are slightly less susceptible than younger larvae. With *Culex tarsalis* Coquillett, early to medium aged 4th-stage larvae appear to be less susceptible than late 3rd- to early

Table 1.–Summary of 1973 aircraft trials with Altosid® SR-10 formulation against field populations of *Aedes nigromaculis* and *Aedes melanimon*.

Field test no.	Date (1973)	Dose (lb AI/acre)	No. acres	Larval stages treated	Final mortality of field population by stage treated (%)			
					1	2	3	4
73-6[a] [c]	5-31	.025	30	2,3,4		100	100	100
73-10[b]	6-15	.025	40	2,3,4		99	100	100
73-12[a]	6-20	.020	26	1,2,3,4	95	99	100	100
73-19[b]	7-13	.020	40	1,2,3,4	95	98	100	100
73-20[a] [d]	7-18	.025	30	1,2,3,4	95	100	100	100
73-25[b]	8-14	.016	40	1,2,3,4	90	95	95	100
73-27[b]	8-16	.014	20	2,3,4		80	85	97
73-43[b]	9-19	.020	30	3,4			100	100

[a] *Aedes nigromaculis* only.

[b] *Aedes melanimon* were 20% or less of the total population.

[c] .037 ppm in water immediately after treatment (four 600 ml samples). <.0005 in water 24 hours after treatment (four 600 ml samples).

[d] Adverse conditions: high, dense vegetation and 10-12 mph wind.

Table 2.–Residues of Altosid® on pasture grass following aerial applications in Fresno County, California.

Sample collection time	Moisture content (% water)	Aliquot #1 (ppm)	Aliquot #2 (ppm)	Average (ppm)
		Gilio pasture, .023 lb AI/acre, 9-10-73		
1-hour (pre)	73.4	N.D.[a]	N.D.	N.D.
1-hour (post)	72.6	.49	.16	.33
1-day	75.3	.031	.082	.057
3-days	75.1	.022	.027	.024
5-days	74.7	.027	.016	.022
7-days	66.2	.00072	.00050	.00061
9-days	75.2	N.D.	N.D.	N.D.
		Reynolds pasture, .025 lb AI/acre, 9-12-73		
1-hour (pre)	75.9	N.D.	N.D.	N.D.
2-hours (post)	61.3	.071	.10	.087
1-day	67.0	.039	.022	.030
3-days	72.4	.0062	.0056	.0059
5-days	68.8	.0041	.0082	.0062
7-days	73.1	.0056	.0028	.0042
9-days	74.0	N.D.	N.D.	N.D.

[a] N. D. = not detectable; minimum detection limit .0005 ppm; recovery from grass samples spiked with 0.1 ppm was 97.4%.

Table 3.–Biological activity of TH6040 against *Culex* larvae and pupae (in % inhibition of adult emergence).

Species	C. p. quinquefasciatus[a]						C. tarsalis[b]		
Stage(s)[c]	M-L-4	E-M-4	3	2	1	P	M-L-4	E-M-4	L-3,E-4
No. tests[d]	3	3	1	2	1	2	1	3	2
Conc. (ppm)									
.0001	0	0	0	0	0		0	0	4
.0004	0	6	16	0	0		10	3	18
.001	56	92	92	23	92	4	55	33	90
.004	98	100	100	100	100	4	100	93	100
.01	100	100	100	100	100	8	100	100	100
.04	100	100	100	100	100	27	100	100	100
.1	100	100	100	100	100	80	100		100
.4						81			
1.0						90			

[a] Laboratory strain. OP-susceptible.

[b] Laboratory strain. OP-resistant.

[c] E = early; M = medium; L = late; E-M = mixture early to late; the number refers to larval instar; P = pupae.

[d] In each test, each concentration was run in duplicate.

Table 4.–Biological activity of TH6040 against *Aedes* larvae and pupae (in % inhibition of adult emergence).

Species	A. melanimon	A. nigromaculis			A. taeniorhynchus					
Strain	OP-S	OP-R			OP-S					
Stage(s)[a]	L-4	M-L 4	E-4	L-3 E-4	E-M 4	E-4	L-3 E-4	2	1	P
No. tests[b]	1	1	1	2	2	1	1	1	1	2
Conc. (ppm)										
.0001	0	0	0	8	0	0	0	0	0	
.0004	0	0	18	45	0	0	0	0	0	
.001	45	31	92	89	0	0	0	20	57	0
.004	64	63	100	100	86	92	91	100	100	-
.01	100	100	100	100	96	100	100	100	100	0
.04	100	100	100	100	100	100	100	100	100	5
.1	100	100	100	100	100		100	100	100	36
.4										77
1.0										85

[a] E = early; M = medium; L = late; E-M = mixture early to medium; the number refers to the larval instar; P = pupae.

[b] In each test, each concentration was run in duplicate.

4th-stage larvae. As previously mentioned, mortality occurs during a molt, in the pupal stage at lower concentrations, and in the partial emergence of abnormal adults at still lower concentrations. The results against *Aedes* species are shown in Table 4. Larvae of the black salt marsh mosquito, *Aedes taeniorhynchus* (Weideman), are slightly less susceptible than *A. melanimon* or *A. nigromaculis*, but a degree of activity is apparent against all. Thus, the laboratory data show that TH6040 has a high degree of biological activity against the most economically important species of *Aedes* and *Culex* in California.

Field tests with TH6040 on .059 acre pasture plots against natural populations of *C. tarsalis* are summarized in Table 5; rates of .025 and below were used in these hand-treated plots, but these were too low to give sufficient residual activity. Bioassay of water from the treated plots shows that by 24 hours post-treatment the TH6040 content of the field water had greatly declined, and no activity was present at 48 hours.

Table 6 summarizes aircraft trials with TH6040 against natural populations of *A. nigromaculis*, *A. melanimon* and

Table 5.–Summary of .059 acre pasture tests with TH-6040 against natural populations of *Culex tarsalis* (all stages present).

Field test no.	Date (1973)	Dose (lb AI/acre)	Days control (98% +)	Bioassay of water at collection time 0	24	48	72
73-15	7-3	.025	3	100	0	-	-
73-17	7-10	.01	3	100	0	0	-
73-22	7-25	.005	2	100	31		
73-31	8-20	.0025	0	-	10	0	

Table 6.–Summary of aircraft trials with TH6040.

Field test no.	Date (1973)	Dose (lb AI/acre)	Stages[a] treated	Acres	Days control (98% +)	Final mortality of Aedes by stage treated (%) 2	3	4
73-3[b]	4-27	.1	C.t. 1,2,3,4	3	10			
73-8	6-13	.025	C.t. 1,2,3,4	15	2			
73-8	6-13	.025	A.m. 2,3,4	15		20	20	20
73-16	7-6	.025	C.t. 1,2,3,4	15	1			
73-24	8-10	.03	A.n. 2,3,4	32	-	100	100	100
73-26	8-15	.02	A.n. 3,4	13	-		70	100

[a] *C. t. = Culex tarsalis; A. m. = Aedes melanimon; A. n. = Aedes nigromaculis;* the numbers refer to larval stages present.

[b] 0.4172 lb AI/gallon EC was used in this test; in all other tests a 25% WP was used.

Table 7.–Biological activity of H24108 against mosquito larvae in the laboratory (in % inhibition of adult emergence).

Species	C. p. quinquefasciatus			A. nigromaculis
Stage(s)[a]	2	E-4	L-4	E-4
No. tests[b]	1	2	2	1
Conc. (ppm)				
.001	0			
.004	0	0		0
.01	0	0	0	0
.04	71	28	0	13
.1	100	100	59	100
.4	100	100	93	100
1.0	100	100	100	100

[a] E = early; L = late; the number refers to the larval instar.

[b] In each test, each concentration was run in duplicate.

C. tarsalis. These data further substantiate that 0.25 lb AI/acre is too low a rate against field populations of *Aedes* or *Culex* species. A rate of 0.1 lb AI/acre gave excellent results but was devastating to beneficial, nontarget organisms (Miura and Takahashi 1974, unpublished data). It appears that a rate of .03 to .05 lb AI/acre would allow for operationally feasible results; further tests for the latter rates are planned.

H24108--The biological activity of H24108 against larvae of *A. nigromaculis* and the southern house mosquito, *Culex pipiens quinquefasciatus* Say, is shown in Table 7. In comparison to the other compounds discussed, H24108 is less active; however, as its structural type is different and until its cost-performance ratio is established, it deserves further attention. Early 4th-stage larvae are more susceptible than late 4th-stage larvae in contrast to the results of tests using compounds having the juvenile hormone-type structure. As mentioned previously, mortality occurs in the pupal stage.

A .059 acre pasture plot containing large numbers of *C. tarsalis* larvae was treated with 0.2 lb AI/acre H24108. On the second and third days following treatment, adult emergence from the treated pond was about 50% below the control; after that time, emergence from the treated pond was normal. It is possible that higher rates would provide feasible results. Further trials are planned.

In summary, one juvenile hormone-type compound (Altosid) has progressed from the synthesis level to commercial production. Other compounds having similar biological effects but no structural similarity are also known. The mode of action of juvenile hormone-type compounds is not known, but they may act through preventing metabolism of the natural hormone rather than producing direct effects themselves (Slade and Wilderson 1973). Further evidence to support the latter possibility was recently presented by Brooks (1973) who showed that juvenile hormone analogs inhibit insect epoxide hydrase, an enzyme that may be responsible for the metabolism of natural juvenile hormone.

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INSECT DEVELOPMENT INHIBITORS: FORMULATION RESEARCH ON ALTOSID®

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ABSTRACT

A 1 m^2 "disposable pond" was developed for testing chemical control agents and for research on formulations. When water is treated with Altosid®(isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate) formulations, the active ingredient tends to "plate-out" onto the sides and bottoms of ponds and is available to mosquito species which browse-feed, but appears to be largely unavailable to filter feeders. By formulating Altosid in large diameter (100μ), microencapsulated particles, the "plating-out" process occurs at an increased rate. A formulation of Altosid on charcoal-base, protected particles (Altosid 515225) causes more of the active ingredient to remain in the upper water layers and greatly increases the biological activity against *Culex tarsalis* Coquillett. The Altosid 515225 formulation does not cause significant residue problems in water or on vegetation following field application, and no undesirable side effect on nontarget organisms was observed. This study demonstrates that further formulation research on Altosid may allow the development of improved formulations without simultaneously producing undesirable effects.

INTRODUCTION. The importance of properly formulating insecticides is exemplified by Altosid® (isopropyl 11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate). Techical Altosid has high biological activity against 4th stage *Aedes nigromaculis* (Ludlow) larvae at concentrations as low as 0.00001 ppm in the laboratory; however, since the late 4th-stage larva is the sensitive stage, the technical material must be present at the appropriate time (Schaefer and Wilder 1972). In field tests EC formulations were effective at rates down to 0.0125 lb AI/acre when 4th-stage larvae were treated, but earlier stage larvae were not affected. The half-life of technical material or the EC formulation of Altosid was only about 2 hr under field conditions (Schaefer and Dupras 1973). Under field conditions there is very limited residual activity of Altosid following application of either technical material or EC; therefore, operational use is limited since field populations develop asynchronously. Numerous test formulations of Altosid were evaluated during 1972 with the hope of improving residual biological activity. One formulation (Altosid SR-10) was found that offered several days of residual activity against *A. nigromaculis* under field conditions; this formulation has allowed the commercial development of Altosid. Chemical analysis of water from treated fields, however, showed a rapid decline in Altosid concentration, and by 24 hr only trace amounts could be found (Schaefer and Dupras 1973). In addition, the SR-10 formulation was not effective against field populations of *Culex tarsalis* Coquillett at doses up to 0.2 lb AI/acre, but doses as low as 0.020 lb AI/acre gave good results against mixed-stage larvae of *A. nigromaculis* and *Aedes melanimon* Dyar (Schaefer and Wilder 1973; Schaefer et al. 1974).

This report includes results of 1973 studies on: (1) the distribution of Altosid SR-10 in water with time, (2) an evaluation of other formulations of Altosid that might be effective at still lower levels and also against *C. tarsalis*, and (3) the distribution of residues of promising formulations and possible harmful effects on nontarget aquatic organisms.

MATERIALS AND METHODS.–Among those formulations of Altosid studied during 1973, the following are of sufficient interest to be discussed here: (1) Altosid SR-10--a liquid suspension of polymer-Altosid particles with an average diameter of one micron. The technical Altosid is encapsulated in an amide, polymeric matrix with the final product being 10% AI by weight or 0.8579 lb AI/gal., (2) Altosid 0127-145B--the same formulation as Altosid SR-10 but with an average particle diameter of 100 microns, (3) Altosid 515225--a charcoal-base material of wide particle size range, inpregnated with 20% Altosid, coated with a UV screen and an antioxidant and then suspended in a water system containing a polymeric material.

Tests on 1 m^2 Ponds at Fresno--A series of "disposable ponds" was constructed to allow evaluation of new formulations, under comparable outdoor conditions, that rarely occur on larger field plots, e.g., due to differences in the rate of water loss, scum formation, etc. These were built according to the diagram in Fig. 1A; excavations were lined with 2 or 3 layers of clear polyethylene sheeting and the bottoms and sides were covered with commercial nursery sod. (Note: Care must be taken to determine prior insecticide treatments of the sod.) Each pond was filled with either 60 or 70 gallons of water and a calibrated depth gauge was marked. The following day the pond level was raised back to the calibrated level and treatment was made. Dosages were applied which, based on laboratory data, were projected to provide adequate results under field conditions. Using this system water samples can be collected before and at predetermined intervals after treatment for chemical and/or bioassay in the laboratory. Also, larvae from laboratory colonies can be added to the ponds daily and adult emergence can be monitored daily by placing a screen-cage cover over each pond (Fig. 1B). Even in very hot weather, the water in each pond remained long enough to collect samples for 5 or 6 days. At the conclusions of a test, any remaining water was pumped out, whence a sample of the sod could be removed for residue analysis; the "used sod" was then removed and discarded. The upper polyethylene sheet was replaced, new sod added, and the pond refilled in preparation for another test.

Chemical Analysis of Altosid--Duplicate water samples of 600 ml each were always collected. Analyses of water samples were done as previously described (Schaefer and Dupras 1973). Samples from the pond top areas were collected from the upper 2 in. of the water surface with white, enamel dippers and then poured into graduate cylinders (for chemical analyses) and/or into Pyrex® storage dishes for bioassay (Schaefer and Wilder 1972). Samples from the

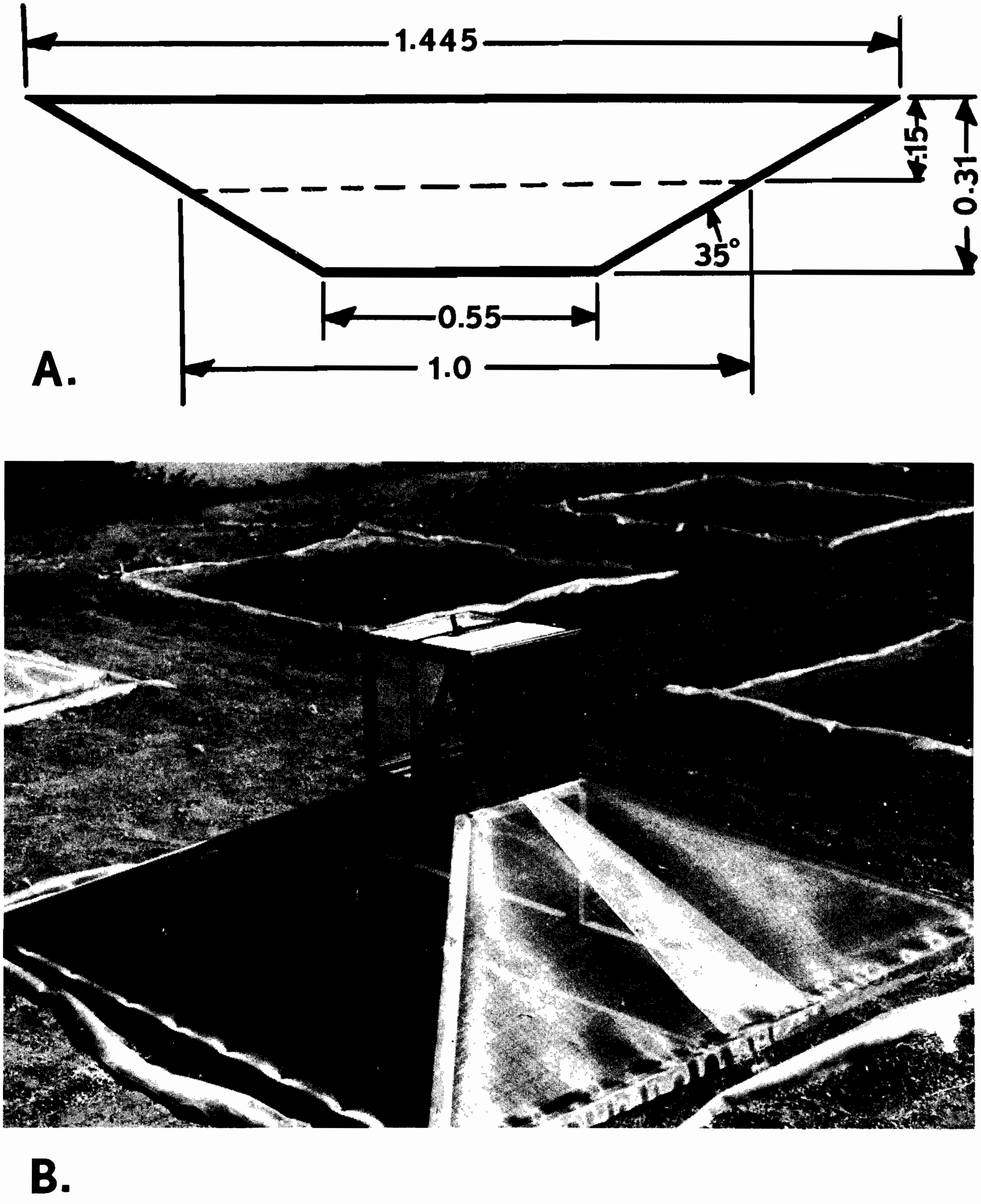

Figure 1.– A.--Diagram showing dimensions of a 1 m^2 pond (in meters).
B.--Photograph of 1 m^2 ponds, also showing screened cage covers.

pond bottoms (and in one case from the sides) were collected by aspiration through the equalizing arm of a cylindrical separatory funnel (SGA Scientific, Inc., No. JF8234) and then drained into the same containers as described above. Analyses of soil and vegetation were as described by Miller et al. (1974).

Distribution of Altosid in Water with Time--A 1 m^2 pond treated with 0.2 ppm of Altosid SR-10; at 2 hr, 24 hr, and 24 hr intervals thereafter for 8 days. Water samples for chemical and bioassay were collected from the top, bottom, and sides of the treated pond, and from a control. At the end of the 8-day test period, a sample of the sod was extracted and analyzed for Altosid residue.

Effect of Particle Size on the Distribution of Altosid--Since the SR-10 formulation is composed of quite small particles (average diameter of 1 micron), it was of interest to study the distribution of a formulation that had an average particle size of 100 microns (Altosid 0127-145B). One 1 m^2 pond was treated with 0.2 ppm of Altosid 0127-145B and another with the SR-10 formulation. Daily water samples for chemical and bioassay were collected as described earlier from the top and bottom of each pond. Samples from the sides were not taken as they proved to be the same as for the bottom in the test described above.

Tests with Altosid 515225. Distribution in Water with Time--The first test with this charcoal-base formulation was conducted in the 1 m^2 ponds. One pond was treated with 0.1 ppm Altosid 515225 and another with the same amount of the SR-10 formulation. Both top and bottom water samples were collected daily for both chemical and bioassay. At the end of the experiment, samples of the sod from each pond were collected and analyzed for Altosid.

Outdoor Bioassay of Altosid 515225--In order to eliminate holding samples of the treated water within the confines of the laboratory during the bioassay, a different type of test was devised. A 1 m^2 pond was treated with 0.2 ppm Altosid 515225 on 8/29/73. On this date and each day thereafter, 400 4th-stage southern house mosquito larvae, *Culex pipiens quinquefasciatus* Say, were added to the treated pond and to a control pond. The ponds were covered with screen cages, and each day the number of successfully emerging adults was estimated. (Counting was rather difficult and when over 30 adults were present, the counts were approximations.) Also, on each day 20 pupae were collected from each pond and then held in the laboratory to determine the percentage of successful adult emergence. On 9/11/73 the above test was repeated, but daily additions of larvae to the ponds became unnecessary after a few days as the ponds became infested by natural populations of the same species. In this second test no screen cages were placed over the ponds, and 20 pupae from each pond were collected daily and held in the laboratory to determine adult emergence.

Field Tests with Altosid 515225 on 0.059 Acre Pasture Plots--Each of these plots is 110′ long and 23′ wide and is located 15 miles south of Bakersfield, California. Treatments were made with a hand sprayer, applying preweighed samples of Altosid 515225 in a total of 1.5 gallons of water per plot. Rates of 0.025 to 0.0071 lb AI/acre were evaluated.

Aircraft Trials with Altosid 515225--Two aircraft trials were conducted using Altosid 515225 against mixed population of *A. nigromaculis* and *A. melanimon.* In the first test a dose of 0.015 lb AI/acre was applied on a pasture containing 2nd-, 3rd-, and 4th-stage larvae; in the second test 3rd- and 4th-stage larvae were treated with 0.010 lb AI/acre. In both tests the specified rate was applied in a total of 1 gal water/acre. In order to determine the residual of Altosid 515225 on vegetation, samples of pasture grass from the second test above (0.01 lb AI/acre) were collected immediately before and after treatment, and at 1, 3, 5, 7, and 9 days following treatment. The samples were held at -20°C, or below, until analysis. Duplicate 100 gm aliquots from each sampling time were extracted and analyzed. Also, four 600 ml water samples were collected for chemical analysis from the same field immediately before treatment and at 1, 24, and 48 hr following treatment.

Effects of Altosid 515225 on Nontarget Aquatic Organisms--As the distribution of Altosid 515225 in water was found to differ greatly from that of the SR-10 formulation, and as it also has greater field activity against *C. tarsalis,* it was conceivable that it might not have the same high degree of safety to aquatic nontarget organisms as previously demonstrated for Altosid SR-10 (Miura and Takahashi 1973). Therefore, in field tests conducted with Altosid 515225, aquatic nontarget organisms were observed for possible side effects. Also, 23′ by 52′ ponds containing large numbers of aquatic nontarget organisms were treated with relatively high doses (0.1 and 0.2 lb AI/acre) of Altosid 515225. Methods for sampling nontarget organisms were as previously described (Miura and Takahashi 1973, 1974).

RESULTS AND DISCUSSION.–Distribution of Altosid in Water with Time--The distribution of Altosid following treatment of a 1 m^2 pond is shown in Table 1. It is apparent from both the bioassay and from the chemical data that Altosid settles or "plates-out" along the sides and bottom; e.g., at 168 hr there was ten times as much in samples from the sides or bottom as from the top. We believe that this explains the difference in biological activity of Altosid against different species of mosquitoes. The mosquito larvae, such as *A. nigromaculis,* which were observed to browse-feed on debris, are highly sensitive, but *C. tarsalis,* which has been shown to be a filter feeder (Pucat 1965) is much less affected by field applications of Altosid (Schaefer and Wilder 1972). Thus, a search was begun to find a formulation that would allow a suspension of "protected" Altosid to be picked up by filter feeding larvae.

Effect of Particle Size on the Distribution of Altosid--Table 2 shows the distribution of Altosid 0127-145B and SR-10 in water following treatment with 0.2 ppm. It is apparent that the larger particle formulation "plates-out" more quickly as confirmed by both chemical and bioassay. The residue of Altosid in the sod at the completion of the test was twice as great with the 0127-145B formulation as compared to the SR-10. Thus, the larger particle size is inferior to the SR-10 formulation with respect to suspensibility and residue.

Distribution of Altosid 515225 in Water with Time--The amounts of Altosid remaining in the top water were much greater with the 515225 than for the SR-10 formulation by

Table 1.–Assay of Altosid® from water collected from the top, bottom, and sides (at water level) of a 1 m^2 pond treated with 0.2 ppm of the SR-10 formulation on July 3, 1973.

Sampling place	Sample collection time (hours post-treatment) 2	24	48	72	96	120	144	168	192	216
					Chemical assay (ppm)[a/]					
top	0.135	0.0106	0.0025	0.0009	0.0007	0.0002	0.0002	0.0002	0.0003	<0.0001
side	.086	.010	.0030	.0016	.0016	.0010	.0010	.0022	.0012	.0014
bottom	.069	.0089	.0024	.0020	.0010	.0011	.0011	.0024	.0009	.0013
					Bioassay (% inhibition of adult emergence)					
top	100	96	82	88	78	56	36	14	0	—[b/]
side	100	100	100	100	98	84	93	82	82	—[b/]
bottom	100	100	100	100	100	80	98	80	74	92

[a] After the pond dried up, a sample of the hybrid bermuda sod (composite of soil plus vegetation) was analyzed and contained 0.0012 ppm Altosid.

[b] Only shallow water on the bottom remained.

Table 2.–Assay of Altosid® from water collected from the tops and bottoms of 1 m^2 ponds treated with SR-10 and 0127-145B formulations (each treated with 0.2 ppm on July 23, 1973).

Sampling place	Sample collection time (hours post-treatment) 2	24	48	72	96	120	144	168	192	216
					Altosid 0127-145B[a/]					
					Chemical assay (ppm)					
top	0.41	0.0037	0.0009	0.0003	0.0004	0.0002	0.0001	0.0001	<0.0001	<0.0001
bottom	.16	.0040	.0007	.0010	.0015	.0007	.0005	.0002	.0003	.0003
					Bioassay (% inhibition of adult emergence)					
top	100	100	50	24	28	9	17	7	0	10
bottom	100	96	73	96	91	74	80	67	41	17
					Altosid SR-10[b/]					
					Chemical assay (ppm)					
top	.13	.011	.0014	.0008	.0010	.0002	.0002	.0001	<.0001	<.0001
bottom	.068	.0025	.0012	.0011	.0004	.0005	.0006	.0004	.0003	.0005
					Bioassay (% inhibition of adult emergence)					
top	100	100	84	85	85	48	59	9	15	0
bottom	100	100	86	98	93	80	68	71	48	13

[a] After the pond dried up, a sample of the hybrid bermuda sod (composite of soil plus vegetation) was analyzed and contained 0.32 ppm Altosid.

[b] After the pond dried up, a sample of the hybrid bermuda sod (composite of soil plus vegetation) was analyzed and contained 0.14 ppm Altosid.

Table 3.–Assay of Altosid® from water collected from the tops and bottoms of 1 m^2 ponds treated with SR-10 and 515225 formulations (each treated with 0.2 ppm on August 20, 1973).

Sampling place	Sample collection time (hours post-treatment) 2	24	48	72	96	120	144	168	192	216	240	264
						Altosid 515225[a/]						
						Chemical assay (ppm)						
top	0.48	0.074	0.013	0.0032	0.0014	0.0022	0.0022	0.0044	0.0031	0.0039	0.0026	0.0017
bottom	.18	.016	.0027	.0011	.0009	.0007	.0006	.0010	.0009	.0022	.0011	.0028
						Bioassay (% inhibition of adult emergence)						
top	100	100	100	84	96	86	80	54	40	36	48	32
bottom	100	100	100	90	100	85	86	73	73	65	74	88
						Altosid SR-10[b/]						
						Chemical assay (ppm)						
top	.23	.041	.0026	.0010	.0002	.0003	.0005	.0004	.0006	.0005	.0005	.0005
bottom	.080	.011	.0029	.0027	.0010	.0016	.0024	.0016	.0018	.0033	.0016	.0019
						Bioassay (% inhibition of adult emergence)						
top	100	100	98	88	84	82	39	44	52	71	32	48
bottom	100	100	100	94	98	90	100	88	89	88	86	88

[a] After the pond dried up, a sample of the hybrid bermuda sod (composite of soil plus vegetation) was analyzed and contained 0.20 ppm Altosid.

[b] After the pond dried up, a sample of the hybrid bermuda sod (composite of soil plus vegetation) was analyzed and contained 0.20 ppm Altosid.

Table 4.–Development of *Culex pipiens quinquefasciatus* in an untreated 1 m^2 pond and one treated with 0.2 ppm of Altosid 515225 on August 29, 1973.

Date (1973)	Control pond No. adults emerging in outside cage	Control pond % final mortality of pupal collection[a/]	Treated pond (0.2 ppm Altosid 515225) No. adults emerging in outside cage	Treated pond (0.2 ppm Altosid 515225) % final mortality of pupal collection[a/]
8/30	0	4	0	98
8/31	0	0	0	100
9/1	18	0	0	100
9/2	4	0	1	100
9/3	14	5	4	100
9/4	∿ 50	5	3	100
9/5	∿ 50	5	3	100
9/6	∿ 100	5	5	100
9/7	∿ 50	5	4	100
9/8	∿ 200	-	2	-
9/9	∿ 75	10	16	78
9/10	∿ 100	15	25	76
9/11	∿ 100	0	75	70
9/12	∿ 50	0	20	40
9/13	∿ 200	0	300	45
9/14	∿ 300	0	300	35

[a] Includes dead pupae and abnormal adults.

chemical analysis (Table 3); however, during the last five days of the experiment there was little difference in the bioassay data of top water from either formulation. This indicates that, while the amount of Altosid in the top pond water was higher with the 515225 formulation as shown by chemical analysis, the amount available to mosquitoes (not tightly adsorbed onto the charcoal particles) may not have been greater then for the SR-10 formulation. The latter may not be a valid conclusion, however, since there is an unlinear response between concentration and % final mortality in this type of bioassay (Schaefer and Wilder 1972). By the end of the experiment, the amount of Altosid in the sod from the two treated ponds was the same.

Table 5.–Summary of 0.059 acre pasture tests with Altosid 515225 against natural, mixed populations of *Aedes nigromaculis* and *Aedes melanimon.*

Field test no.	Date (1973)	Dose lb AI/acre	Larval stages treated	Final mortality of field population by stage treated (%)		
				2	3	4
73-33a[a]	8/30	0.025	3,4		100	100
73-33b	8/30	.017	3,4		100	100
73-33c	8/30	.0125	3,4		99(+)	100
73-36a	9/6	.0125	2,3	95	98	
73-36b	9/6	.010	2,3	90	95	
73-36c	9/6	.0083	2,3	90	95	
73-36d	9/6	.0071	2,3	—[b]	95	

[a] In test 73-33 the population was 80% *Aedes melanimon* and 20% *Aedes nigromaculis.*

[b] Plot dried up before larvae that were 2nd-instar at the time of treatment had pupated.

Table 6.–Summary of 1973 aircraft trials with Altosid 515225 against mixed populations of *Aedes nigromaculis* and *Aedes melanimon.*[a]

Field test no.	Date (1973)	Dose lb AI/acre	No. acres	Larval stages treated	Final mortality of field population by stage treated (%)		
					2	3	4
73-35	9/5	0.0125	50	2,3,4	96	96	99
73-41	9/16	.0100	50	3,4		90	95

[a] *Aedes melanimon* were 20% or less of the total population.

Table 7.–Residues of Altosid® on pasture grasses following aerial application of 0.01 lb AI/acre of 515225 on September 16, 1973, in Stanislaus County, California.[a]

Sample collection time	Moisture content (% water)	Aliquot #1 (ppm)	Aliquot #2 (ppm)	Average (ppm)
1-hour (pre)	69.1	N.D.[b]	N.D.	N.D.
1-hour (post)	62.7	0.12	0.13	0.13
1-day	51.6	.040	.034	.037
3-days	58.6	.021	.016	.019
5-days	51.3	N.D.	N.D.	N.D.
7-days	58.0	N.D.	N.D.	N.D.
9-days	52.7	N.D.	N.D.	N.D.

[a] Water residue taken pre and 1, 24, and 48 hr post-treatment (four 600 ml samples each) showed 0.0035 ppm at 1 hr after treatment and nondetectable at other sampling times (detection limit = 0.0005 ppm).

[b] Detection limit = 0.001 ppm. ppm.

Outdoor Bioassay of Altosid 515225--Table 4 shows the numbers of adults emerging daily from the treated and control ponds as well as the final mortalities of pupal samples that were collected daily and then held in the laboratory. It is apparent that the Altosid 515225 treatment provided about 10 days of satisfactory control. However, the possibility existed that the screen-cage covers may have reduced the intensity of natural UV light and thereby protected the Altosid 515225 formulation. Therefore, in the second test initiated on 9/11/73, no screen covers were used. Cool weather prevailed during the second test period, but there was a final mortality of 100% for all pupal samples collected from the treated pond for a 20-day period. Emergence of adults from pupae collected from the control pond was normal.

Field Tests with Altosid 515225 on 0.059 Acre Pasture Plots--The results against *Aedes* species are shown in Table 5. Rates of 0.0125 to 0.025 lb Al/acre gave good control when 3rd or 4th-stage larvae were treated. At lower rates (0.0071 to 0.010 lb Al/acre) there was little apparent difference between doses, which was surprising. A dose of 0.0125 lb AI/acre gave good results following treatment of 2nd - 3rd-stage larvae. One plot containing large numbers of all stages of *C. tarsalis* was treated with 0.1 lb AI/acre Altosid 515225 and there was no successful emergence of adults for 72 hr, after which time insufficient water remained to allow evaluations. The latter result was very encouraging, as previous work with the SR-10 formulation with rates up to 0.2 lb Al/acre proved inadequate for controlling *C. tarsalis* (Schaefer and Wilder 1973).

Aircraft Trials with Altosid 515225--In order to premix the Altosid 515225 formulation to insure good dispersal in the aircraft tank, it was necessary to vigorously shake batches of about 225 grams each in a gallon jar containing 0.6 to 0.75 gal water each. The batches were then poured into 50 gal of water that had previously been placed in the tank, and finally water was added to achieve the correct final concentration. Such a procedure cannot be considered as operationally feasible but did allow for aircraft applications of this formulation. Results against the field populations are shown in Table 6; the 0.0125 lb AI/acre treatment gave excellent results against 2nd-, 3rd-, and 4th-stage larvae. These results are better than those obtained with the SR-10 formulation at this dose range (Schaefer et al. 1974). A dose of 0.01 lb Al/acre against 3rd- and 4th-stage larvae was not as promising.

Residues of Altosid in water and on vegetation, following treatment with the 515225 formulation at 0.01 lb AI/acre by aircraft, were very limited and of short duration (Table 7). Thus, the 515225 formulation does not appear to cause any significant residues.

Effects of Altosid 515225 on Nontarget Aquatic Organisms -- In tests with Altosid 515225 on pastures (Tables 5 and 6), no toxic effects on nontarget organisms associated with pasture habitats were observed. In pond tests where rates of 0.1 and 0.2 lb AI/acre were applied, the following nontarget organisms were observed for effects of the treatments. Rotatoria (*Asplanchna* sp.); Crustacea, Cladocera (*Daphnia* and *Moina* spp.); Eucopepoda (*Cyclops* and *Diatomus* spp.); Conchostraca (*Eulimnadia* sp.); and Podocopa (*Cypricerus* sp.); Insecta, Ephemeroptera (*Callibaetis* sp.); Diptera (*Goeldichironomus holoprasinus* Goldi) and (*Chironomus stigmaterus* Say), Coleoptera (*Tropisternus lateralis* (F.) and *Hydrophilus triangularis* Say). No effect on any of these organisms was observed with the exception of the Chironomidae; emergence of both species of midges was slightly reduced. Midges are listed in this paper as nontargets because the treatments were specifically directed against mosquitoes. As midges are a pest problem, the effects of the treatment against them cannot be considered as deleterious.

Both *Physa* and *Lymnaea* snails and *Pardosa* spiders were frequently found in the habitats where tests were conducted; no apparent toxic effect of Altosid 515225 was observed on these animals. Thus, Altosid 515225 appears to be very safe with respect to nontarget organisms at the rates evaluated.

While the Altosid 515225 formulation does not have the physical characteristics which would allow operational use, tests with it demonstrate the potential of improved formulations of Altosid that could allow lower use rates as well as improved performance against filter feeding species of mosquitoes.

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INSECT DEVELOPMENT INHIBITORS: BIOLOGICAL ACTIVITY OF RE17656, RE17937 and RE18286 AGAINST MOSQUITOES (DIPTERA: CULICIDAE) AND NONTARGET ORGANISMS

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ABSTRACT

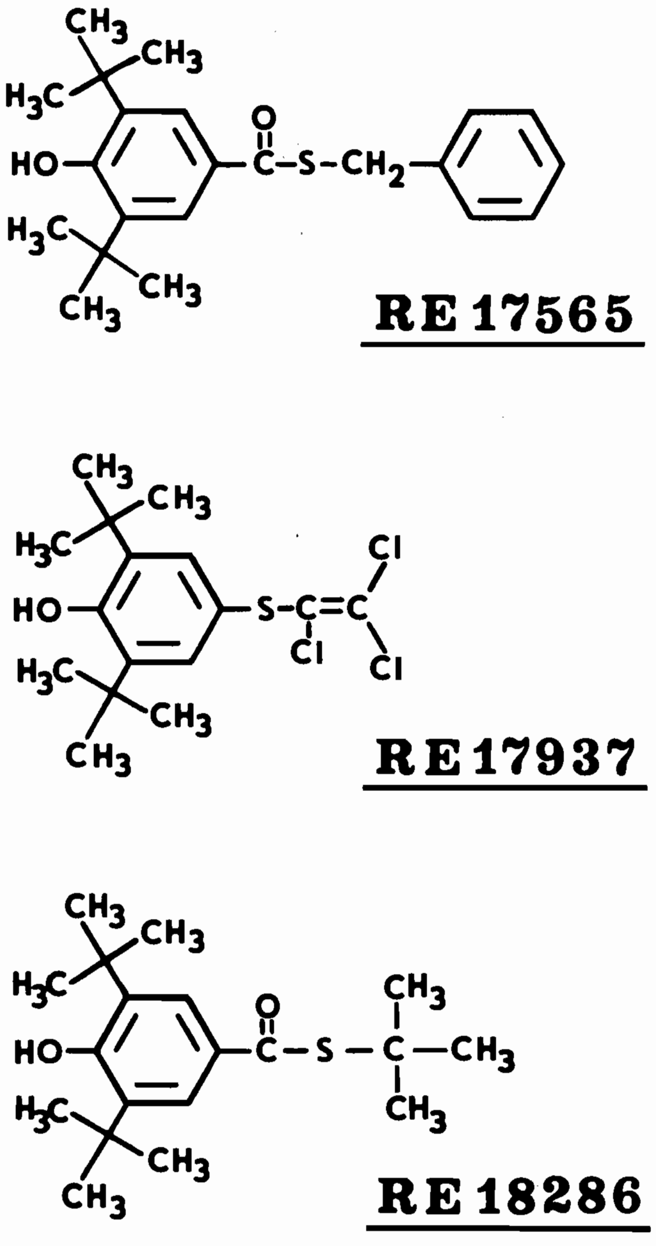
RE17565, RE17937 and RE18286 are new *tert*-butyl substitute phenol compounds, related to the structure of MON585. They offer promising activity against economically important species of California mosquitoes. When sensitive larvae (early to medium aged, 4th-stage) are exposed, there is no direct toxicity but mortality occurs during the prepupal and pupal stages. Affected pupae do not pigment but die as albinos. At the doses which are effective against mosquitoes, no deleterious side effects were observed against microcrustaceans, important predatory insects or mosquito fish.

INTRODUCTION.–MON585 [2, 6-di-*tert*-butyl-4-(α,α dimethylbenzyl) phenol] does not cause direct intoxication of mosquito larvae, but when 4th-stage larvae are exposed to effective concentrations mortality occurs in the pupal stage. Affected pupae do not melanize but die unpigmented (Sacher 1971). In the first paper of this series (Schaefer and Wilder 1972), the biological activity of MON-585 against several species of mosquitoes was described. In the laboratory, treatment of *Aedes nigromaculis* (Ludlow) larvae at 0.1 ppm resulted in pupal mortality of 90-95%; in field tests [against the same species] excellent control was obtained when 1.5 or 2.0 lb AI/acre were applied by helicopter. While MON585 has not been advanced to commercial development, its unique type of biological activity remains of interest, particularly it's effectiveness against organophosphorus-resistant strains of mosquitoes. Recently we have evaluated three new *tert*-butyl substituted phenols that exhibit similar biological activity. This report summarizes 1973 laboratory and field research on these compounds.

MATERIALS AND METHODS. The methods for laboratory and field evaluation of developmental inhibitors against mosquitoes were the same as previously described (Schaefer and Wilder 1972, 1973) except where noted otherwise. Methods for laboratory and field evaluation of side effects on nontarget, aquatic organisms were as described previously (Miura and Takahashi 1973, 1974) except as noted otherwise. Each field test has been assigned a Field Test Number, and these are referred to as F.T.Nos. in the text. Since it is impractical to include all the details recorded for each test, e.g., temperature, wind, skycover, water depth, etc., this information will be kept on file and is available to interested persons.

The structures of the three new *t*-butyl substituted phenols, RE17565, RE17937 and RE18286, are given in Fig. 1.

Field Studies.–Tests on 1 m^2 Ponds at Fresno--Each pond contained 70 gallons of water and was sprayed with a predetermined amount of the test compound in 25 ml of water. Water samples were collected daily and bioassayed by adding 20 early 4th-stage larvae of *Culex pipiens quinquefasciatus* Say. The daily water samples were taken from the upper 2-3 inches except in one experiment where additional samples were also pipetted from the bottom of the pond.



RE 17565

RE 17937

RE 18286

Figure 1.–Structures of RE17565, RE17937 and RE-18286.

Tests on 0.059 Acre Pasture Plots--These plots are 110′ long and 23′ wide and are located 15 miles south of Bakersfield. Treatments were made with a hand sprayer, applying RE17937-4E or RE17565-50 WP in 1.5 gallons of water per plot. The water was 10-12 inches deep at the time of application and the vegetation was predominantly bermuda grass. After pupation had begun in the field population, daily samples of larvae and pupae (several hundred in each) were collected and held in the laboratory in order to monitor adult emergence. Also, 1 ft^3 screen cages were placed over pupae in the field plots and the daily emergence of adults was monitored. After the ponds were irrigated, eggs of *A. nigromaculis* and *Aedes melanimon* Dyar hatched quickly; the species composition varied between tests but an 80:20 ratio, respectively, was typical. Such natural mixtures of these two species are typical on irrigated pastures in Central California. When tests with *Culex tarsalis* Coquillett were desired, the *Aedes* were not treated and within a few days the ponds were infested with *Culex* egg rafts. Pond levels were raised the day before treatment to a 10-12 inch depth so that sufficient water would be present to allow completion of the test.

Aircraft Trials.–Only RE17565 was available in sufficient quantity to conduct small-scale aircraft tests. Only lower doses (0.1 and 0.2 lb AI/acre) were tested because of the limited amount of 50 WP available. The first test (F. T. No. 73-23) was run on July 9, 1973. A 12-acre pasture containing 3rd- and 4th-stage larvae of *A. nigromaculis* was sprayed with 0.1 lb AI/acre in one gallon of water (Kings County). A second test (F. T. No. 73-44) was conducted on September 27, 1973 (Kern County); an eight-acre duck club pond containing all stages of *C. tarsalis* larvae was treated with 0.2 lb AI/acre in one gallon of water.

Effects on Nontarget, Aquatic Organisms.–The following nontarget aquatic organisms, which frequently coexist in mosquito breeding habitats, were studied with respect to potential deleterious effects from RE17565 and RE17937; Rotifera (rotifers)- *Asplanchna* spp.; Cladocera (water fleas) --*Daphnia* spp., Moina spp.; Eucopepoda (copepods)--*Cyclops* spp., *Diatomus* spp.; Podocopa (seed shrimp)--*Cypricerus* spp.; Conchostraca (clam shrimp)--*Eulimnadia* spp.; Hemiptera (water boatmen)--*Corisella* spp.; (backswimmers) --*Notonecta unifasciatus* Guerin; Ephemeroptera (mayflies) --*Callibaetis* spp.; Odonata (dragonflies)--*Orthemis* spp., *Pantala* spp.; Coleoptera (diving beetles)--*Thermonectus basillaris* (Harris), *Acilius* spp., *Laccophilus* spp., (water scavenger beetles)--*Hydrophilus triangularis* Say, *Tropisternus lateralis* (F.), *Helophorus* spp., *Enochrus* spp.; Diptera (midges)--*Chironomus attenuatus* Walker, *Chironomus stigmaterus* Say, *Goeldichironomus holoprasinus* (Goeldi); Cyprinodontiformes (mosquito fish)--*Gambusia affinis* (Baird and Girard); Volvocaceae (flagellates)--*Eudonna* spp., *Volvox* spp. Preliminary toxicities against many of these organisms were determined by treating populations in small containers with acetone solutions of the technical materials. Microcrustacea maintained outdoors in 5-gallon aquaria were treated with RE17565, and the population density measured for 23 days following treatment. In field tests on mosquito breeding habitats, frequent observations and counts of existing nontarget organisms were made before and following treatment.

RESULTS.–Laboratory Studies--The biological activities of the three compounds in the laboratory against *C. p. quinquefasciatus*, *C. tarsalis* and *A. nigromaculis* are shown in Tables 1-3, respectively. The data show that all three compounds are most active when early to medium aged, 4th-stage larvae are treated. Mortality occurs in the prepupal and pupal stages and melanization does not occur. Pupae that successfully pigment are not affected by the treatment. On *C. tarsalis* the medium-to-late 4th-stage larvae are fairly insensitive to all three compounds (Table 2), and earlier stage larvae show equal sensitivity. RE17565 appears to be as effective against OP-resistant larvae as for susceptible ones (Table 2). There is little difference between the biological activities of RE17565 and RE18286 against *A. nigromaculis* larvae (Table 3), but RE17937 shows lesser activity.

Field Studies.–Tests on 1 m^2 Ponds--The results are given in Table 4. It is readily apparent that RE17565 was the most active compound, and the test of August 30, 1973 demonstrates that it gradually "plates-out" of the water.

Table 1.–Biological activity against *Culex pipiens quinquefasciatus*[a] 4th-stage larvae (in % inhibition of adult emergence).

Compound	RE17565	RE17937		RE18286		
Larval age[b]	E-M	E-M	E	E-M	E	M
No. tests[c]	4	3	1	2	1	1
Concn. (ppm)						
0.0004	0	0		0		
.001	0	0	0	0	0	0
.004	77	26	4	10	19	50
.01	98	88	100	92	100	96
.04	100	100	100	100	100	100
.1	100	100	100	100	100	100
.4	100	100				
1.0	100	100				

[a] Laboratory strain, OP-susceptible.

[b] E = early, M = medium, E-M = mixture of early to medium.

[c] In each test, each concentration was run in duplicate.

Tests on 0.059 Acre Pasture Plots.–The results of the tests against *Aedes* are shown in Table 5 and are surprising in view of laboratory and 1 m^2 pond data. It is apparent the RE17565 does not effectively control *Aedes* larvae at the rates tested, as would be expected from the laboratory data. Furthermore, RE17937, which had lesser activity than RE17565 against *Aedes* larvae in the laboratory, shows greater activity in the field. The residual activity of RE17565 diminishes much more quickly than would be expected based on the 1 m^2 pond data.

Table 2.—Biological activity against *Culex tarsalis* larvae (in % inhibition of adult emergence).

Compound	RE17565							RE17937		RE18286		
Strain	OP-S	OP-S	OP-S	OP-S	OP-R	OP-R	OP-R	OP-S	OP-R	OP-S	OP-R	OP-R
Larval age[a/]	M-L-4	E-M-4	E-4	L-3 E-4	M-L-4	E-4	L-3 E-4	M-L-4	E-M-4	M-L-4	L-3 E-4	E-M-4
No. tests[b/]	3	2	1	2	1	1	4	1	2	1	1	1
Concn. (ppm)												
0.0001	0	0	0	8	0	0	0		0		0	0
.0004	5	1	0	6	15	20	9		17		0	0
.001	34	25	40	19	52	51	52	8	47	11	32	0
.004	73	58	95	74	100	100	98	63	98	59	90	80
.01	77	94	100	100	96	100	100	71	100	65	100	98
.04	81	100	100	100	100	100	100	81	100	75	100	100
.1	82	100	100	100	100	100	100	85	100	65	100	
.4		100	100	100	100	100	100					
1.0		100		100	100							

[a] E = early, L = late, E-M = mixture of early to medium, M-L = mixture of medium to late; the number refers to the larval stage.
[b] In each test, each concentration was run in duplicate.

Table 3.—Biological activity against *Aedes nigromaculis*[a] larvae (in % inhibition of adult emergence).

Compound	RE17565				RE17937			RE18286			
Larval age[b/]	M-4	E-M-4	E-4	L-3 E-4	M-4	E-M-4	L-3 E-4	L-4	M-4	E-4	L-3 E-4
No. tests[c/]	1	1	2	3	1	1	1	1	1	1	1
Concn. (ppm)											
0.0001			0	0				0			
.0004	0	14	0	6				0	0	0	6
.001	71	21	24	20	0	0	0	6	65	22	11
.004	100	100	97	59	51	17	22	56	90	92	81
.01	100	100	100	85	84	64	81	53	100	100	98
.04	100	100	100	100	97	100	100	55	100	100	100
.1	100	100	100	100	100	100	100	75		100	100
.4			100			100		79			

[a] Laboratory strain, OP-resistant.
[b] E = early, M = medium, L = late, E-M = mixture of early to medium; the number refers to the larval stage.
[c] In each test, each concentration was run in duplicate.

Table 4.–Bioassay of water samples from 1 m^2 ponds at Fresno, California (in % inhibition of adult emergence of *Culex pipiens quinquefasciatus*).

Date (1973)	Concn. (ppm)	Sample collection time (hr post-treatment)							
		2	24	48	72	96	120	144	168
				RE18286					
5/30	0.2	100	100	88	10	0	0	0	8
6/14	.1	--	100	100	8	0	--	20	0
				RE17937					
5/30	.2	100	100	7	8	0	0	0	0
				RE17565					
5/30	.2	100	100	100	82	89	51	51	16
6/14	.1	--	100	100	15	12	98	87	0
8/30	.2								
	Pond top	100	100	100	100	100	10	4	6
	Pond bottom	100	100	100	100	100	86	100	100

Table 5.–Summary of 0.059 acre pasture tests with RE17565 and RE17937 against natural mixed populations of *Aedes nigromaculis* and *Aedes melanimon*.

Field test no.	Date (1973)	Dose (lb AI/acre)	Larval stages treated	Final mortality of field population by stage treated (%)		
				2	3	4
			RE17565			
73-30	8/20	0.2	4	--	--	50
73-36	9/6	.3	2,3	70	85	--
			RE17937			
73-30	8/20	.1	4	--	--	60
73-33	8/30	.2	3,4	--	45	90
73-36	9/6	.3	2,3	95	99	--

Table 6.–Summary of 0.059 acre pasture tests with RE17565 and RE17937 against natural populations of *Culex tarsalis* (all stages present).

Field test no.	Date (1973)	Dose (lb AI/acre)	Days control (98% +)	Bioassay of water[a] at collection time (hr)		
				0	24	48
			RE17565			
73-15	7/3	0.2	3	100	55	0
73-22	7/25	.1	2	100	0	0
73-22	7/25	.05	0	100	0	0
73-31	8/20	.1	0	—	35	0
			RE17937			
73-17	7/10	.4	5	100	0	0
73-22	7/25	.2	3	100	0	--

[a] In % inhibition of adult emergence of *Culex pipiens quinquefasciatus.*

The results against *C. tarsalis* larvae are summarized in Table 6. RE17565 and RE17937 have similar activity against *C. tarsalis* at 0.2 lb AI/acre and both show very limited residual activity in water based on the bioassay data. RE17937 shows good activity at 0.4 lb AI/acre.

Aircraft Trials.–Following the first application of RE-17565 at 0.1 lb AI/acre, there was an approximate final mortality of 60% for larvae that were 4th-stage at the time of treatment and 50% for those that were 3rd-stage. Following the second aerial application (F. T. No. 73-44), flooding of water through parts of the field continued after treatment and may have affected the results. The normal emergence of *C. tarsalis* adults was reduced by about 50% for 4 days after treatment. Larvae which were late 4th-stage at the time of treatment were unaffected, as expected.

Effects on Nontarget, Aquatic Organisms.–Laboratory data on the effects of RE17565 on nontarget organisms are shown in Table 7; it appears quite safe to microcrustaceans such as cladocerans and copepods, as well as to important predators such as beetles and fish. Counts on nontarget organisms, following a field application of 0.2 lb AI/acre of RE17565, showed no deleterious effects (Table 8); similar studies, following a field application of 0.4 lb AI/acre of RE17937, also revealed no adversities (Table 9). Treatment of 5-gallon aquaria containing microcrustacean populations with 0.005 ppm of RE17565 also showed no adverse effects (Fig. 2). Thus, RE17565 and RE17937 appear to be quite safe with respect to the commonly occurring aquatic nontarget organisms that exist in mosquito-producing habitats.

DISCUSSION AND SUMMARY.–Special care must be taken in evaluating these compounds (RE17565, RE17937 and RE18286) under field conditions as the predominant mortality occurs in the prepupal stage; these sink into small depressions in the bottoms of ponds or pastures and are very

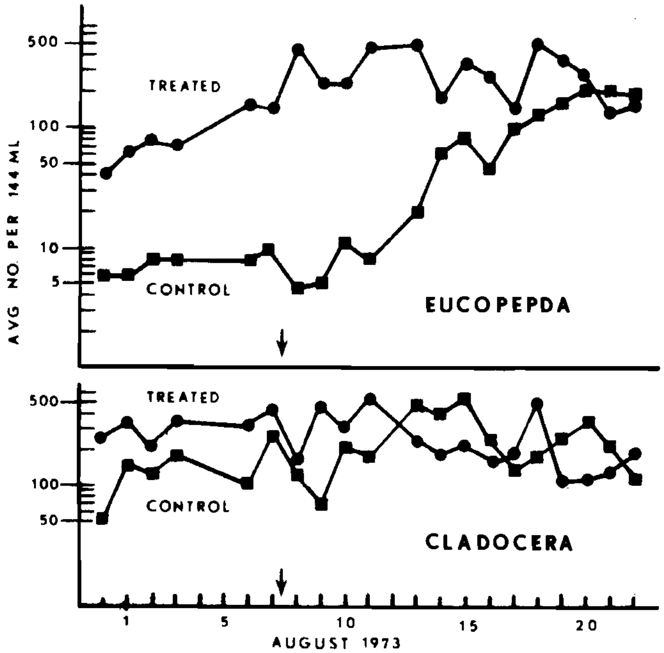


Figure 2.–Effects of RE17565 (0.005 ppm) against microcrustaceans. Arrow indicates application day.

Table 7.—Biological activity of RE17565 in the laboratory against organisms associated with mosquito breeding habitats.

Organism	Stage	Mortality (%)	Concn. (ppm)	Exposed (hr)	No. test	No. reps	No. organism/ container
Cladocera	Mixed	50	0.8	24	1	3	50+
Cladocera	Mixed	50	.45	144	1	3	50+
Eucopapoda	Mixed	50	5	24	1	3	12+
Conchostraca	Mixed	0	.25	48	1	3	10
Notostraca	Juvenile	7-13	1	24-120	1	3	10
Podocopa	Mixed	5	1	96	2	3	30+
Ephemeroptera	Nymphs	10	.1	168	1	1	64
Odonata	Nymphs	0	1	144-168	2	3	1, 5
T. basillaria	Adults	0	1	264	1	1	8
Laccophilus spp.	Adults	0	1	168	1	3	15
H. triangularia	Larvae	0	1	120	1	3	1
G. affinis	Mixed	0	.01	144	1	3	5

Table 8.—Biological activity of RE17565 at 0.2 lb AI/ acre applied as 50% WP formulation on July 3, 1973, against pond organisms (F. T. No. 73-15).

	No. organisms collected/6 dips								
Organism July	1	2	3	5	6	9	10	11	12
Cladocera	486	2,805	487	337	488	1,196	595	2,867	2,454
Eucopepoda	14	39	44	13	23	127	75	71	188
Podocopa	0	0	1	0	0	0	0	6	1
Rotifera	9	1	0	2	9	25	57	0	0
mayfly N	6	9	17	2	33	99	33	16	37
chironomid L	1	0	1	2	5	4	16	1	13
Volvocaceae	114	0	942	1	507	64	16	14	26
	No. organisms collected by a 1 ft^3 trap								
Corisella spp. N		0	0		1		0	0	1
N. unifasciata N		1	0		0		0	2	0
mayfly N		5	25		28		28	27	13
dragonfly N		1	1		0		2	4	4
H. triangularis L		2	2		0		2	2	2
H. triangularis A		2	0		3		0	0	0
T. lateralis L		0	1		0		0	4	0
T. lateralis A		9	8		1		0	0	1
Helophorus spp. A		0	3		8		4	10	0
Enochrus spp. A		0	2		1		0	0	0
T. basillaris A		1	5		1		0	0	0
Acilius spp. L		0	0		1		3	2	1
Laccophilus spp. A		0	1		0		0	0	1

N = nymphs, L = larvae, A = adults.

difficult to see. Also, the dead and dying prepupae and pupae are readily eaten by scavengers and predators. Therefore, effects of these compounds are easily underestimated. The absence of viable pupae is an indication of good results.

Table 9.—Biological activity of RE17937 at 0.4 lb AI/ acre applied as 50% WP formulation on July 10, 1973, against nontarget organisms (F. T. No. 73-17).

	No. organisms collected/6 dips					
Organism July	10	11	12	16	17	19
Cladocera	121	9	81	6,206	8,153	14,371
Eucopepoda	41	34	57	35	53	61
Podocopa	0	0	1	0	1	0
mayfly nymphs	34	8	21	11	16	31
chironomid larvae	1	1	1	1	5	7

Conversely, the percentage of emergence of normal-appearing pupae is a poor criterion since those pupae which appear normal (active and pigmented) have escaped the effects of the treatment and would be expected to emerge normally. This is in sharp contrast to evaluating compounds having the juvenile hormone-type biological activity, where pupal mortality is a good indication of effect.

RE17565 showed lesser activity in field tests than expected from the laboratory bioassay data and from the 1 m^2 pond tests. This is probably due to either adsorption onto organic matter or to microbial deactivation and is currently being studied. RE17937 showed lesser activity in laboratory bioassay tests and lesser residual in 1 m^2 pond tests but proved to be as active as RE17565 against *C. tarsalis* and showed greater activity against *A. nigromaculis.*

Preliminary toxicological data provided by the manufacturer indicate a high degree of mammalian safety; all three compounds showed approximate acute oral toxicities to male rats of 2,000 mg/kg (LD_{50}). The acute dermal toxicities were approximately the same value to male rats as well.

ACKNOWLEDGMENT.—This study was supported in part by a Grant-in-aid from Chevron Chemical Corporation.

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STRUCTURE-ACTIVITY RELATIONSHIP OF BRANCHED-CHAIN CARBOXYLIC ACIDS AGAINST MOSQUITO LARVAE

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ABSTRACT

Larvae of the southern house mosquito, *Culex pipiens quinquefasciatus* Say, produced overcrowding factors under overcrowded conditions. These factors, showing toxicity and growth-retarding activity against younger larvae of mosquitoes, were determined to be straight- and branched-chain hydrocarbons and branched-chain aliphatic carboxylic acids. To ascertain the activity of substituted carboxylic acids against mosquito larvae, twenty 2-ethyl, 2-butyl, and 2-hexyl carboxylic acids having even numbers of carbon atoms with total number of carbons from 12 to 24 were synthesized, and their structure-activity relationship studied. In the synthesis, diethyl malonate was alkylated twice with appropriate alkyl bromides in ethanol in the presence of sodium ethoxide and the resulting diethyl dialkylmalonates were saponified and decarboxylated to give the desired 2-substituted carboxylic acids.

The carboxylic acids were bioassayed against first-instar larvae of *C. p. quinquefasciatus*. The biological activity was measured in terms of inhibition of emergence of adults resulting from treated larvae. In the 2-ethyl substituted carboxylic acid series, the activity increased as the length of the main chain of the carboxylic acids increased with 2-ethyl=octadecanoic acid showing the maximum activity (LC_{50} 2.2, LC_{90} 6.2 ppm). As the chain lengths increased further, the activity decreased. In 2-butyl substituted carboxylic acids, the activity increased as the chain length increased, maximum activity shown by 2-butyldodecanoic acid (LC_{50} 6.4, LC_{90} 13.0 ppm) and 2-butyltetradecanoic acid (LC_{50} 6.0, LC_{90} 13.0 ppm). After a sharp decrease, the activity increased again as shown by 2-butyleicosanoic acid. In 2-hexyl substituted carboxylic acids, the same trend was obtained. The activity increased to the maximum in 2-hexyldecanoic acid (LC_{50} 3.8, LC_{90} 10.0 ppm), then decreased up to 2-hexylhexadecanoic, and increased again as shown by 2-hexyloctadecanoic acid. In general, the 2-substituted carboxylic acids having the total number of carbons from 14 to 18 showed the maximum activity against the mosquitoes. However, 2-ethyloctadecanoic acid exhibited an exceptional activity and was by far the most active compound among those synthesized and bioassayed.

The branched-chain carboxylic acids are especially effective against younger larvae. Since these acids are insoluble in water, it is possible that their biological activity can be increased greatly by formulating them with suitable surfactants.

MOSQUITO CONTROL STUDIES IN NORTHERN CALIFORNIA WITH JUVENILE HORMONE ANALOGUES: A PROGRESS REPORT

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For the past two years, the applicability of juvenile hormone analogues (JHA) has been explored for use in mosquito control in urban and rural situations in northern California. The purpose of this progress report is to briefly summarize the results of ongoing studies at the University of California at Davis on the effects of JHA on nontarget insects, its use in slow release formulations, its effectiveness against mosquito larvae in rice paddies and adult mosquitoes in a state of facultative diapause.

SLOW RELEASE FORMULATION STUDIES.–Storm drains/catch basins constitute a major breeding source of *Culex pipiens* in many urban areas throughout California. As an alternative to repetitive treatment of catch basins for *C. pipiens* control, work was initiated on a slow release formulation of JHA. In the initial test in 1972, a single application of ZR-515 incorporated in polyurethane foam gave 100% control for 58 days (Dunn and Strong 1973). During the past summer the work was expanded to include 50 catch basins treated with ZR-515 and R-20458 incorporated in different materials. Unfortunately, no wild population of mosquitoes was established in these basins even after 70 days. Evaluation by using caged laboratory-reared larvae in the basins was also unsuccessful because of the muck and fluctuating water levels. In spite of these difficulties, work is being continued this summer in the catch basins to improve on the duration of mosquito control achieved in the 1972 studies.

From an ecological standpoint, polyurethane foams are not the most desirable material. A search was begun for an inexpensive compound that dissolves very slowly. Dissolution systems having near-linear release rates would avoid the undesirable logarithmic release rate phenemenon that is characteristic of a diffusion system. A combination of these materials dissolved much too rapidly (Table 1). Various additives such as HiSil® and Silanox® failed to retard dissolution. Since plaster of paris seemed to crumble slowly, it was deemed a likely candidate and has been the basis of further refinements.

Using C^{14} labeled R-20458, release rates were monitored from various concoctions and this information was fortified with biological data obtained from bioassaying treated water with fourth instar *Aedes aegypti* larvae. Two systems of bioassay evaluation were used: 5 gallon crocks filled with water from a nearby creek and 1 square yard earthen ponds using irrigation water. Each of the 12 ponds was 1 foot deep with individual water depth regulators and planted with rice. The results of the C^{14} release rate study and the bioassay were consistent (Figure 1). With the plaster-rubber formulation, release was slower and correspondingly the percentage kill lasted considerably longer than with the Silastic® formulation. Presently, the main point is to determine the release rate necessary to maintain mosquito control over a 3-4 month period. This information will greatly assist in selecting the best JHA formulation for mosquito control with the slow release approach and form the basis for next summer's evaluation work in catch basins.

Table 1.–Dissolution formulations screened for feasibility as slow release carriers. (PEG=Polyethylene glycol, PPG=Polypropylene glycol, MW=Molecular weight)

PEG		MW 20,000
PEG	+	10%, 50%, PPG MW 1200
PEG	+	20%, 50%, PPG MW 4000
PEG	+	10%, 25%, 50% HISIL
PEG	+	1%, 5% CABOSIL
PEG	+	20% PPG MW 4000 + 1% CABOSIL
PEG	+	SILANOX COATING
PEG	+	20% CHARCOAL
PEG	+	10%, 33% PLASTER OF PARIS
PLASTER OF PARIS		

Table 2.–ZR-515 aerial applications to rice plots in Colusa County, 1973.

Treatment	% Adult Emergence Post Treatment Samples[1] Day 1		Day 3	
	C. tarsalis	*A. freeborni*	*C. tarsalis*	*A. freeborni*
Control-1	81	62	91	--
Control-2	--	--	--	25
SR10-1	--	0	57	12
SR10-2	55	0	63	0
Charcoal-1	58	--	88	--
Charcoal-2	63	0	--	0
Granular-1	86	--	96	30
Granular-2	--	--	--	15

[1] All samples of $N > 15$ 4th instar larvae.

LARVICIDAL STUDIES IN RICE PADDIES.–During August, 1973, ZR-515 was applied aerially to rice paddies in Colusa County to test its efficacy in controlling *Anopheles freeborni* and *Culex tarsalis.* Three formulations were tested at 0.1 lb/acre: (1) the micro encapsulation SR10, (2) a granular and (3) a 20F charcoal formulation. The initial tests involved laboratory-reared third and fourth instar *A. freeborni* larvae in screened cages placed strategically throughout the treated and untreated control rice

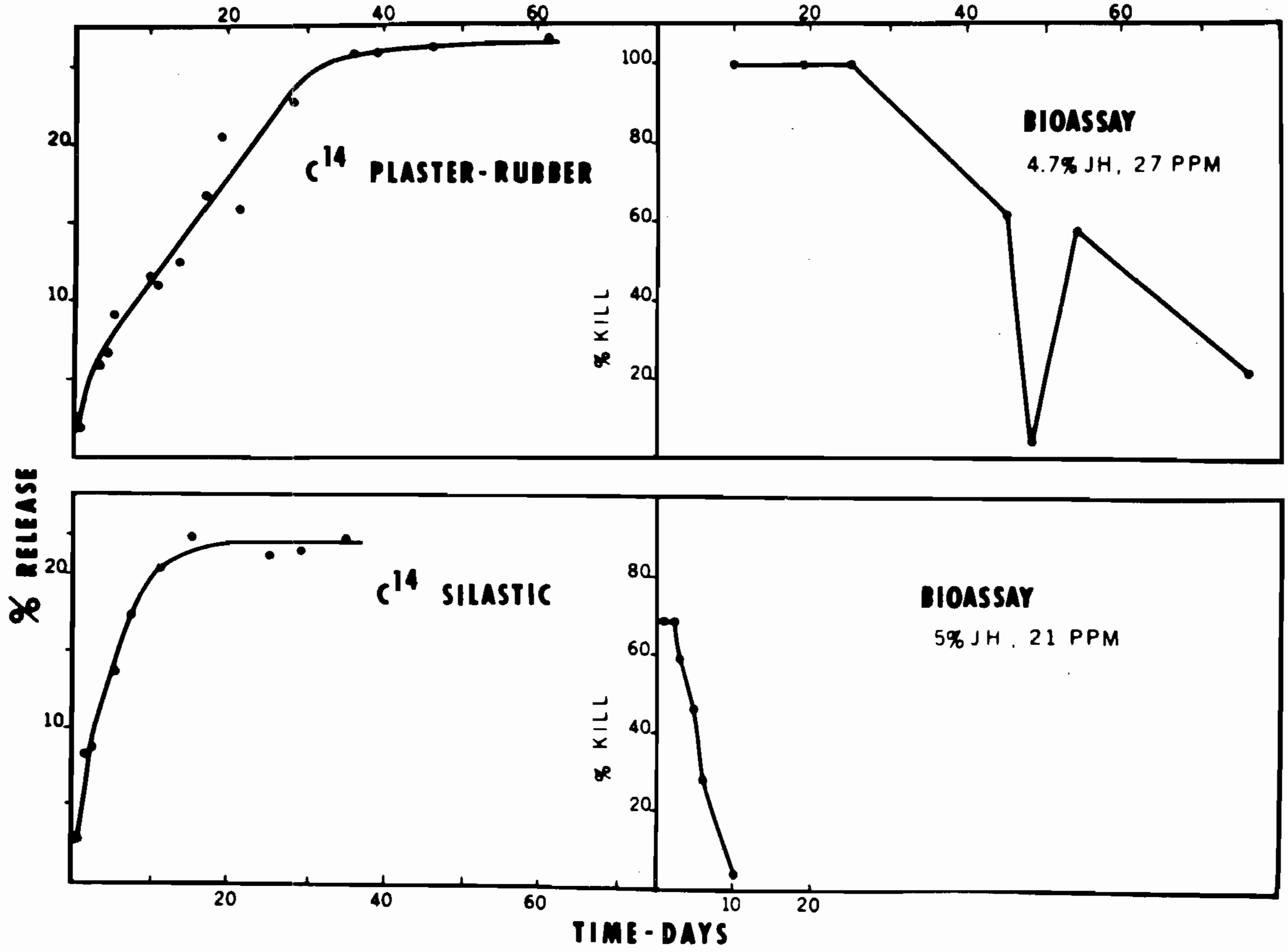

Figure 1.—Release rates of R-20458 from two slow release formulations determined by C^{14} labeling and by bioassay.

paddies. Aquatic insect predators, however, penetrated these cages and eliminated the test larvae. A different test procedure was employed with the use of field larval populations. In contrast to aedine breeding in an irrigated pasture situation, larval density in rice paddies is often very low. For this reason, large dip nets rather than enamel dippers were utilized. At least 5 people waded through the paddies and continued sampling until sufficient numbers of larvae could be collected and brought to the laboratory to observe the following: mortality, emergence, rotation of the male genitalia and degree of ovarian development of the surviving females. Water samples and larvae were taken 1, 3, 5, and 9 days after treatment. For simplicity only the emergence data for the first two days is shown (Table 2). Mortality was not overwhelming. Only on day 1 was significant control of *A. freeborni* achieved by SR10 and the charcoal formulation. Because the rice was 1-2 feet high at the time of application, we had expected the granular material to out-perform the liquid sprays. This was not the case. Of the 500 males examined, only two did not rotate their genitalia; one from a control and one from a treated plot. The results of the water residue analysis (not yet available) will enable us to determine whether or not the inconsistencies in our test can be attributed to failure of the test materials to penetrate the rice canopy or merely to the ineffectiveness of the material.

Table 3.—Preliminary evaluation of R-20458 on nontarget *Tropisternus lateralis*.

Rate PPM	No. Treated[1]	Emergence[2]
0.0	6	100
0.001	9	67
0.01	10	70
0.1	8	43
1.0	8	43

[1] Treated individually as late 3rd instar larvae.

[2] Adults emerging appearing morphologically normal.

STUDIES ON NONTARGET INVERTEBRATES.—Previous studies to evaluate the effects of JHA on nontarget organisms have been extensive but inconclusive. In northern California rice fields two beetles, *Tropisternus lateralis* and *Hydrophilus triangularis*, are very abundant and predaceous on mosquito larvae. These studies were extremely laborious because of the cannibalistic behavior of these larvae, the long pupation period and our efforts to avoid the short term acute toxicity approach to evaluation.

In very preliminary tests, the beetle larvae were treated by putting them in water containing known concentrations of JHA. The larvae were allowed to pupate in sand and subsequent adult emergence scored 25-35 days later. *Tropisternus lateralis* was treated with R-20458 at rates of 10^{-3} ppm to 1 ppm (Table 3). Due primarily to the low numbers treated the 43% emergence is significantly different from the controls at only the .15 level. Larvae of *H. triangularis* were treated with SR10 at the same rates with no lethal effect up to 1 ppm (Table 4). However, one of the unexplained effects of JHA was observed. The treated larvae did not crawl into pupation chambers in the sand as soon as the untreated larvae. In addition, some abnormal adults were found in both *T. lateralis* and *H. triangularis*. Attachment of a pupal exuvia to the tip of the abdomen and wrinkled elytra were the most common abnormalities observed. Further replications will be made this next season before any conclusions are made as to the effects of these materials on the two nontarget species studied.

Table 4.—Preliminary evaluation of ZR-515 on nontarget *Hydrophilus triangularis*.

Rate PPM	No. Treated[1]	% Normal Emergence[2]	**% Larvae Pupating**[3]
0.0	7	75	100
0.001	8	71	71
0.01	7	86	71
0.1	8	86	50
1.0	7	71	0

[1] Treated individually as late 3rd instar larvae.

[2] Those adults emerging appearing morphologically normal.

[3] Counted after 2 days.

DIAPAUSE TERMINATION STUDY.—If diapause could be artificially terminated in *A. freeborni* we would have a unique tool in mosquito control. JHA materials have previously been shown to break diapause in adult alfalfa weevil (Bowers and Blickenstaff 1966), cereal leaf beetle (Connin et al. 1967), lady bugs (Hodek et al. 1973), and others, but not in mosquitoes. During the past fall diapausing adult female *A. freeborni* were treated with R-20458 and ZR-515. The material was applied topically or fed in a sugar solution, the mosquitoes held seven days and then offered a blood meal. The degree of diapause termination was determined by the extent of blood feeding and subsequent oogenesis.

In the tests with R-20458, 46% of the control mosquitoes completed oogenesis after a blood meal. Adult females treated with 1 µg and 10 µg resulted in 93% and 100%, respectively, of the blood engorged females completing oogenesis. Note, however, that the percentage of blood feeding did not increase (Table 5). With females treated with ZR-515, however, the percentage of blood feeding increased dramatically with increased dosage of this com-

Table 5.—Diapause termination in *Anopheles freeborni* with Stauffer's R-20458 in November, 1973.

Treatment	No. Treated	% Mortality After Treat.	% Blood Feeding[1]	Number Dissected		% with Ovarioles III to V	
				Blooded	Unblooded	Blooded	Unblooded
Control	400	6	46	40	151	46	1
1 µg	221	10	22	40	58	93	34
10 µg	230	46	33	14	6	100	0

[1] Based on the number that survived after blood was offered.

Table 6.—Diapause termination in *Anopheles freeborni* with Zoecon's ZR-515 in October, 1973.

Treatment	No. Treated	% Mortality After Application	% Blood Feeding[1]	Number Dissected		% with Ovarioles III to V		No. of Eggs/♀ (No. ♀)
				Blooded	Unblooded	Blooded	Unblooded	
Topical Control	267	40.1	25.4	20	36	20	0	151 (4)
1 ug	273	73.6	55.9	32	8	93.7	0	154 (6)
10 ug	346	81.5	77.2	20	1	95.0	0	125 (5)
Sugar Control	198	2.5	21.9	32	34	9.3	0	200 (1)
20 ppm	252	2.4	27.5	39	7	69.2	0	162 (6)
200 ppm	291	5.5	41.1	22	0	81.8	–	163 (5)

[1] Based on the number that survived after blood was offered.

pound (Table 6). In addition, it appears that diapause in females treated with ZR-515 can be terminated by exposing the females to JHA by both contact and ingestion.

Diapause in both *C. tarsalis* and *Anopheles punctipennis* that undergo gonotrophic concordance was not terminated by ZR-515.

This is suggestive of a difference in the hormonal systems that control diapause in mosquitoes such as *A. freeborni* that undergo reproductive changes and in mosquitoes such as *C. tarsalis* and *A. punctipennis* that undergo changes in the biting drive. Additional studies will hopefully elucidate our initial findings and determine the feasibility of using JHA under field conditions to terminate diapause.

ACKNOWLEDGMENTS.—We thank the Colusa, Sutter-Yuba, and San Joaquin Mosquito Abatement Districts for their kind assistance throughout the studies. Portions of this work were supported by Stauffer Chemical Company, Mountain View, California, and Zoecon Corporation, Palo Alto, California.

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FIELD EVALUATION OF TWO INSECT GROWTH REGULATORS AGAINST CHIRONOMID MIDGES IN WATER SPREADING BASINS

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Chironomid midges have been a nuisance problem for many years within the Southeast Mosquito Abatement District. The District covers a large urbanized portion of Los Angeles County and the majority of the breeding sites are located adjacent to homes and industry. Sites are found primarily in concrete and natural flood control channels, recreational lakes including water hazards on golf courses, and water conservation spreading basins.

The control methods used have consisted mainly of the application of various species of benthic foraging fish in impounded water areas, water management in areas such as water spreading basins, and chemical control in areas such as flood control channels, forebays involved with water spreading operations, and street gutters.

Various organophosphorous compounds have been used to control chironomid midges in those areas where physical and biological means were not feasible. Usually, chemical control of midges requires higher dosages to achieve reduction below nuisance threshold levels than is needed for mosquito control. In many cases several midge species were present in one source, and control would be directed at the species with the highest population. Frequently the midge population could not be kept below the nuisance threshold for any reasonable length of time, due to differential susceptibility of the various species of midges present to the toxicant applied. This problem was compounded by the development of resistance by several species to many of the compounds. Also, a new species of Chironomous (not yet given a species name) occurred in the Southeast Mosquito Abatement District which was highly resistant to all of the organophosphorous compounds used by the District except malathion (Pelsue and McFarland, 1971). The District discontinued the use of malathion for midge control in the early 1960's due to the development of resistance by mosquitoes, and its high toxicity to fish. Fish normally do not occur in the sources where the target species is located, but many of the sources empty into the tidal net where fish kills could occur.

With the occurrence of this highly resistant species, the District had to resume the use of malathion to provide relief to the residents during the midge season. During the summer season the new species predominates in most of the flood control channels and water spreading forebays in the District.

In 1970, the Southeast Mosquito Abatement District initiated research for the control of chironomid midges by granting $10,000 to the University of California at Riverside to fund research headed by Drs. Mir Mulla and Fred Legner to explore chemical and biological means of controlling chironomid midges in the particular habitats common to Los Angeles County. At this same time, Orange County Mosquito Abatement District also granted research funds to UCR for the same purpose. These Grants have produced much valuable data, and new chemical and biological control agents have been discovered that show promise in the control of chironomid midges.

With the emergence of insect growth regulators (IGR's) as mosquito control agents (Schaefer and Wilder, 1972), it appeared that these might be active against chironomid midges. Preliminary laboratory and field data by Mulla and Kramer (in press) and Norland (1973) indicated that Altosid® (ZR-515) showed a high level of acitivity against several species of the Chironomidae. During the early part of 1973, a new compound called TH-6040 became available for experimentation. Preliminary laboratory results of this compound also indicated a high level of activity against chironomid midges.

The purpose of this study was to field test Altosid SR-10 and TH-6040 for the control of midges in water spreading basins. The project was begun during the summer of 1973 and the results presented are of a preliminary nature.

MATERIALS AND METHODS.—Experiments were performed at the Rio Hondo Spreading Grounds of the Los Angeles County Flood Control District, located in Pico Rivera. These plots of simulated water spreading basins were made available by the Flood Control District for this research. Test plots were approximately 40 feet square and 2 feet in depth. The bottom substrate was sandy loam to gravel. This soil type allows rapid penetration of water into the underground aquifers. Since these simulate water spreading basins, water is continually flowing into the basins and being percolated into the ground.

Initially four of the available eighteen basins were chosen for the tests. Three were used as test basins and one as a check. The basins were filled in early July and midge sampling was begun in the latter part of July. Samples were collected in cone-shaped emergence traps each of which sampled an area of approximately 50 square centimeters. Two emergence traps were placed per test basin and samples were collected two to three times a week with each sample representing a single trap night. Data presented showing adult emergence are an average of the two traps per test basin. The traps were moved after each sample night and were removed from the basins when no samples were taken. The samples were returned to the laboratory, identified and counted. The number of samples collected pre- and post-treatment varied but at least three samples were collected pre- and post-treatment.

Experiment 1: Three basins were treated with TH-6040, 1-(4-chlorophenyl)-3-(2, 6-difluorobenzoyl)-urea, (25% wettable powder) at the rate of 0.05 pounds actual per acre. One basin was used as a check. July 31, 1973. Water temperature ranged from 26°C - 31°C; pH range was 8.3-8.9.

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Experiment 2: Approximately one week after Experiment 1, the same three basins were treated again with TH-6040 (25% WP) at a rate of 0.05 ppm, August 8, 1973. Water temperature ranged from 25° - 30°C; pH ranged from 8.75-9.3.

Experiment 3: Two weeks after Experiment 2 was completed, adult emergence had totally recovered. Another test basin was added making four test basins and one check basin (untreated). In this experiment, two basins were treated with Altosid SR-10, isopropyl (E, E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate, and two basins were treated TH-6040 (25% WP) at the rate of 0.1 ppm on August 28, 1973. Water temperature ranged from 22°-25°C, pH ranged from 8.7-9.6.

Experiment 4: On October 16, 1973. A treatment was made consisting of a combination of Altosid SR-10 plus 1% Abate sand-core granules. Dosage rate for each material was 0.1 ppm. Each material was replicated once. Water temperatures ranged from 21°-22°C and pH ranged from 8.5-8.9.

The TH-6040 and Altosid SR-10 were applied as a dilute spray in water using a pressurized sprayer and the Abate as granules applied with a U.S. Borax granule spreader. Post-treatment samples were collected the night following treatment and twice or three times weekly thereafter until population recovery occurred.

RESULTS AND DISCUSSION.—Experiment No. 1, reported in Table 1, using TH-6040 at 0.05 pound per acre indicated a rather poor reduction in midge population, which lasted only a few days. This may be related to the high-dilution rate in the experimental ponds as the water is continually being replaced. The chironomid midge species most abundant during the first experiment were *Dicrotendipes californicus* (Johannsen), *Cricotopus* sp., *Tanytarsus* sp., and *Chironomus attenuatus* complex. Following the treatment, the populations affected most were *Tanytarsus* sp. and *Cricotopus* sp. which did not fully recover by the time of the second experiment. *Dicrotendipes californicus* and *Chironomus attenuatus* both recovered rapidly in 3 days but not to the pre-treatment level.

Figure one shows the totals of the three most numerous species recorded in the first two experiments. In Experiment 2, TH-6040 was again applied at a rate of 0.05 ppm with results similar to those recorded in Experiment 1. *Cricotopus* sp. dropped after the first experiment and remained low during the second experiment. The *Dicrotendipes* population was high before the second experiment but then dropped dramatically for a few days after treatment, then increased quickly to a level above the pre-treatment average. The *Tanytarsus* sp. population was also relatively high during the pre-treatment period, also dropped after treatment, but did not recover to its pre-treatment level, remaining low for several days compared to *D. californicus*. *Cricotopus* sp. remained low during the test and *Procladius culiciformis* began to appear during the post-treatment period. In Experiment II (Table 1), during the test period no reduction was recorded but there was a dramatic drop in the population to almost zero the first trap night after treatment; then the population began to rise.

Experiment III consisted of comparing Altosid SR-10 with TH-6040 at a dosage rate of 0.1 ppm. *Dicrotendipes californicus* was the predominant species with *Tanytarsus* sp. present in relatively high numbers. *C. attenuatus* and *Procladius culiciformis* were also present at low levels. With each compound, adult emergence was initally reduced to nearly zero. In the TH-6040 plot, the population recovered more rapidly than in the Altosid SR-10 plot, as shown in Figure 2. Figure 3 shows the pre- and post-treatment population levels for the species most abundant during the treatment period. For each test compound, other species such as *Paralauterborniella* sp., *Ablabesmyia* sp. and *Cricotopus* sp. were also present at low levels. Figure 3 shows that both compounds performed well against the midge species present. However, in the TH-6040 test, *Tanytarsus* sp. emergence was not as effectively inhibited as in the Altosid test. There may be some selective toxicity, TH-6040 did not appear as toxic to *Tanytarsus* sp. as to the other species present. This may be also true for *Procladius culiciformis*. This may be advantageous with *P. culiciformis* since this species is a predator on other midge species. Altosid SR-10 was not effective in inhibiting emergence of *Tanytarsus* sp. as compared to the other species present, but Altosid was more effective than TH-6040 against *Tanytarsus* sp. *Dicrotendipes californicus* emergence was effectively inhibited by both compounds and population recovery was initially low, dropping even lower, and never really fully recovered as shown in Figure 1.

In Experiment IV, Altosid SR-10 and TH-6040 were used, but Abate granules were also applied at the same time, to determine if an effective larvicide could prolong the action of the adult emergence inhibitors. This experiment was predicated on the idea that even though there was a large dilution factor occurring, if one could initially prevent adult emergence for a short period of 2-3 days while also killing larvae present in the test plot, a longer recovery time could be achieved. Figure 4 shows that a longer recovery period did occur using a larvicide along with adult emergence inhibitors.

In this test ten pre-treatment samples were taken with an average population level of over 100 adults per trap night. Fourteen post-treatment samples were taken, with the population initially dropping to below 0.5 midges per trap night and increasing slightly to over one midge per trap night. The post-treatment period represents more than one month. The population level remained well below the nuisance threshold until the experiment was terminated. Table 2 shows the cumulative average pre- and post-treatment counts for Experiment IV, indicating excellent reduction in both test plots as compared with Experiment III. Figure 5 compares the predominant midge species present indicating that there was little or no selective toxicity occurring with the addition of Abate to the IGR compounds. The evidence is by no means conclusive that the IGR-Abate combination prolonged the population recovery period, but does indicate a greater effect on the midge population than either IGR compound used alone. Since the experiment was performed close to the end of the midge season and temperatures were starting to decline, this may have contributed to a prolongation of the recovery period. Further experiments are planned to supplement the data presented here. Abate was not applied alone to compare its singular effect on the larval population or its ability to reduce adult emergence.

Table 1.–Results of two experiments using TH-6040 25% WP at 0.05 pounds actual per acre and 0.05 ppm.

Experiment No. 1 – Experimental Period 7/27 – 8/3/73

Test Pond	Material[a]	Pre-treat Ave/TN[b]	Post-treat Ave/TN[c]	Percent Reduction	Average
A	TH-6040 25%WP	125	125	54	59%
B	TH-6040 25%WP	324	92	75	
C	TH-6040 25%WP	344	205	48	
D	Check	328	370		

[a] Dosage Rate 0.05/acre treatment date 7/31/73.

[b] Pre-treatment based on 3 trap nights, 6 days.

[c] Post-treatment based on 2 trap nights, 2 days.

Experiment No. 2 – Experimental Period 8/3 – 8/16/73

Test Pond	Material[a]	Pre-treat Ave/TN[b]	Post-treat Ave/TN[c]	Percent Reduction	Average
A	TH-6040 25%WP	108	161	0	
B	TH-6040 25%WP	115	159	0	
C	TH-6040 25%WP	122	157	0	
D	Check	160	186		

[a] Dosage rate 0.05 ppm treatment date 8/8/73.

[b] Pre-treatment based on 6 trap nights.

[c] Post-treatment based on 5 trap nights, 6 days.

Table 2.–Comparative results of two experiments using TH-6040 and Altosid alone and in combination with 1% Abate Granules, at a dosage rate of 0.1 ppm for each material.

Experiment No. 3 – Experimental Period 8/17 – 9/14/73

Test Pond	Material[a]	Pre-treat Ave/TN[b]	Post-treat Ave/TN[c]	Corrected % Reduc.	Average
A	TH-6040 25%WP	164	37	76	64
B	TH-6040 25%WP	134	62	54	
C	Altosid SR-10	154	23	83	86
D	Altosid SR-10	148	18	88	
E	Check	117	104		

[a] Dosage rate 0.1 ppm.

[b] Pre-treatment based on 2 trap nights, 12 days.

[c] Post-treatment based on 6 trap nights, 20 days.

[d] Percent reduction based on 100 - $(\frac{C}{T} \times \frac{T}{C})$ 100

Experiment No. 4 – Experimental Period 9/17 – 11/9/73

Test Pond	Material[a]	Pre-treat Ave/TN[b]	Post-treat Ave/TN[c]	Corrected % Reduc.	Average
A	TH-6040+Abate	58	0.75	91	94
B	TH-6040+Abate	92	0.42	97	
C	Altosid SR-10+Abate	82	0.32	98	98
D	Altosid SR-10+Abate	46	0.39	98	
E	Check	93	12.4		

[a] Dosage rate TH-6040 .1 ppm Abate = 0.1 ppm.

[b] Pre-treatment based on 12 trap nights, 30 days.

[c] Post-treatment based on 14 trap nights, 24 days.

[d] Percent reduction based on 100 - $(\frac{C}{T} \times \frac{T}{C})$ 100.

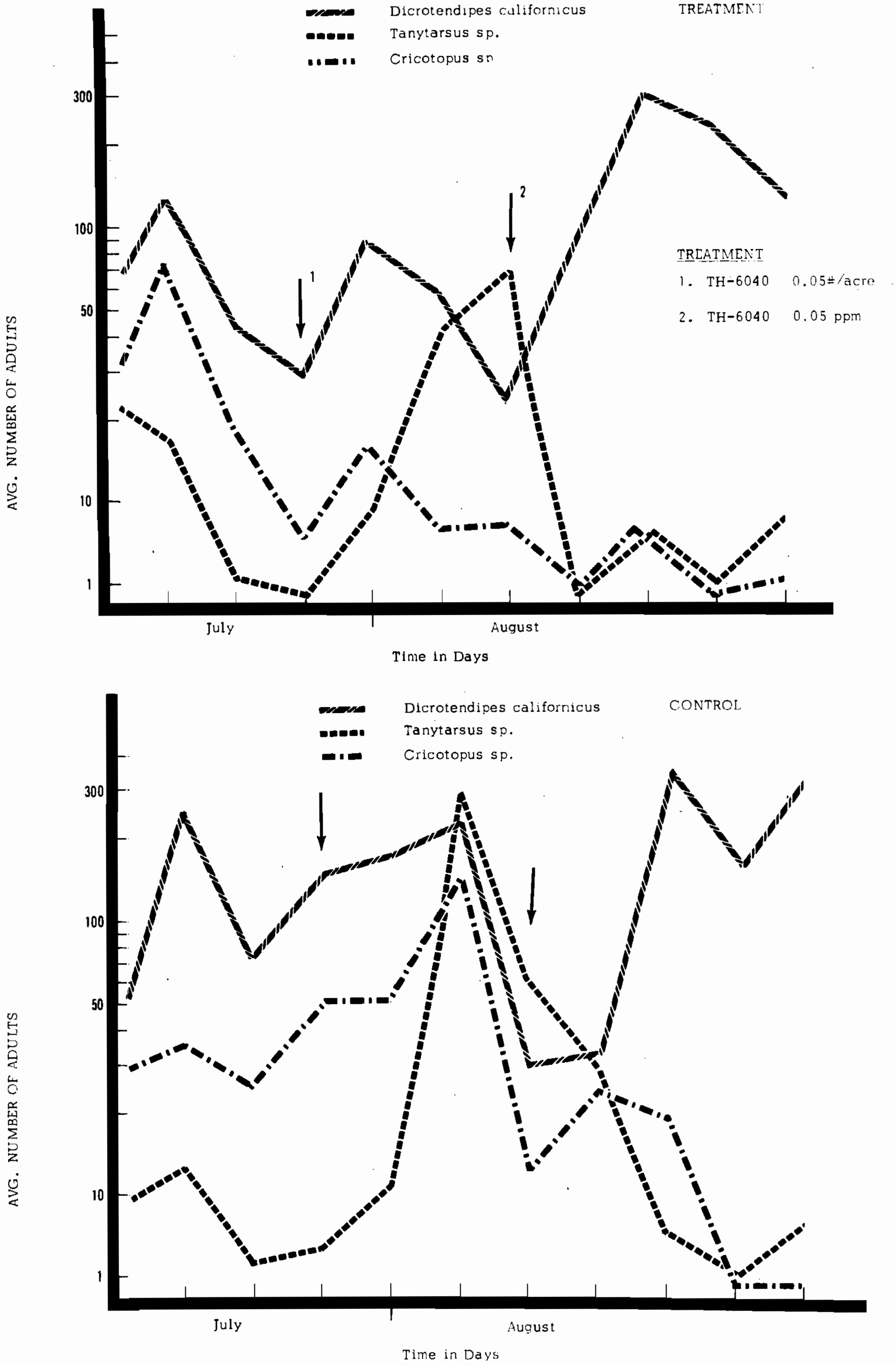

Figure 1.–Comparison of Experiments 1 and 2 showing the average effect of each treatment on the three most prominent species present. Treated plots verses control plot.

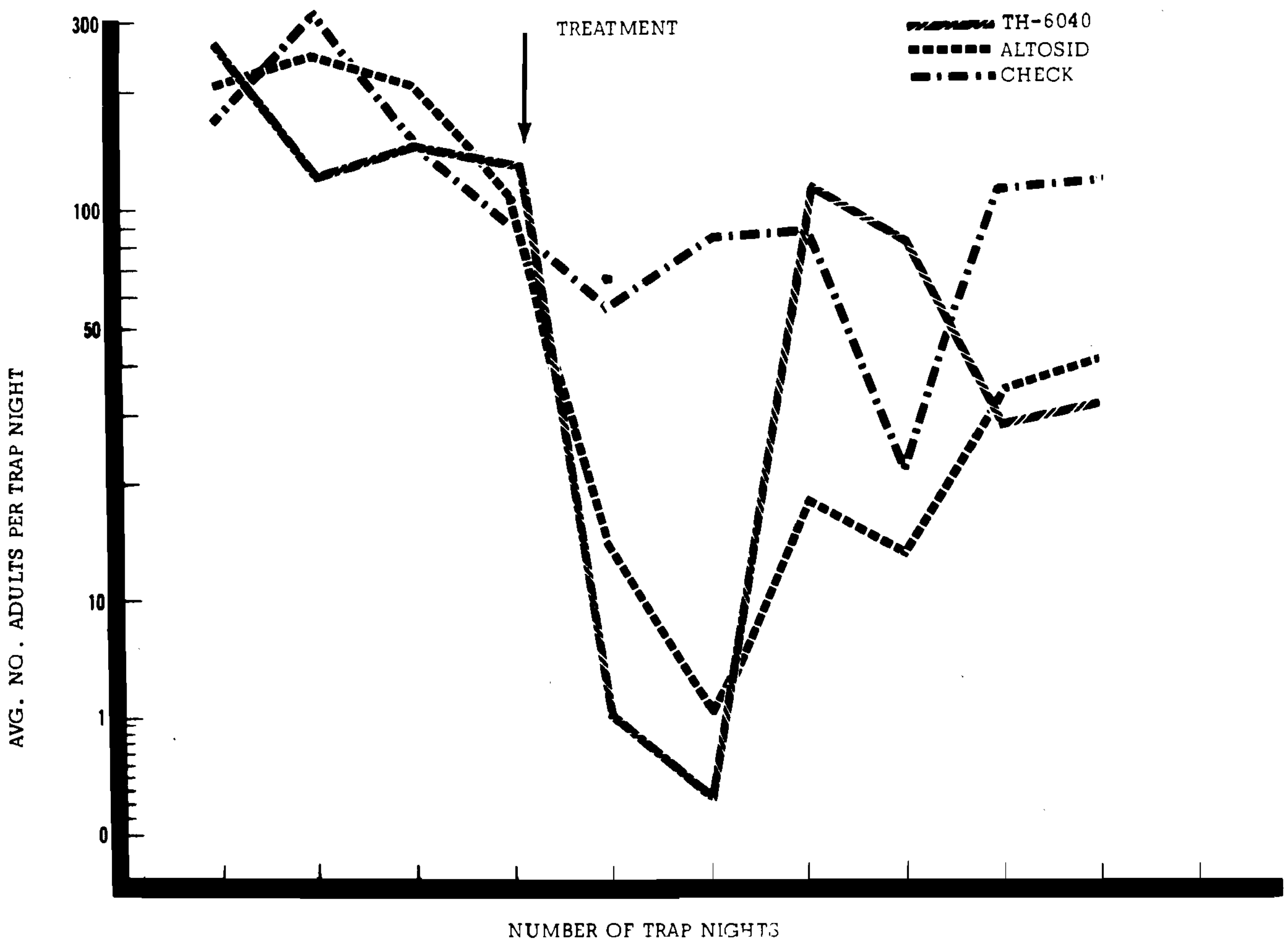

Figure 2.–Pre- and Post-treatment adult population levels for TH-6040 and Altosid SR-10 treated test ponds.

In comparing Experiments III and IV, it is clear that Abate included in combination with the IGR compounds will prolong the period of population recovery. There appear to be only slight differences between TH-6040 and Altosid in their effects on chironomid midge adult emergence under water spreading conditions.

Since both IGR compounds used in the Experiments are emergence inhibitors, it appears that they are well suited to midge control as the adults are the targets. Previously, larviciding was the only feasible means of preventing adult emergence, usually causing a greater environmental hazard by toxicity to fish and predatory arthropods. Although the impact of IGR compounds was not assessed quantitatively on predator populations present in test ponds, it was observed that these populations were not appreciably reduced; however, some mortality was observed in the TH-6040 ponds to dragon fly and mayfly naiads, with little effect on water beetles and notonectids.

ACKNOWLEDGMENTS.–The Authors acknowledge the cooperation of the Los Angeles County Flood Control District, particularly the Water Conservation Division, for the use of the test basins and the manipulation of water in those basins. We also thank Dr. Mir Mulla, Professor of Entomology, U.C. R., for providing experimental materials.

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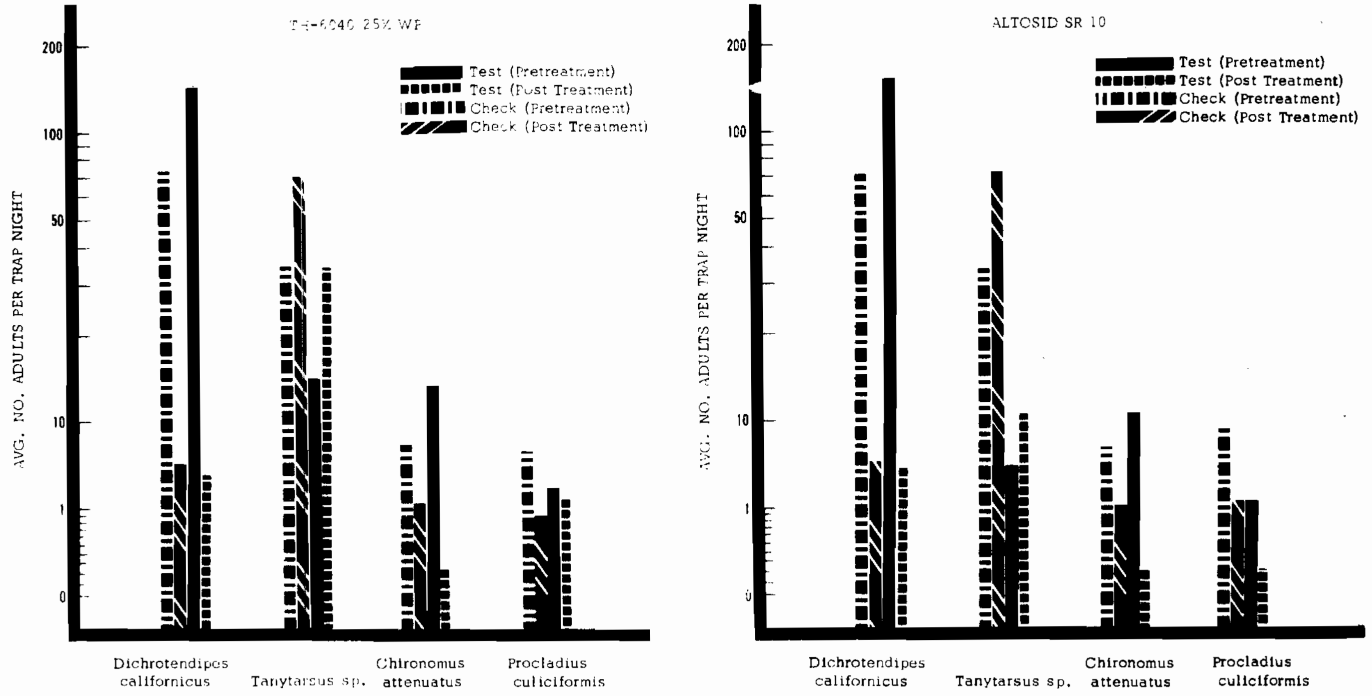

Figure 3.—Pre- and Post-treatment population levels for predominant midge species present in test basins for Experiment Number 3.

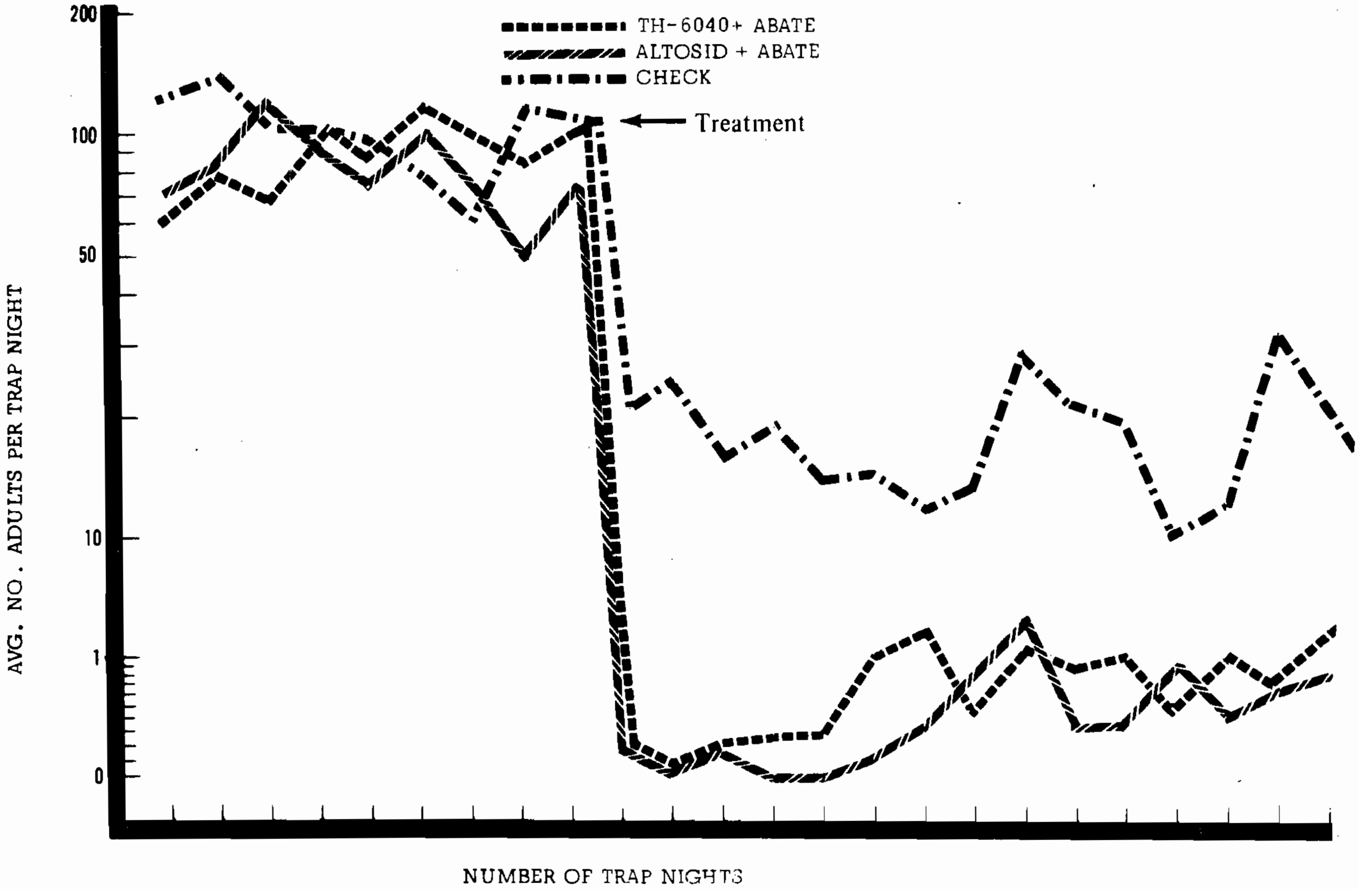

Figure 4.—Pre- and Post-treatment levels showing averages of all species for each test compound for experiment number 4.

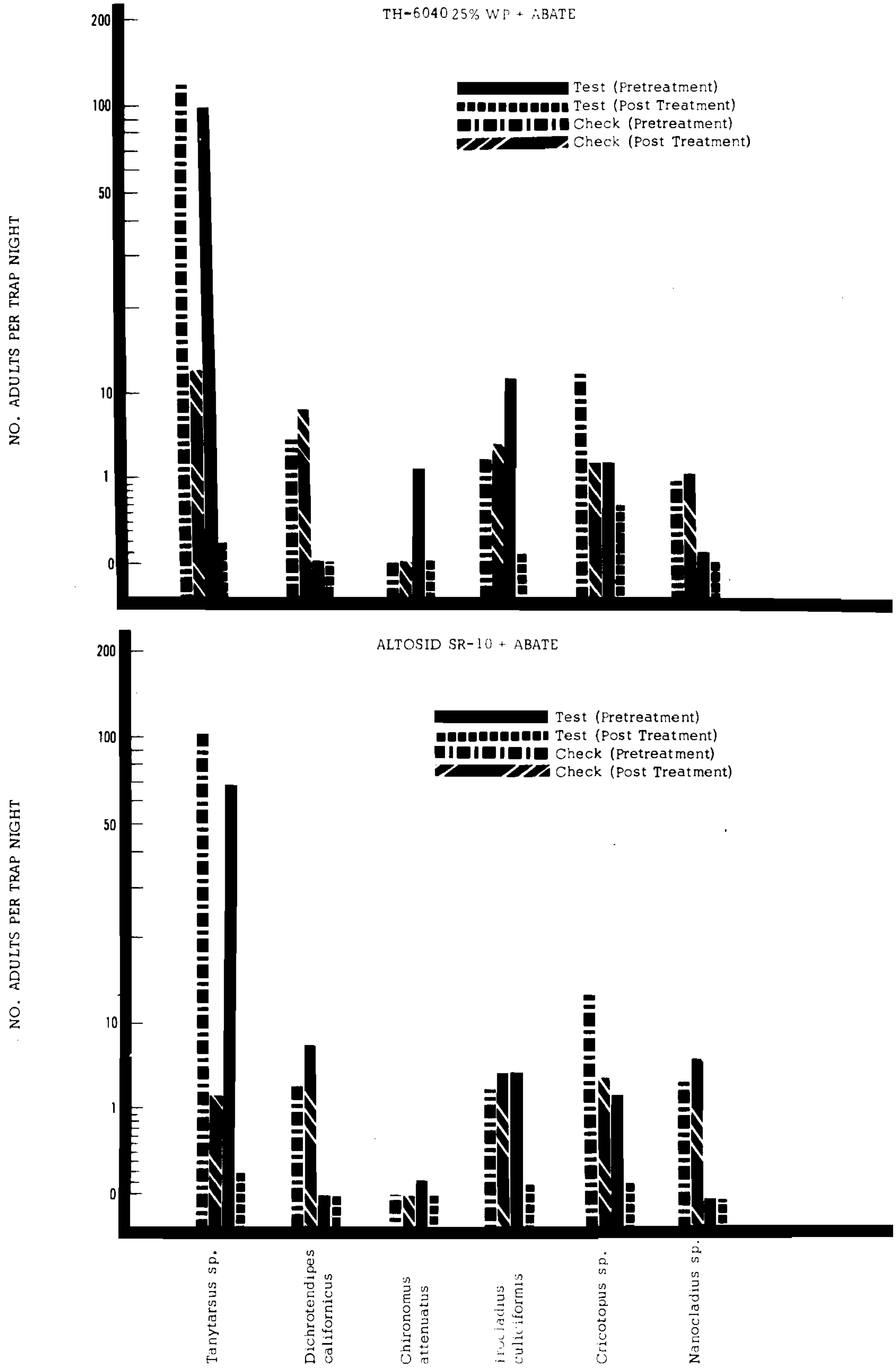

Figure 5.—Relative abundance of the predominant midge species, Pre- and Post-treatment, for Experiment 4. Represents the average for each species during the experimental period.

TIME-SEQUENCE RESPONSE OF *CULEX TARSALIS* FOLLOWING EXPOSURE TO INSECT GROWTH REGULATORS

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Extensive tests were performed on the mode of action of the insect growth regulators (IGR) Altosid® and to a lesser extent of Stauffer R-20458 [*trans* 1-(4′-ethylphenoxy)-6, 7-epoxy-3,7-dimethyl-2-octene] on *Culex tarsalis*. The results, presented in summary below, help to elucidate several aspects of the biological and physical properties of this and other IGR compounds previously not clearly understood.

Sequential effects of treatment of 4th instar larvae.— Most researchers on IGRs are confronted with the question as to what meaningful data should be recorded in bioassays of such compounds. It is generally agreed that the 24-hour mortality count, which is considered the end point of toxicity for most conventional insecticides, does not apply to IGRs. The results of many tests led to the following conclusions: Larval mortality following treatment of 4th instar larvae is not dose-dependent and should not be considered in the total effect of the treatment. The effects on pupae (based on proportion of undeveloped pupae) and adults (recorded as incomplete development, attachment to puparium, or inability to fly) were dose-dependent (Figure 1). Thus, the "total effect" of treatment must be based on the number of adults successfully emerging in relation to the number of pupae obtained within a certain period after treatment. This period must correspond to the known stability of the chemical in the water medium.

Precision of the "critical period" of larval sensitivity.— When the total effect of treatment of larvae by Altosid is plotted against dose, the slope of the line obtained is surprisingly flat as compared to that of conventional insecticides. A low slope generally indicates considerable heterogeneity in the amount of active principle reaching a sensitive site within the insect at a critical time of development. It is, therefore, evident that many individuals either escape treatment or are insufficiently exposed. We hypothesized that the observed heterogeneity of effect was due to (a) rapid hydrolysis of the chemical in water, (b) narrowness of the "critical period" of sensitivity of 4th instar larvae. The importance of these questions in practical control is self-evident. They were also important in our investigations of the potentiality for resistance (Georghiou et at. 1974) since in such studies it is necessary to ensure that every individual contributing to the next generation has indeed been exposed to the appropriate dose of the chemical.

The rate of loss of Altosid (tech) in water was investigated by exposing late 4th instar larvae for 30 minutes to treated water at various intervals after application. A rapid loss of activity from a 66% effect to 23% effect was measured during the first 16 hours (Table 1).

The question of differential sensitivity of larvae within the 4th instar was examined in a long series of test of which only the final set is described below: Large numbers of 4th instar larvae were exposed to 50 ppb Altosid for periods of 15′, 30′, 60′, 120′ or continuously until pupation. After exposure they were transferred to clean water, pupae were isolated as they were formed, and records were taken on the percentage of adult emergence vs. the time interval between larval exposure and pupation. The results indicate that the peak of sensitivity occurs at approximately 20 hours before pupation. The "critical period" is most clearly evident in the results of short exposures (15′) and extends from about the 10th to the 30th hour before pupation. Similar results were obtained with three strains of *C. tarsalis* (Bakersfield, Coachella and Altosid-selected) and with *C. quinquefasciatus*.

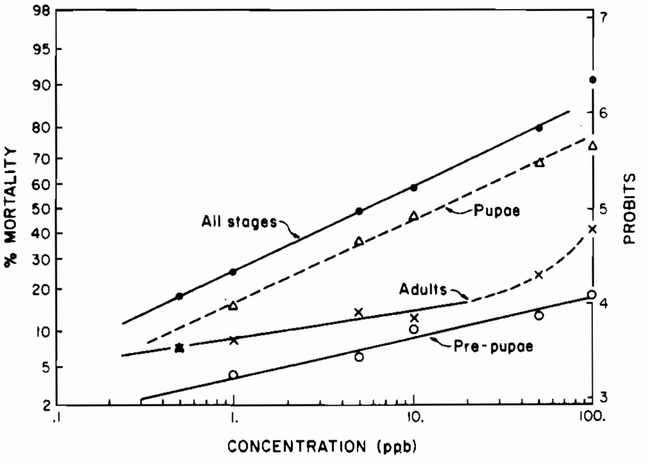

Figure 1.—Relative sensitivity of pre-pupae, pupae, and adults to treatment by Altosid applied against 4th instar larvae.

Table 1.—Rate of loss of activity of Altosid (tech) bioassayed at various intervals by 30-min. exposure of 4th instar *C. tarsalis*.[a]

Interval (hrs) between appl. to water and larval exposure[b]	% Mortality
0	66
1	67
2	71
4	61
8	51
16	23

[a] Coachella strain; 200 larvae/test; Altosid 50 ppb. Each test in separate pan.

[b] Based on individuals pupating within 3 days after exposure.

By taking into account only the individuals which pupate between 10 and 30 hours after treatment, i.e. those which were within the "critical period" of sensitivity at the time of treatment, it is possible to attain a considerable measure of reproducibility in the results of tests. Thus, by clarifying this aspect of the mode of action of Altosid we have introduced a considerable measure of accuracy and reproducibility in evaluations of IGR compounds. The procedure has potential uses in residue bioassays, screening new materials, determining rates of loss of activity, and other work on IGR compounds.

Rate of loss of activity of "thick-wall" and "thin-wall" formulations of IGR R-20458.–By the use of the procedure described above, investigations were carried out on the rate of loss of activity of IGR R-20458 provided in two formulations, "thick-wall" and "thin-wall", by the Stauffer Chemical Co., Palo Alto, California. Figure 2 indicates that the thin-wall formulation showed higher initial activity, possibly due to a higher rate of release of the compound. However, activity declined more rapidly so that by the 4th day of incubation the thick-wall formulation was more active. Therefore the latter should be able to provide the longer residual activity required on asynchronous populations.

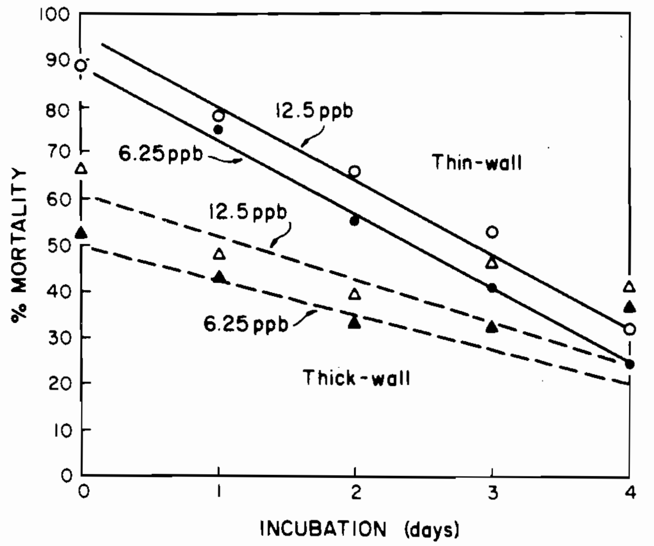

Figure 2.–Rate of loss of activity of R-20458 "thick-wall" and "thin-wall" encapsulated formulations evaluated by continuous exposure of 4th instar *C. tarsalis* larvae.

Effect of larval treatment on adult longevity, fecundity and fertility.–Records obtained on the rate of pupation of larvae following exposure to Altosid indicated that there was no difference between treated and control groups. However, extension of the study to the post-emergence period indicated that treatment of 4th instar larvae affected the adult adversely in terms of longevity, fecundity and fertility. The results of detailed records obtained daily on two treated groups (0.25 and 0.5 ppb representing LC_{35} and LC_{53} levels, respectively) and one control group led to the following conclusions: At 0.25 ppb, longevity of females was reduced by 18% and of males by 17% relative to the control. At 0.5 ppb corresponding reductions were 24% and 20%. Fecundity and fertility were also depressed more strongly by the 0.5 ppb treatment. Thus total egg production per female was reduced by 54% and egg hatchability by 43% relative to the control, representing an overall reduction of the F_1 generation by 73%. It is obvious that such suppression of the biotic potential of treated populations constitutes a considerable "bonus effect" of IGR application. Similar effects of Altosid on longevity, fecundity and fertility were noted by Cerf in house flies (Cerf 1973).

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A NEW CONCEPT IN MOSQUITO IDENTIFICATION: THE CIRCULAR MOSQUITO KEY

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The Circular Mosquito Key is intended to serve as an aid in field and laboratory identification of common mosquitoes. It is not intended as a replacement for more complete keys for precise laboratory identification. Due to the design of the key, only about eight species of mosquitoes can be represented. The most commonly encountered and important species in an area are usually on the key. There are presently four keys available for different regions of the state. They are: (1) North Coastal, (2) South Coastal (3) Central Valley, and (4) Southern Interior. In some districts with overlapping geographical areas, it may be necessary to utilize more than one key.

USE.--The key has two sides. One is for identifying adult female mosquitoes. The opposite side is for identifying fourth instar larvae. The key is used by turning the smaller wheel until the triangular mark on its margin corresponds with the mark on the inner wheel. Minor adjustments may be necessary to provide a clear picture.

Characteristics necessary for proper identification are found in the various cut-outs in the wheel. In addition, the adult wheel has two cut-outs entitled "Identification Information" and "Biological Notes." Information in these sections should provide additional assistance in identification. Arrows are used to point out some of the most important characteristics on the larval key. Some larval habitat data are presented under the "Biological Notes" section on the adult key. The common names used on the key are not all "accepted," but are used by many workers in the field.

COMPARISON OF LIGHT TRAP AND HUMAN-BITING CAPTURES OF ANOPHELINES IN EL SALVADOR

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ABSTRACT

Captures of *Anopheles* (primarily *A. albimanus*) by the standard New Jersey, the CDC miniature, and a new ultra violet/updraft light trap were compared in several field locations. Concurrent human-biting collections of *A. albimanus* were usually much higher than the best trap catch. However, when the *A. albimanus* population density was low, the ultra violet trap catch often equalled and occasionally exceeded the human-biting collection. The parous rate of trap-caught females averaged nearly twice that of females coming to bite, suggesting that newly emerged and older females respond differently to the two types of attractant.

THE ZOOGEOGRAPHY OF MOSQUITOES IN FLORIDA

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ABSTRACT

Major point made is that Florida's zoogeography is very simple: it is where the western hemisphere's NEARCTIC region meets its NEOTROPICAL region in the Atlantic Ocean. The line is traditionally placed between Florida and Cuba, but I prefer placing it between mainland Florida and the Florida Keys because the climate of the Keys fulfills the two criteria most often used to define the tropics climatologically: (1) no record of frost, and (2) January isotherm above 70°F.

Florida's 67 mosquito species are predominantly nearctic forms. Twenty-four (24) species find their southern limits in Florida and seventeen (17) their northern limits. Roughly 60% of our mosquitoes are of nearctic origin and 40% of neotropical. Most faunistic and floristic studies of Florida as a whole have concluded that the ratio of tropical to non-tropical origin is not too far removed from 50-50.

THE LIKELY MOSQUITO VECTORS OF DOG HEARTWORM IN A COASTAL AREA OF SOUTHERN NEW JERSEY

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ABSTRACT

During the summer of 1973, an effort was made to locate the natural vectors of dog heartworm in a coastal area of Southern New Jersey where the disease constitutes a serious veterinary problem. Mosquitoes were collected weekly from June through September. Data were collected on population succession, parity rates, longevity and incidence of filarial parasites. Infective stage filariae were recovered and preserved for species identification. Developing forms of the parasite were recorded but not saved.

Gross morphological characteristics of the infective stage larvae indicated that more than one species of filarial worm was present in the mosquito population. All specimens have been submitted for species identification but confirmation of *Dirofilaria immitis* had not been received at the time of this writing.

Data indicated that *Aedes vexans* and *Aedes sollicitans* showed a very low vector potential for the transmission of filarial worms. Both of these species regularly contained developing filarial larvae in the malpighian tubules but neither species appeared to live long enough to sustain complete development to the infective stage.

Aedes cantator and *Culex salinarius* showed a moderate vector potential. In each case, infective stage filarial larvae were found in a very small percentage of wild-caught mosquitoes in mid-summer. Data indicated that very few mosquitoes lived long enough to sustain complete development to the infective stage.

Aedes canadensis was the only mosquito collected during this investigation which showed a high vector potential for the transmission of filarial worms. The species appeared to be extremely long lived and infective stage filarial larvae were found in every collection of wild-caught specimens from mid-June through September. The incidence of filarial parasites showed a direct correlation with parity rates and peak transmission periods occurred when mosquito populations were at their lowest.

INDUCED TRANSLOCATIONS IN *CULEX TARSALIS* FOR POSSIBLE USE IN CONTROL SYSTEMS

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ABSTRACT

Six radiation-induced heterozygous chromosomal interchanges have been cytologically identified in laboratory strains of the encephalitis vector, *C. tarsalis*. One, now in the homozygous condition, is considered as a possible vehicle that could serve a dual function: act as an autocidal mechanism for reducing a field population, and as a transport device for introducing desirable genotypes into the population. Since individuals from different field populations as well as colonized strains of *C. tarsalis* vary greatly in their susceptibility to infection with Western Equine Encephalitis virus and/or their ability to transmit WEE virus, a strain selected for refractoriness in either ability is being considered as a potential genotype to genetically combine with the homozygous translocation described.

MORTALITY OF *CULEX TARSALIS* MOSQUITO LARVAE OBTAINED IN LABORATORY STUDIES USING VARIOUS COMBINATIONS OF *BACILLUS THURINGIENSIS* (HD-1) WITH TWO GROWTH REGULATORS

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ABSTRACT

In laboratory studies, mortality of *Culex tarsalis* mosquito larvae has been obtained by the use of combinations of narrow spectrum control agents. These control agents are:

1. *Bacillus thuringiensis* (HD-1). Effective larvicidal activity was obtained when applied to early larval instars ($\sim 10^6$ spores/ml). Application of adequate aqueous concentrations results in larval mortality, supplemented by delayed pupation and reduced levels of viable adult emergence.
2. Monsanto 585. When dispersed as microdroplets at a sufficient level (nominally 0.1 μg/ml), this compound affects early 4th instar larvae to produce early pupal mortality, i.e., mortality immediately following the larval-pupal transition, supplemented by delayed pupal mortality and defective adult emergence in survivors.
3. Altosid SR-10. This is a microencapsultated formulation which is effective as a colloidal aquatic dispersion at an aqueous level of nominally 0.1 ug/ml when introduced prior to pupation. This results in late pupal mortality, i.e., pupae die just prior to the pupal-adult transition. Pupal mortality is supplemented by the emergence of increased numbers of defective adults.

Combinations of these three larval control agents are suggested since each demonstrates a different primary mode of action, i.e., larval mortality, mortality at the larval-pupal transition, and mortality at the pupal-adult transition, thus potentially requiring three independent mutations for significant resistance to develop against combinations of these control agents.

INTRODUCTION.--The rapid development of resistance in mosquitoes resulting from the sequential use of broad spectrum chemical insecticides indicates that future control may require the combined use of multiple, narrow spectrum control agents to obtain a beneficial parasite-predator-pest balance.

In laboratory tests, we successfully killed *Culex tarsalis* mosquito larvae by three narrow spectrum control agents employed in combination. We plan to conduct similar trials in the field. These control agents are:

1. *Bacillus thuringiensis* (HD-1).
2. Monsanto 585.
3. Altosid SR-10

Combinations tested against *Culex tarsalis* larvae yielded larval or pupal mortality in excess of 90%. These combinations demonstrated an independent or simple additive mode of action. Therefore, we expect that these combinations should minimize the appearance of resistant mutants following extended field usage.

For field application, various practical problems remain to be solved, including aquatic persistence, bio-degradation, and photo-degradation. Potentially successful methods are presented for the resolution of these problems.

Each of the three agents is discussed separately and data are presented suggesting that such compounds be employed in pairs or in a balanced triple formulation.

The recent publication of Reeves and Garcia (1970) on the pathogenicity of bi-crystalliferous isolate (BA068) of *Bacillus thuringiensis* (BT), coupled with our isolation of an equally toxic crystalliferous strain from an infected colony of *Aedes dorsalis* at Fort Baker, California, suggested screening of available strains of BT for mosquito larval toxicity (Norris 1969) in view of the contradictory data reported in the literature (Kellen and Lewallen 1960, Liles and Dunn 1959, Rogoff and Ignoffo 1969, Shaikh and Morrison 1966).

Contrary to published data, isolates of *B. thuringiensis* grown for 48 hours at 30°C on nutrient agar (Difco) possessed larvicidal activity comparable to BA068 against certain strains of mosquito test larvae, i.e., *Culex tarsalis*, *Aedes dorsalis* and *Aedes taeniorhynchus*. No activity was observed against *Culex pipiens* (Singer 1973). Isolates having larvicidal activity are summarized in Table 1; isolates which were not larvicidal are shown in Table 2. These data show that larvicidal activity is not associated with any single sero type.

To determine the mode of action of BT in relation to field stability, in particular to photo-degradation, a series of tests of BA068 was undertaken as a function of U.V. (2537Å) exposure. These data are summarized in Figure 1. Although viability of the irradiated test suspension was reduced from 3.6 x 10^9/ml to 3.8 x 10^5/ml, a reduction of four orders of magnitude, no measurable reduction in larval toxicity could be observed with *Culex tarsalis*. The toxicity of this BT spore is not correlated with its viability and is not associated with an infectious process but probably is associated only with the endotoxin. Additionally, the spore endotoxin showed a high order of photo-stability.

The experimentally observed dose response can be mathematically defined as a "single-hit" dose response, i.e., there is a single critical chemical site which if interfered with or chemically blocked can result in death of the larvae. Such a dose response has a regression line of unit slope (Goldberg).

If the BT test spore is present prior to the introduction of larvae, bio-degradation may also occur. Using a nutrient liquid containing 10^8 bacteria/ml the ED_{50} dose of the initial BT test challenge was evaluated as a function of the number of days during which the BT spores were present prior to the introduction of test larvae. These data are summarized in Figure 2, showing that bio-degradation occurs in an aquatic environment which supports microbial flora. A

Table 1.–Summary of screening of test culture of BT for larvicidal activity against *Culex tarsalis* (KL) 1st instar test larvae: Isolates found to demonstrate larvicidal activity against *C. tarsalis* (KL) 1st instar test larvae.

Original Designation	Serotype	Code Number
kurstaki	H3a, 3b•	(2536-9693TW)
kurstaki	"	(2819-9763F)
aizawai	H7•	(1850-9762C)
tolworthi	H9•	(NPI-460)
sotto	H4a, 4b	(NPI-180-5-2)
thuringiensis	H1	(NPI-186-104)
thuringiensis	"	(NPI-185-104)
thuringiensis	"	(NPI-201-113)
thuringiensis	"	(NPI-197-105)
thuringiensis	"	(NPI-198-105)
thuringiensis	"	(NPI-199-105)
sotto	H4a, 4b	(NPI-194-101)

• Cultures marked with an asterisk have had recent confirmation. The remainder are based on information available before 1963, and therefore, there is a possibility those classifications are not accurate

parallel test of aqueous stability in distilled water at room temperature over a period of weeks showed no loss in larval toxicity.

The sensitivity of test larvae is affected by larval age at the time of challenge. "Larval age" is defined as the time span of 1st through 4th instar, since pupae are not sensitive to BT. Figure 2 also indicates that the larvicidal activity of BT is highest against the first two larval stages. Even if the problem of aquatic bio-degradation were solved, the current strain of BT would be of possible practical use only if applied prior to the aquatic development of the 3rd larval instar.

Preliminary unpublished studies show that larvicidal activity of BT is dependent on water temperature, efficacy increasing with higher water temperatures. Therefore, most of the tests were conducted at a temperature of 25°C (77°F).

The application problems encountered with BT are associated primarily with aquatic bio-degradation. We are investigating microencapsulated formulations (Ignoffo and Batzer 1971, Markin and Hill 1971, Miller and Gordon 1972, Rann and Jackson 1966) which should minimize this factor, in an effort to find one which will retain a high level of larval toxicity. To date we have not succeeded. In addition, new strains of BT or other microbial species may be selected which will have higher levels of toxicity and a broader or different spectrum of activity (Davidson and Singer 1973, Singer 1973).

A summary of laboratory findings with Monsanto 585 follows:

Sacher (1971) and Jackob (1972), and Jakob and Schoof (1971, 1972) note that metamorphosis was blocked in the early stage of pupation and the resulting pre-pupae die in a characteristic compact, stalky and unmelanized form (Sacher 1971). They also observed that the larvae would develop normally if M-585 was introduced during the late 4th instar. Since this mode of action differs from that of BT, the authors selected M-585 for use in combination with BT (HD-1).

Table 2.–Summary of screening of test culture of BT for larvicidal activity against *Culex tarsalis* (KL) 1st instar test larvae: Isolates found to be inactive against *C. tarsalis* (KL) 1st instar test larvae.**

Original Designation	Serotype	Code Number
kurstaki	H3a, 3b•	(720619A)
B thuringiensis var. ?	H5a, 5b	(730130-1)
thuringiensis	H1•	(1840-182C)
finitimus	H2•	(NPI-451)
subtoxious	H6•	(NPI-456)
entomocidus	H6•	(NPI-457)
aizawai	H7•	(NPI-458)
morrisoni	H8•	(NPI-459)
darmastadiensis	H10•	(NPI-461)

• Cultures marked with an asterisk have had recent confirmation. The remainder are based on information available before 1963, and therefore, there is a possibility those classifications are not accurate.
••Selected from 64 negative results to illustrate range of serotype.

A formulation was prepared as follows: 0.1 ml of technical M-585 (3 lb/gal) was added to 1 ml of acetone. This solution was forcibly injected into 100 ml of distilled water using a 2 ml syringe fitted with a 27 gauge needle. This procedure produced a colloidal suspension which upon 10-fold dilution was used for U.V. exposure studies, thus reducing screening effects due to turbidity.

Data from two experimental series are summarized in Figures 3 and 5. Figure 3 shows dose/response data of test challenges of Monsanto 585 when the micronized chemical was added to the larval environment at selected days between the 1st to the late 4th stages of development. No degradation occurred as shown by the observed dose responses when the chemical was introduced prior to the critical phase or "deadline" during the early 4th instar. The dose response observed corresponds to a "2-hit" model, i.e., two independent chemical sites simultaneously blocked in order to obtain a lethal response. A single hit dose response compared with the observed 2-hit response has been added for reference (Figure 4). The single and two hit curves become closely asymptotic for dose response levels greater than 90%. From a practical point of view, an additive mode of action results.

Figure 5 summarizes the dose/response data when M-585 micronized aqueous suspension was exposed for up to four hours of 2537Å radiation, simulating the effects of prolonged exposure to sunlight. The U.V. levels were approximately three times as great as those used in the reported BT studies. The authors estimate that four hours of exposure to 2537Å corresponds to one week of daily exposure to average sunlight. A 2-fold reduction or less is detectable from a four hour test exposure to 2537Å.

Zoecon's SR-10 microencapsulated formulation was exposed in parallel with M-585 so the data are presented in Figure 6 for direct comparison. This chemical showed no significant sensitivity to photo-degradation.

In the laboratory studies, Monsanto 585 showed good persistence against bio-degradation and to sunlight as simulated by a 2537Å germicidal lamp, measured by bioassay using *Culex tarsalis* larvae.

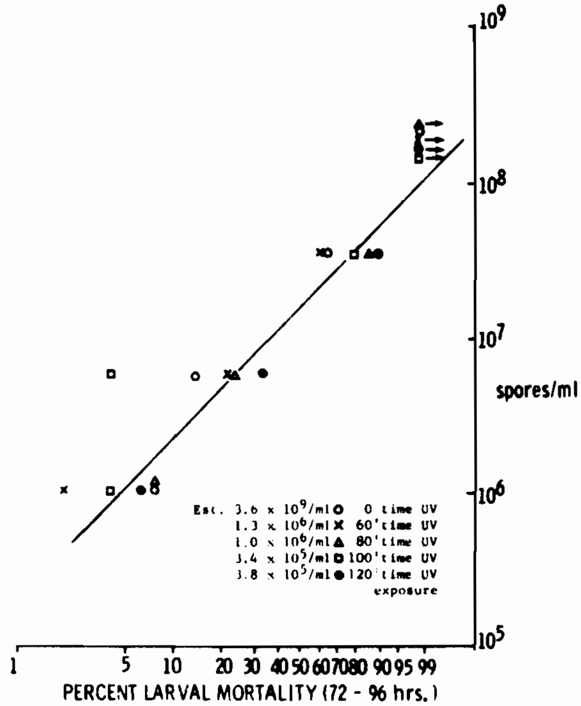

Figure 1.–Mortality in *Culex tarsalis* larvae, challenged at the 1st instar with BA068, which has been exposed for various time periods to germicidal ultra violet radiation (2537Å).

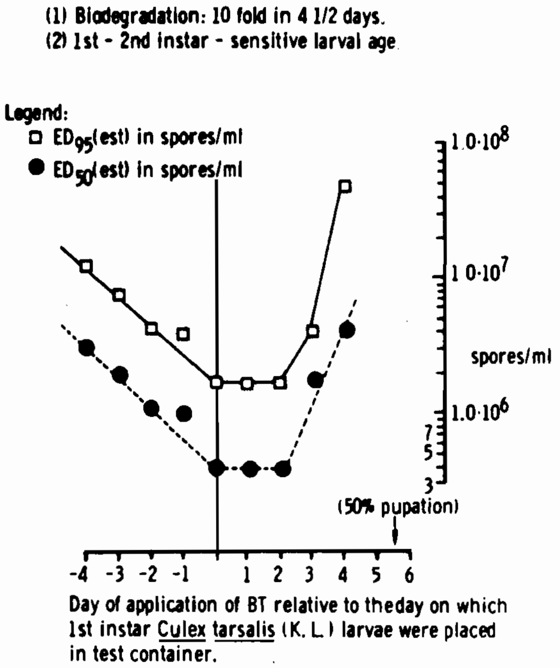

Figure 2.–Estimated ED_{50} and ED_{95} dose, using technical I.M.C. BT (HD-1) for test challenge, as a function of time of application relative to the time at which 1st instar *Culex tarsalis* larvae were placed in the test container.

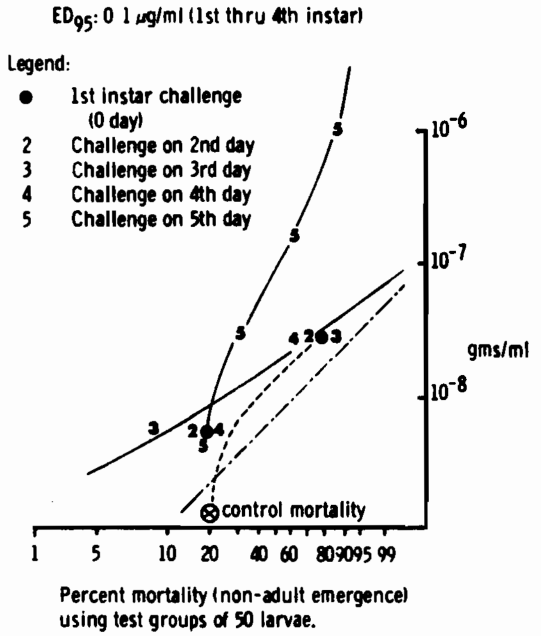

Figure 3.–Dose response of Monsanto M-585 growth regulator applied on 0, 2, 3, 4, 5th day of larval growth of *Culex tarsalis* (KL) 1st instar larvae: 50% pupation observed on the 6th day.

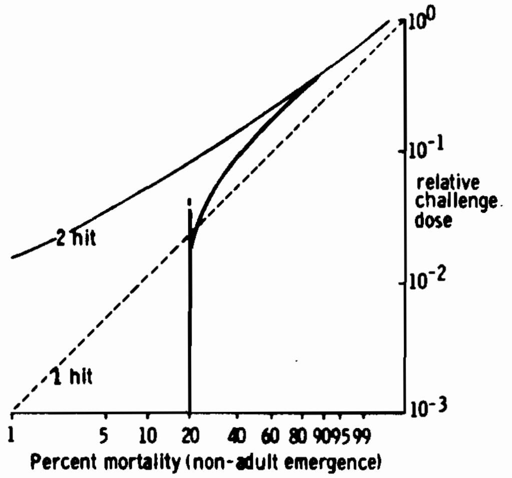

Figure 4.–One and two hit dose response curves as a function of challenge dose: correction resulting from 20% control mortality.

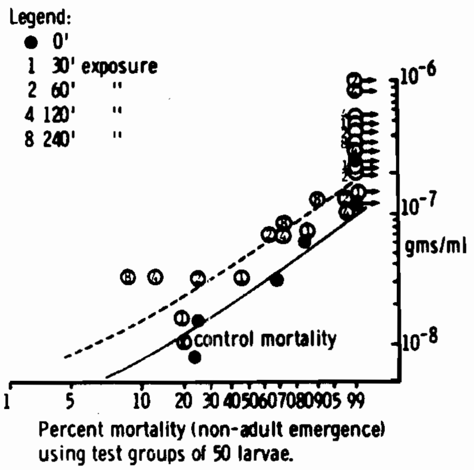

Figure 5. Stability of an aqueous suspension of M-585 exposed at 28 cm to a 30 W (GE 30T8) germicidal lamp (2537Å) for periods of 0 to 240 minutes, using 1st instar *Culex tarsalis* (KL) test larvae.

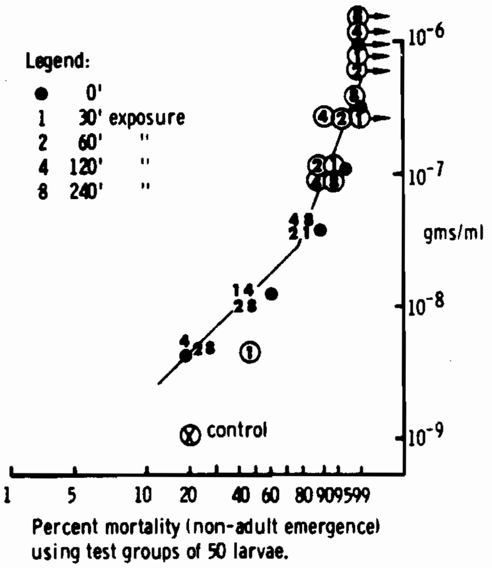

Figure 6.–Stability of an aqueous suspension of SR-10 exposed at 28 cm to a 30 W (GE 30T8) germicidal lamp (2537Å) for periods of 0 to 240 minutes, using 1st instar *Culex tarsalis* (KL) test larvae.

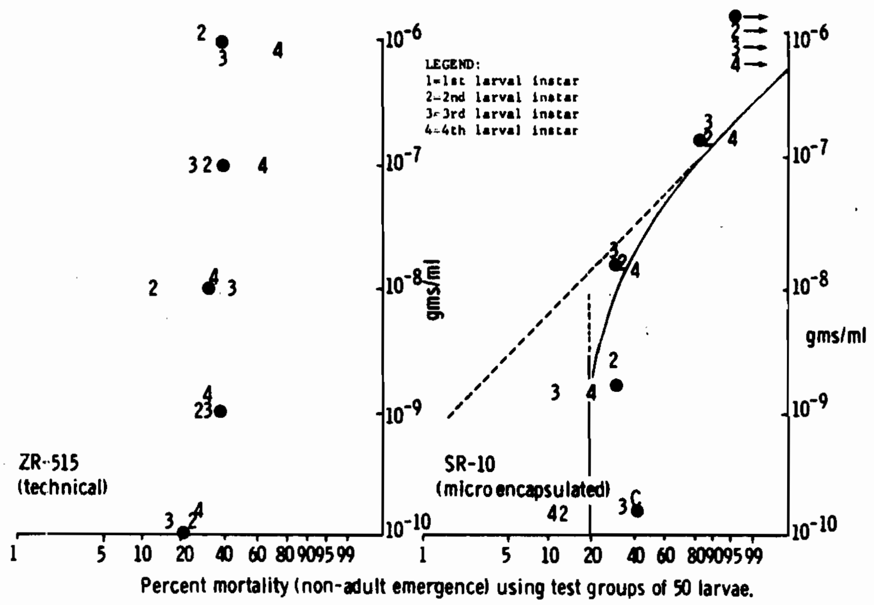

Figure 7.–Dose response to Zoecon ZR-515 and SR-10 growth regulator formulations as a function of mean larval instar age, using *Culex tarsalis* (KL) test larvae.

Laboratory data for the Zoecon growth regulator in technical and microencapsulated formulation are presented in Figure 7. Thomas Graves, who developed this formulation, by suitable microencapsulation was able to reduce biodegradation to a negligible level for laboratory aquatic exposure of one to two weeks. The level of microbial flora was ∿10^8 bacteria/ml.

These data indicate that the activity of the microencapsulated formulation is independent of larval instar age. The application of SR-10 within a few hours prior to pupation was usually as effective as aquatic application made during the 1st instar. Aquatic persistence of the material is more important than early challenge. This chemical is optimally effective when present just prior to pupation. It is not effective when application occurs following pupation.

Following larval challenge with SR-10, the pupae at first appear to be healthy but are unable to complete emergence. Deformed adults also occur but it is doubtful that this effect is important in practical control. One observes a single hit dose response thus suggesting a single but different chemical site of interaction than that of the protenaceous endotoxin of *Bacillus thuringiensis.*

SUMMARY.–The development of narrow spectrum agents should tend to reduce the development of resistance by field use of multiple control agents. By also employing the most advanced field application technology, it should be feasible to reduce dosage rates and keep control costs within reasonable limits, also minimizing the ecological side effects which occur when excessive quantities of toxic chemicals or microbial agents are used in control operations.

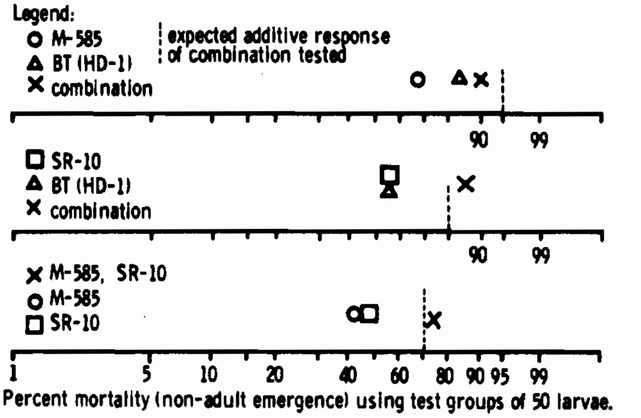

Figure 8.–Response of *Culex tarsalis* (KL) 1st instar larvae challenge to IMC BT (HD-1) in combination with Zoecon SR-10 or Monsanto M-585, and to M-585 in combination with SR-10.

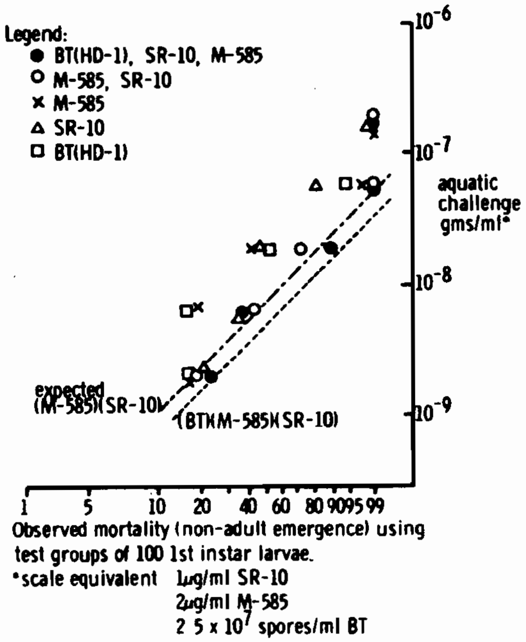

Figure 9.–Dose response of IMC BT (HD-1), Zoecon SR-10, and Monsanto M-585, in single, double and triple formulation.

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LABORATORY AND FIELD EVALUATION OF INSECT GROWTH REGULATORS AGAINST MOSQUITOES

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Insect growth regulators, which include juvenile hormone type compounds, overcrowding factors, and trans-specific regulating factors, have been recently made the subject of intensive studies for mosquito control. These materials, possessing good margin of safety to man and nontarget organisms, show good promise for mosquito control in the near future. A variety of unrelated compounds, including growth and morphogenetic regulating factors, have become available for investigations. The efficacy of these against mosquitoes is discussed below.

OVERCROWDING FACTORS.—There are a number of waste products produced by mosquitoes which are detrimental to their own kind. Chemical identification of a few of these in detectable quantities in larval cultures are: substituted fatty acids and substituted and straight chain hydrocarbons.

Several substituted fatty acids were synthesized and evaluated for effectiveness against mosquito larvae. Positions of the substituent and chain length were very important in investing biological activity. Among the four analogs studied, 2-n-butyl substituent on a 12-carbon acid showed the greatest activity. The same substituent on a 10-carbon acid was much less active. Young mosquito larvae exposed to 0.1-1.0 ppm remained young (no further growth) for many days before they died. Substituted long chain hydrocarbons were found to be much more active than their straight chain analogs. A good feature of these compounds is that they induce considerable prolongation of larval stages of mosquitoes. The feature might lend itself for practical consideration in the control of floodwater mosquitoes where temporary water will dry up before completion of the larval cycle.

To determine the most effective compounds in this group, various analogs having different chain lengths and substituents have to be synthesized and evaluated against mosquito larvae.

JUVENILE HORMONES, THEIR ANALOGS AND MIMICS.—A number of highly effective compounds have become available in the past year or two. Some of these materials are related to the terpenoid insect juvenile hormone compounds, while others quite unrelated to these induce growth abnormalities and inhibition of development similar to that of insect juvenile hormones.

Studies on the efficacy of juvenile hormone analogs and mimics were conducted in the laboratory, using larvae of the common house mosquito *Culex pipiens quinquefasciatus*.

The growth regulators R0-8-5497 and R0-20-3600 and R-20458 yielded good inhibition of adult emergence at 0.1 ppm. TH-6038 and TH-6040, two compounds closely related to the herbicide dichlorobenil, yielded complete inhibition of adult emergence at 0.05 and 0.01 ppm, respectively, when 4th stage larvae were exposed. The latter compound is a difluro analog, and has 10-20 times greater activity. Another distantly related compound, HE-24108, produced complete inhibition of emergence at 0.05 ppm. It is of interest to note that bulk of mortality in TH-6038 and TH-6040 occurred in the larval stage, but the other produced mortality in the pupal or adult stage. TH-6040 also manifested high level of activity against pupae, inducing mortality in both the pupae and adults.

GROWTH REGULATORS/MOSQUITOES IN FIELD.—R0-20-3600 (EC$_4$) was evaluated against *Culex tarsalis* in field plots at 0.25-1.0 lb/acre. The life cycle of this species is asynchronous (overlapping broads) and, therefore, behaves differently from *Aedes* species which are mostly synchronous. This material yielded about 95% inhibition of emergence of *C. tarsalis* during the first 24 hours and only 80% inhibition 48 hours later. The longevity of this compound and Altosid or ZR-515 was essentially the same, both being very short-lived under the experimental conditions. There was no difference between 0.25, 0.5 and 1.0 lb/acre rates. This indicates that the most important consideration is the availability of larvae in a stage susceptible to the growth regulator.

It is of interest to determine the exposure duration of susceptible larvae in fields where ZR-515 was applied at 0.1 ppm. Larvae exposed for various periods four hours after treatment suffered the greatest inhibition of adult emergence after an exposure of 2-3 hours. Shorter exposure did not inhibit adult emergence completely.

The inhibition of emergence or developmental events were studied by isolating a sample of 4th instars from each treatment in floating units (Figure 1). The larval chamber was provided with sections of strainer cloth screens to facilitate exchange of food and water but not larvae between inside and outside. A ventilated adult retaining chamber was affixed to the top of the larval chamber.

EFFECT OF GROWTH REGULATORS ON MOSQUITO FISH.—Replicated ponds stocked with five pairs of *Gambusia affinis* were treated with weekly applications of the growth regulator Altosid (ZR-515) and R0-20-3600 for a total of six treatments. Altosid EC$_{50}$ was applied at the rate of 0.25 and 0.5 lb/acre active ingredients, while R0-20-3600 was applied at 0.5 and 1.0 lb/acre active ingredient. After six weekly treatments, the ponds were drained and all adult and young fish were recovered from the treated and untreated ponds. The five pairs of fish reproduced equally in all treatments and yielded between 500-600 fish after six weeks in each pond. There was no significant difference between any of the treatments and the checks. No mortality of fish was observed during the study period.

Similarly, TH-6040 (at 0.1-0.5 lb/acre) and R-20458 (at 0.5 and 1.0 lb/acre), two other insect growth regulators were applied to stocked ponds weekly for nine weeks. After this period, the number of progeny produced in treated and untreated ponds was essentially the same.

From these preliminary data, it can be concluded that these four growth regulators at the indicated dosages will not interfere with the reproduction and survival of the mosquito fish.

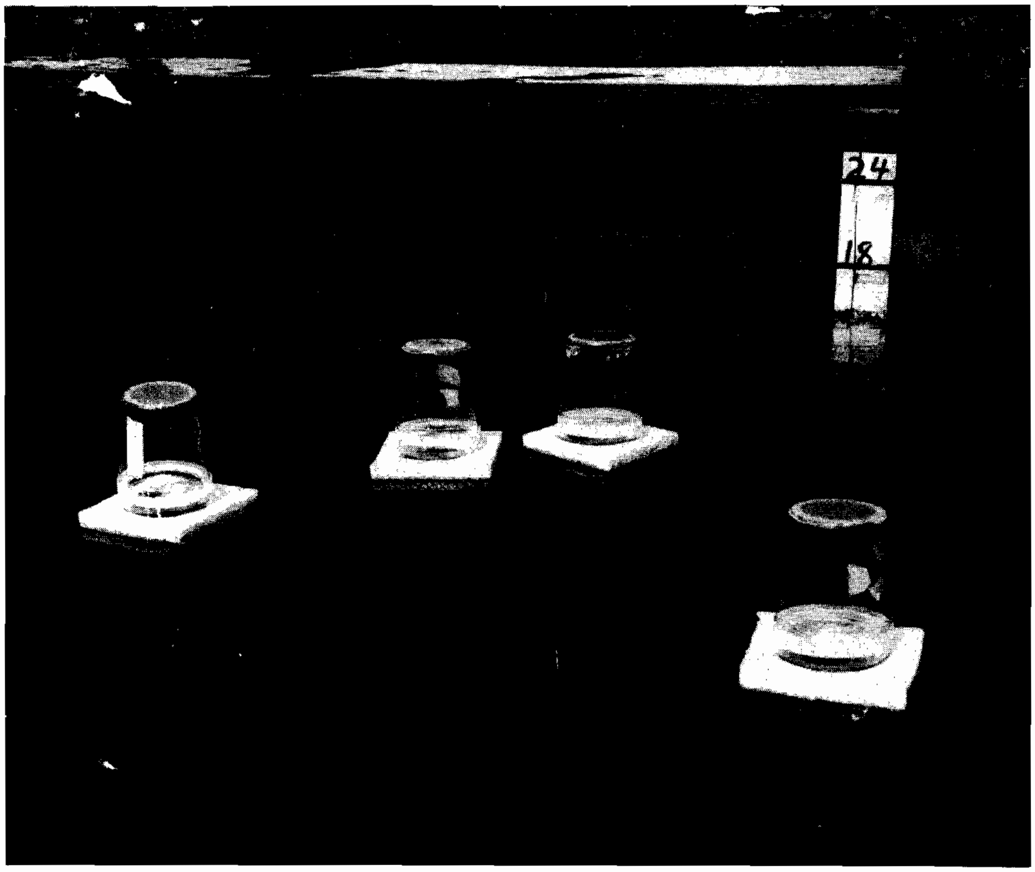

Figure 1.—Test units holding 4th-instar mosquito larvae for assessment of developmental events and emergence of adults. These units are employed in studies on the efficacy of insect growth regulators against mosquitoes.

TOXICITY OF TH-6040 TO FRESHWATER CRUSTACEA AND THE USE OF A TOLERANCE INDEX AS A METHOD OF EXPRESSING SIDE EFFECTS ON NONTARGETS

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TH-6040 (Other designations: PH 60-40, ENT-29054, and OMS 1804.) [1-(4-chlorophenyl)-3-(2,6-diflourobenzoyl)-urea] is an experimental insecticide with a wide range of biological activity. The mode of action of the compound against various insects including the yellow fever mosquito has been reported as the inhibition of chitin synthesis during molting, thus interfering in the formation of endocuticular deposition (Mulder and Gijswijt 1973, Post and Vincent 1973).

Its potential use as a mosquito larvicide (Jakob 1973, Schaefer et al. 1974) led to concurrent studies of its effects on the nontarget organisms commonly associated with mosquito breeding habitats (Miura and Takahashi 1974). This paper gives results of toxicity studies on freshwater crustaceans in the laboratory and outdoors in artificial containers. Also, the term "Tolerance Index (TI)" is proposed for comparing relative toxicity of any particular toxicant to nontarget and target organisms.

MATERIALS AND METHODS.—Mosquitoes (target organisms) used in this study were either from a laboratory colony (*Culex tarsalis* Coquillett) or were collected in the field (*Aedes nigromaculis* (Ludlow)). The nontarget organisms tested were collected in the field; they were maintained in separate containers, as colonies, or were acclimatized for 1 to 2 days in the laboratory (temperature ca. 22-26°C) before being used for a test.

Methods used for testing organisms in the laboratory were similar to those used by Schaefer and Wilder (1972) for mosquitoes and Miura and Takahashi (1973) for nontargets.

A simulated field test was conducted outdoors in four glass aquariums (5 gal. capacity). Two were tested with 0.05 ppm TH-6040 (technical material in acetone) and two others were left as controls. Techniques used for pre- and post-treatment population counts were previously described (Miura and Takahashi 1973).

Tolerance index (TI_{50}) is used to express the relative toxicities of a toxicant to nontarget organisms. It is defined as the ratio between the LC_{50} of a nontarget and that of a target (mosquito species) as follows:

$$TI_{50} = \frac{LC_{50} \text{ of a nontarget}}{LC_{50} \text{ of a target}}$$

Usually the LC_{50}'s and LC_{90}'s values are obtained from dosage-mortality lines on probit sheets. The relative toxicities of a toxicant against nontargets are better expressed by using both TI_{50} and TI_{90} values. Simplified hypothetical relationships between TI values and nontarget population trends are summarized in Table 1, and these relationships can also be expressed by using dosage-mortality lines (Fig. 1).

LC values for specific target and nontarget organisms are constant for a given compound, but the TI values for nontargets vary according to the target organisms. The TI value simply expresses the toxicity in terms of how much it deviates from that of the target. Thus, it measures relative side effects of toxicants against nontarget organisms.

RESULTS AND DISCUSSION.—Figures 2, 3 and 4 summarize the results of the simulated field studies. The water flea population was markedly reduced three days after treatment, but the population slowly recovered (Figure 2). Typical symptoms were lessened filter feedings and movements; reproduction was also suspended for a week.

Copepod populations responded to the treatment in the same way as did the water flea population, but the magnitude of reduction was small and the interval of the recovery period was short (Figure 3). The seed shrimp population showed no harmful effect (Figure 4).

Water fleas, copepods and shrimp (clam, tadpole and seed) are the most common and abundant organisms found in the mosquito breeding habitats in the San Joaquin Valley (Miura and Takahashi 1974). The side effects of TH-6040 against crustaceans in the laboratory are calculated in terms

Table 1.—Simplified hypothetical relationships between TI values and nontarget populations.

Nontarget	TI_{50}[a]	TI_{90}[b]	Effects on populations
A	>1	>1	Usually safe; safety degrees are proportioned to TI values
B	≦1	≦1	Population may be exterminated
C	≧1	<1	Population may be exterminated
D	≦1	>1	Some mortality; population will recover
E	>1	1	Population may be exterminated
F	<1	1	Some mortality; population will recover

[a] $TI_{50} = \frac{LC_{50} \text{ nontarget}}{LC_{90} \text{ target}}$ [b] $TI_{90} = \frac{LC_{90} \text{ nontarget}}{LC_{90} \text{ target}}$

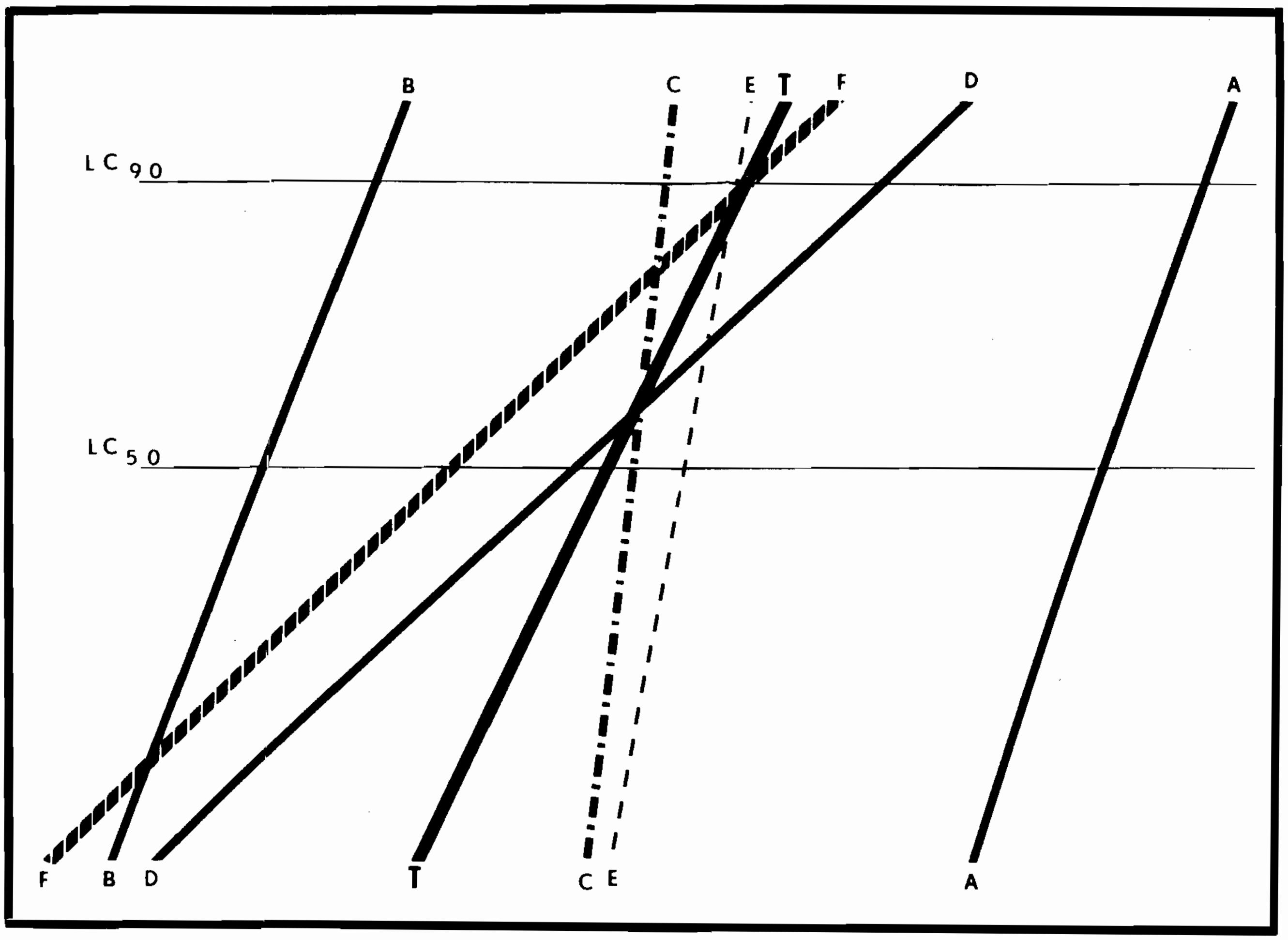

Figure 1.—Hypothetical dosage-mortality relationships of a toxicant (T) to six different nontarget organisms (A, B, C, D, E and F).

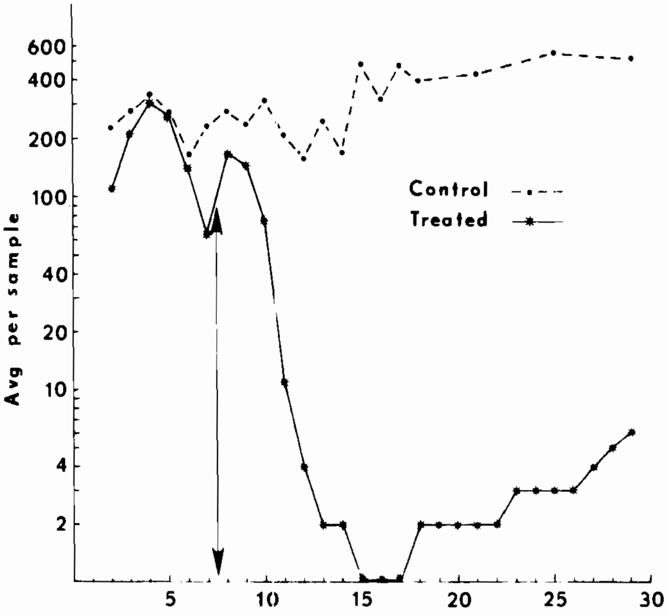

Figure 2.—Biological activity of TH-6040 against water fleas. Samples were taken before and after treatment (↑).

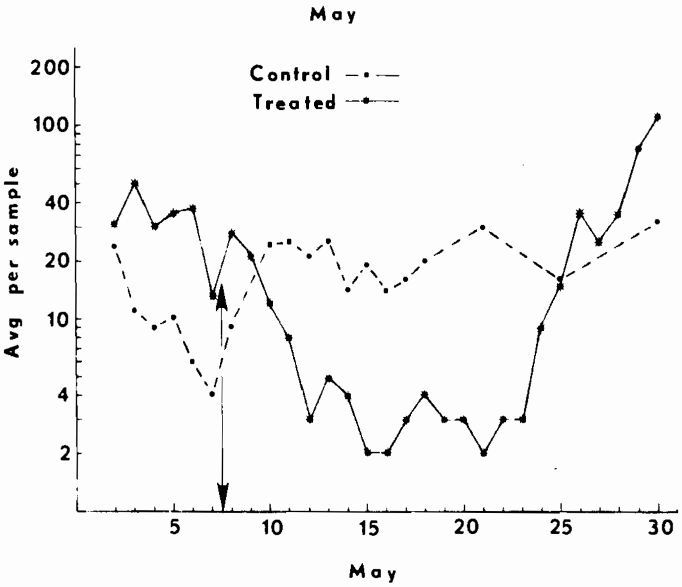

Figure 3.—Biological activity of TH-6040 against copepods. Samples were taken before and after treatment (↑).

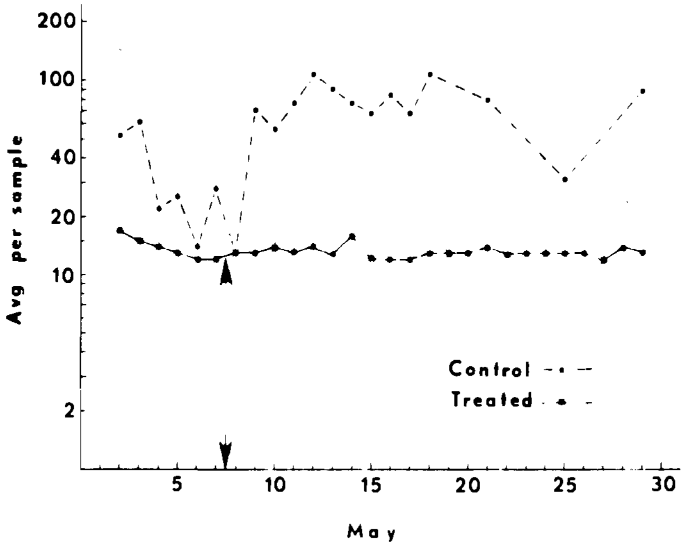

Figure 4.–Biological activity of TH-6040 against seed shrimp. Samples were taken before and after treatment (↑).

of TI values and shown in Table 3. When TH-6040 is used at the required rate for *A. nigromaculis* control, tadpole and clam shrimp populations may be reduced, but will probably recover. When TH-6040 is applied for *C. tarsalis* control, the side effects may not be as severe.

The report by Miura and Takahashi (1974) shows that when TH-6040 (a 25 WP formulation, at 0.25 and 0.01 lb AI/acre) was applied with a hand sprayer to small shallow intermittent ponds, ca. 0.028 acre in size, tadpole and clam shrimp populations were eliminated from the ponds. While water flea populations were markedly reduced, they recovered within a week.

Tolerance index can be used for comparing side effects of two or more toxicants against nontargets. For example, Table 4 summarizes the laboratory study on side effects of Altosid® against crustaceans. In comparison with TH-6040, the biological activity of Altosid is highly selective; when it was applied at the rates for mosquito control, there was no measurable deleterious effect on the fresh water crustaceans.

Table 2.–Biological activity of TH-6040 against fresh water crustacea.

Crustacea	Stage	LC_{50} (ppm)	Exposed (hr)	No. Test
Water fleas	Mixed	1.8×10^{-3}	24	3
Clam shrimps	Mixed	3.8×10^{-4}	24	4
Tadpole shrimps	Juvenile	6.4×10^{-4}	24	3
Copepods[a]	Mixed	10 (Ca.)	24	3
Seed shrimps[a]	Mixed	5 (Ca.)	24	1

[a] The succession of concentrations used was too low to determine the LC_{50} level.

Table 3.–Expected side effect of TH-6040 mosquito larvicide against some fresh water crustacea.

A. Target: *Aedes nigromaculis*	$LC_{50} = 7.2 \times 10^{-4}$,	$LC_{90} = 3 \times 10^{-3}$ (ppm)
Nontarget	TI_{50}	TI_{90}
Water fleas	2.50	2.27
Clam shrimp	.53	.47
Tadpole shrimp	.89	.47
B. Target: *Culex tarsalis*	$LC_{50} = 7 \times 10^{-4}$,	$LC_{90} = 1.4 \times 10^{-3}$ (ppm)
Nontarget	TI_{50}	TI_{90}
Water fleas	2.57	4.86
Clam shrimp	.54	1.00
Tadpole shrimp	.91	1.00

Table 4.—Expected side effect of Altosid® against some fresh water crustacea.[a]

A. Target: *Aedes nigromaculis*	$LC_{50} = 8 \times 10^{-6}$,	$LC_{90} = 1.2 \times 10^{-4}$ (ppm)
Nontarget	TI_{50}	TI_{90}
Water fleas	1.12×10^5	6.66×10^4
Clam shrimp	1.25×10^5	2.08×10^4
Tadpole shrimp	5.87×10^5	4.16×10^4
B. Target: *Culex tarsalis*	$LC_{50} = 3 \times 10^{-4}$,	$LC_{90} = 3.7 \times 10^{-3}$ (ppm)
Nontarget	TI_{50}	TI_{90}
Water fleas	3.00×10^3	2.16×10^3
Clam shrimp	3.33×10^3	6.76×10^2
Tadpole shrimp	1.56×10^4	1.35×10^3

[a] Data are taken from Miura and Takahashi 1973.

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CONVENTIONAL AND NEW INSECTICIDES FOR THE CONTROL OF CHIRONOMID MIDGES

R. L. Norland[1], M. S. Mulla[2], F. W. Pelsue[3] and T. Ikeshoji[4]

INTRODUCTION.–In recent years aquatic midges of the family Chironomidae have posed an increasing nuisance problem for residents of southern California. Population expansion has resulted in the crowding of residential dwellings and business establishments near flood control channels, water spreading basins, and municipal reservoirs. Descriptions of various problem situations were outlined by Grodhaus (1963).

Previously many insecticides were effective in controlling midge larvae. Since the development of resistance to chlorinated hydrocarbons (Lieux and Mulrennon 1956), organophosphates have been employed almost exclusively (Anderson et al. 1965, McFarland et al. 1962, Mezgar 1967, Mulla and Khasawinah 1969, Mulla et al. 1971, 1973). In recent years most of the available organophosphates lost their effectiveness against one or more nuisance species of Chironomidae. In the Los Angeles area *Chironomus* sp. 51 (*Ch. attenuatus* complex) has developed resistance to nearly all available organophosphates and has become the major nuisance species (Pelsue and McFarland 1971). Malathion, which is toxic to fish and ineffective against most midge species in this area, shows fair activity against *Ch.* sp. 51 (Pelsue and McFarland 1971). Tests are currently under way to find safer and more effective compounds for controlling this pest.

This paper reports the findings of laboratory and field evaluations of some newer and some commercially available compounds against *Ch.* sp. 51 and species of other genera of Chironomidae.

METHODS AND MATERIALS.–In the laboratory the insecticides were bioassayed against susceptible and resistant strains of *Chironomus* sp. 51. The susceptible colony has been maintained in our laboratory for several years. The resistant colony of *Ch.* sp. 51 was derived from larvae collected from the Coyote Creek area of southeastern Los Angeles County. One test was conducted using uncolonized larvae brought directly from Coyote Creek. Technical grade materials were dissolved in acetone and administered to 4th-instar larvae in wax-treated paper cups and larval response noted according to the technique of Mulla and Khasawinah (1969).

Field evaluation of Cidial® (ethyl mercaptophenylacetate, 0-dimethyl = phosphorodithioate) was conducted in flood control channels (concrete bottom, 12 ft wide, 1 to 2 in. water) in the Canoga Park area of Los Angeles County. Two sites were chosen to assess the efficacy of 2% Cidial celite granules. The two lengths of channel comprised approximately 1-acre plots and were treated by means of a U. S. Borax Spreader®. Two 3-in x 6-in scoop-dredge larval samples were taken to the downstream limit of each plot. Check samples were obtained above each plot. Samples were taken to the laboratory where they were washed and counted on a midge counting tray. Samples were taken before and 72 hours after treatment.

Granular and emulsifiable formulations of Cidial were compared for midge activity in Westlake, a man-made lake west of Los Angeles. This lake has been used earlier for testing purposes by the authors and is described by Mulla et al. (1971, 1973). Malathion granules and emulsifiable formulations were compared. Cidial 2% celite and malathion 1% 20-mesh sand-core granules were applied by a Borax Spreader from the front of a motorboat. Cidial EC_4 and malathion EC_5 were diluted in one gallon of water and sprayed into the propeller wash of the boat. A series of fingers of the lake served as treatment plots. Two fingers were employed for each treatment and two were left as checks. Two 5-in x 6-in Ekman dredge samples were obtained from each plot. These were washed through a 50-mesh sieve (Bay 1965) and the material on the screen was taken to the laboratory where it was floated and counted (Mulla et al. 1971).

Species composition, determined from adults reared from sampled larvae, was almost exclusively from the subfamilies Tanypodinae and Orthocladiinae. These included *Procladius bellus* (Loew), *Tanypus grodhausi* Sublette, *Tanypus carinatus* Sublette, and *Cricotopus* sp. Only an occasional *Chironomus* sp. 51 and *Ch. attenuatus* Walker were encountered. Samples were obtained before and 1 week after treatment. Fish mortality was also recorded in each plot. The species were bluegill (*Lepomis microchirus* Rafinesque) and large-mouth bass (*Micropterus salmoides* la Cépède).

Chemical descriptions of those compounds which have no common names are as follows:

Azodrin:	3-hydroxy - N-methyl-cis-crotonamide dimethyl phosphate.
Bas 2350-I:	3,5-diethylphenyl-N-methylcarbamate.
Ciba C-9491:	0-(2,5-dichloro-4-iodophenyl) 0,0-dimethyl phosphorothioate.
Dasanit:	0-0-diethyl 0-p-[(methylsulfinyl)-phenyl] phosphorothioate.
Gardona:	2-chloro-1-(2,4,5-trichlorophenyl) vinyl dimethylphosphate.
Lannate:	S-methyl-N-[(methylcarbamoyl) oxy] thioacetimidate.
Landrin:	3,4,5-trimethylphenyl methylcarbamate.
Methyl Trithion:	S[(P-Chlorophenylthio)-methyl] 0,0-dimethylphosphorodithioate.
N-2596:	0-ethyl S-(p-chlorophenyl) ethylphosphorodithioate).

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NIA-18729:	(bioresmethrin) 5-benzyl-3-furyl methyl-2,2-dimethyl-3- (2-methylpropenyl (cyclopropanecarboxylate, (+) trans isomer.
Phostex:	bis (dialkoxyphosphinothioyl) disulfides.
RU-11679:	[d-trans ethanochysenthemate of (5 benzyl-3-furyl alcohol)].
Supracide:	S-[(2-methoxy-5-oxo- Δ^2-1-3-4-thiadiazolin-4-yl) methyl] 0,0-dimethylphosphorodithioate.

RESULTS AND DISCUSSION.—Only two of the compounds listed in Table 1 showed any activity against the OP-resistant *Ch.* sp. 51 colony at 0.1 ppm concentration. Three compounds showing highest activity were re-evaluated against this species to obtain LC_{50} and LC_{90} values. These values along with those obtained from seven other compounds are given in Table 2. Of interest here is the effectiveness of malathion compared to the other standard and widely employed chemicals. The compound's high toxicity to fish, however, is not in its favor. Supracide, although displaying highest activity against this species, is a new organophosphate insecticide and needs further evaluation.

Of the compounds which might become available for the control of midges, Cidial and Supracide appear to be the most promising against *Ch.* sp. 51, but like malathion, these have low margins of safety to fish. Experiments were conducted to study the efficacy of Cidial in two field situations. The results of the flood control channel tests were promising (Table 3). In all cases except one, reduction was 88+% at 72-hr post-treatment.

In the Westlake study (Table 4), formulations of malathion and Cidial were studied. *Ch.* sp. 51 occurs here only occasionally, but nuisance situations arise from adult concentrations of tanypodines and orthocladines. Neither compound proved effective against these groups. Fish mortality resulted from the malathion treatment. Mortality was proportional to dosage rate; however, the emulsifiable formulation was generally more toxic than the granules. Significant mortality also occurred in the Cidial EC_4 treated plots.

Of the insecticides available for use by control agencies against *Chironomus* sp. 51, Cidial would appear to hold the most immediate promise. At effective control rates (0.1-0.25 lb/acre) Cidial is safe to fish when employed in granular formulation.

Among the organophosphate insecticides, Supracide and N-2596 are additional compounds which might prove useful for the control of chironomid midges. It is likely that these organophosphates, like malathion and Cidial, will not readily control tanypodine and orthocladine midges.

Table 1.—Forty-eight hour response of larvae of OP-resistant *Chironomus* sp. 51 (Coyote Creek colony) to various insecticides.[a]

Material	Percent Mortality At	
	1 ppm	.01 ppm
Thionazin	100	0
Phosphamidon	100	0
Azinphosethyl	100	4
Azinphosmethyl	0	0
Diazinon	0	0
Dioxathion	94	0
Ethion	0	0
Phostex	0	0
Carbophenothion	100	0
Methyl Trithion®	0	0
Dasanit®	60	0
Supracide®	100	100
Gardona®	0	0
Azadrin®	0	0
Ciba C-9491	100	0
Lannate	84	-
Methoxychlor	100	0
Landrin	100	0
Lindane	100	0
Bas 2350-I	100	0
NIA 18739	100	0
NIA 24110	100	0
NIA 26021	100	8
N-2596	100	84

[a] Twenty-five 4th-instar larvae per replicate, 2 replicates per concentration.

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Table 2.–Susceptibility of Coyote Creek and a susceptible strain of *Chironomus* sp. 51 to various organophosphate insecticides.

Insecticide	Lethal concentration (parts per billion)					
	Susceptible		Coyote Creek		Resistance Ratio[a]	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
Chlorpyrifos	1.1	1.9	230	450	209	237
Methyl Dursban	0.4	0.7	350	550	875	786
Abate	0.9	1.6	100	180	111	112
Malathion	1.2	2.8	3	6	2	2
Cidial	1.0	1.5	11	20	11	13
Cidial[b]	-	-	28	67	-	-
Primiphos Methyl	-	-	28	65	-	-
Supracide	-	-	0.4	1.0	-	-
NIA 24110	-	-	8.7	11.8	-	-
N-2596	-	-	0.8	1.2	-	-
SBP 1382	-	-	95	213		

[a] Fold of resistance of Coyote Creek midges as compared to the susceptible strain.

[b] Assayed against uncolonized larvae brought directly from the field.

Table 3.- Effectiveness of Cidial 2% celite granules against larvae of *Chironomus* sp. 51 in Los Angeles flood control channels.

Dosage lb/acre	Sample location	Average number larvae/sample[a]		Percent reduction[b]
		Pre-treatment	Post-treatment	
0.1	2	2630	330	92
	3	1070	200	88
0.28	1	1000	82	95
	2	1950	58	98
	3	1950	130	96
	4 (check)	2670	4170	-

[a] 3-4 samples were taken in each location.

[b] $\%\text{ reduction} = 100 - \left(\frac{\text{pretr. ck}}{\text{pretr. tr}} \times \frac{\text{post-tr tr}}{\text{post-tr ck}}\right) 100$ (Mulla et al. 1971)

Table 4.- Effectiveness of malathion and Cidial against chironomid midges in a warm-water lake (Westlake, California 1972.

Material or formulation	Dosage lb/acre	Average number larvae/sample			Bluegill Mortality[a]
		Pre-treatment	1 week	2 weeks	
Malathion 1% G	0.25	42	16	-	48
	0.50	56	52	-	157
Malathion EC_5	0.25	26	18	-	86
	0.50	70	156	-	386
	Check	58	12	-	1
Cidial 2% G	0.25	33	54	42	0.5
	0.50	24	53	35	1.0
	Check	22	15	31	0
Cidial EC_4	0.25	26	16	-	14
	0.50	38	47	-	72
	Check	10	5	-	0

[a] Cumulative average per plot (1.5-2 acres) over a 72-hr period. Slight mortality of largemouth bass also occurred with Cidial EC_4.

EVALUATION OF GOLDEN BEAR 1111 AND CHEVRON 72-R-2569 MOSQUITO LARVICIDE OILS

Frank W. Pelsue[1], Gardner C. McFarland[1] and Charles Beesley[2]

During 1972, field research was initiated to determine the effectiveness of certain larvicide oils in urban mosquito habitats in the Southeast Mosquito Abatement District. Prior to 1972, the District used FLIT MLO operationally in street gutters, catch basins, swimming pools, and other urban sites with some success, but fully satisfactory control was not obtained in some cases. Consequently, studies were performed using FLIT MLO fortified with Richfield Larvicide "A" resulting in an increase in the level of control (Pelsue & McFarland, 1973). In the summer of 1973, the studies were continued using two new larvicide oils, Golden Bear 1111 and Chevron 72-R-2569, in street gutters.

Larvicide oils used in urban mosquito control programs should have low phytotoxicity, good spreadability, and should provide rapid knockdown of mosquito larvae and pupae. They should also be non-corrosive and non-odorous. Golden Bear 1111, Chevron 72-R-2569, and FLIT MLO meet these criteria generally, but in moving water situations where rapid knockdown is important, FLIT MLO is deficient. Golden Bear 1111 appears to be a slightly more aromatic than FLIT MLO or Chevron, but Chevron has an additive to increase toxicity.

Patented petroleum oils designed for mosquito control are becoming more important because of organophosphorous insecticide resistance. For this reason, the District has done field evaluation to determine which oils are best suited for a comprehensive mosquito control program. The purpose of this study was to further evaluate new oils to determine if they would fit into a control program.

MATERIAL AND METHODS.–The study area described by Pelsue and McFarland, 1973, was used for this study. Few changes were made in the methods used in 1973; these are reported below.

The number of larval sample sites was increased from three to five, with one site as check and four test sites. Samples consisted of 3 dips per site, composited. Three larvicide oils were used in the experiments, Golden Bear 1111, Chevron 72-R-2569, and FLIT MLO (as a standard). Golden Bear was replicated five times, Chevron 72-R-2569 three times, and FLIT MLO two times. Samples were taken the day before the treatment, the day of the treatment, and the day following treatment. The samples were taken to the laboratory, counted, and identified. The dosage rate for each of the oils was 5 gallons per acre.

RESULTS AND DISCUSSIONS.–Table 1 shows the results of the tests. Golden Bear 1111 was clearly superior to Chevron and FLIT MLO. Chevron was superior to FLIT MLO. The level of control achieved with Golden Bear 1111 approaches the level required in an urban control program, and appears to be the best suited oil, possessing qualities necessary for control in moving water situations.

Table 1 shows the average numbers of larvae and pupae before and after treatment. The data do not differentiate between mortality of pre-fourth instar and fourth instar larvae; both Golden Bear 1111 and Chevron provided good control of the early instar larvae. However, in both Chevron and FLIT MLO, survival of pre-fourth instar larvae was greater than in Golden Bear 1111. Pupal mortality was good for all the oils tested.

Table 1.–Average number of larvae (all stages) and pupae per dip.

Material	Pre-treatment	Post-treatment	Percent Reduction
Golden Bear 1111	87	4	95
Chevron	43	5	88
Flit MLO	22	5	77
Check[a]	37	54	

[a]Based on 9 pre- and post-treatment samples.

The mosquito species were *Culex tarsalis*, *Culex pipiens quinquefasciatus*, *Culex peus*, and *Culiseta incidens*. There appeared to be no selective toxicity to any of these species.

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[1] Southeast Mosquito Abatement District, 9510 S. Garfield Avenue, South Gate, California 90280.

[2] Contra Costa Mosquito Abatement District, 1330 Concord Avenue, Concord, California 94520.

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FLIT MLO® WITH PYRETHRINS FOR MOSQUITO ADULTICIDING

M. R. Pittman[1], S. M. Silveira[1] and P. A. Gillies[2]

INTRODUCTION.—The area served by the Turlock Mosquito Abatement District includes approximately 190,000 acres of farmland. Many truck and tree crops have high labor requirements during harvest time in late summer, which coincides with peak mosquito populations in this area. Occasionally mosquitoes will migrate into a field being harvested by hand labor from a large source nearby where larval control failed because of insecticide resistance or other operational reasons. When the infestation is severe (in corn, tomatoes, grapes, orchards, etc.), harvest crews may refuse to work until the mosquitoes are brought under control. Economic loss can result if immediate adult mosquito control measures are not taken.

Aedes nigromaculis is the principal species involved. This mosquito breeds primarily in irrigated pastures and to a lesser extent in corn, alfalfa and orchards. In 1972 adults and larvae of *A. nigromaculis* were highly resistant to all available organophosphorous compounds, posing a difficult control problem. Baygon, a carbamate, is effective against *A. nigromaculis* adults, but is limited to use in noncrop areas.

Thus, it was necessary for this District to obtain a safe adulticide that could be used effectively to control organophosphorous resistant mosquitoes in commercial food crops near harvest, as well as in noncrop areas. Aircraft application was chosen in preference to other methods, requiring the selection of a suitable formulation.

During 1973, a series of aerial applications was made using "Pyrocide Mosquito Adulticiding Concentrate for ULV Fogging F7088" (E. P. A. Reg. No. 1021-1185), a formulation containing 12% pyrethrins and 60% piperonyl butoxide, produced by McLaughlin, Gormley and King. FLIT MLO® was used as a carrier and synergist (Chambers, G. V., personal communication). The observations and collection of test data prior to operational applications were made by personnel of the Vector Control Section of the California Department of Health, and the ESSO Research and Engineering Company, in cooperation with personnel of the Turlock Mosquito Abatement District.

MATERIALS AND METHODS.—For the preliminary tests, FLIT MLO was applied at 2 gal/acre, containing 0.0075 and 0.00375 lb/acre pyrethrins. The PA 25-260 aircraft was equipped with 12 No. 8020 Flat Spray TeeJet nozzles positioned at 45 degrees down and aft, operated at a boom pressure of 25 psi. Application was made at a flight speed of 100 m.p.h. and an altitude of approximately 15 ft., producing a 50 foot swath width.

Operational applications were made using 0.005 lb/acre pyrethrins in 0.5 gal/acre FLIT MLO. Twenty-four No. 8002 Flat Spray TeeJet nozzles were positioned at 45 degrees down and forward with 50 psi boom pressure. Swath width and altitude were unchanged.

Table 1.—Percent mortality of caged *Culex pipiens quinquefasciatus* following application of a Flit MLO-pyrethrins formulation in a pasture situation.

	Hours Post-Treatment					
	.0075 lb/ac ai pyrethrins		.00375 lb/ac ai pyrethrins		.005 lb/ac ai pyrethrins	
Stations	1	24	1	24	1	24
1	100KD	100	100KD	100	100KD	100
2	100KD	100	60	70	100KD	100
3	100KD	100	85	92	100KD	100
4	100KD	100	85	100	100KD	100
5	100KD	100	100	100	100KD	100
6	100KD	100	92	100	100KD	100
7	100KD	100	100	100	100KD	100
8	100KD	100	100	100	100KD	100
9	100KD	100	100	93	100KD	100
10	100KD	100	100	100	100KD	100
11	100KD	100	100	100	100KD	100
12	100KD	100	92	75	100KD	100
13	100KD	100	100	100	100KD	100
14	100KD	100	100	100	100KD	100
15	100KD	100	95	100	100KD	100
16	100KD	100	77	77	100KD	100
Control	0	0	0	7	0	0

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[1] Turlock Mosquito Abatement District, Post Office Box 1629, Turlock, California 95380.

[2] California Department of Health, Vector Control Section, 5545 East Shields Avenue, Fresno, California 93727.

Table 2.—Field observations of adult *Aedes nigromaculis* treated with Flit MLO (½ gal/ac) containing pyrethrins (.005 lb/ac ai). Field counts were based on pants-leg counts.

Stations Test Field	Pre	Post (1 hr.)	Post (5 hr.)	Post (12 hr.)
1	2	0	2	1.5
2	2	0	.5	1
3	6	0	0	0
4	3	0	0	0
5	4	0	0	0
6	5	0	0	1
7	5	0	0	1
8	4	0	0	2
Check Field				
1	10	0	0	1.5
2	10	0	0	.5
3	9	0	0	1.5
4	9	.5	0	1
5	4	.5	0	.5
6	6	1	1	3.5
7	11	.5	1.5	6
8	21	4	2	7
9	11	2	1.5	4

Results and Discussion.—Complete data (i.e. exact times, winds, temperatures, grass and foliage cover) are on file at the Turlock Mosquito Abatement District.

Table 1 gives the results of testing 0.0075 lb/acre and 0.00375 lb/acre ai pyrethrins in two gal/acre of FLIT MLO. As a result of these tests, 0.005 lb/acre ai pyrethrins in ½ gal/acre FLIT MLO with appropriate equipment changes and adjustments was found to be the best combination to give the excellent results obtained with controlled field experiments as shown in Tables 1 and 2.

Table 3.—Operational results of aerial applications of Flit MLO (½ gal/ac) containing pyrethrins (.005 lb/ac).

Number of Fields	Crop	Total Acres	Pre-Inspect. Adult/pants-	24 hr. Post-Inspection Adult/pants-
2	Alfalfa	120	5.5	6
2	Slough	40	8	2
1	Corn	10	25	0
2	Pasture	20	2	0
2	Pasture	40	10	1
3	Pasture	50	4	1
4	Pasture	64	5	0
3	Pasture	34	6	0
1	Pasture	14	2	0
1	Pasture	6	2	2
2	Pasture	12	5	1
1	Pasture	4	3	0
1	Pasture	40	20	5
1	Pasture	30	15	3
1	Pasture	20	8	0
5	Pasture	26	10	0
2	Pasture	44	20	0
1	Pasture	30	3	1

Table 3 shows the results observed by operating personnel with 0.005 lb/acre ai pyrethrins in ½ gal/acre FLIT MLO in typical pasture situations with *Aedes* adults.

Table 4 shows that in a grape vineyard, only partial control was obtained, probably due to the dense canopy of vegetation present in this situation.

These field tests indicated that aerial application of FLIT MLO and MGK's "Pyrocide" combination can be an effective and safe adulticide for some crop and noncrop situation.

Table 4.–Percent mortality of caged *Culex pipiens quinquefasciatus* following application of a Flit MLO (½ gal/ac) containing pyrethrins (.005 lb/ac ai) formulation on a grape vineyard .

Station North	Placement –	Position	Mortality	
	of cages		Hours Post-Treatment	
			1	24
1	high open	vertical	100	100
2	low open	vertical	71	86
3	high vine	horizontal	95	95
4	mid-vine	horizontal	0	0
5	mid-vine	horizontal	0	11
6	ground	horizontal	67	93
7	high vine	horizontal	25	81
8	mid-vine	horizontal	90	95
9	mid-vine	horizontal	47	88
10	ground	horizontal	0	19
11	high open	vertical	100	90
12	low open	vertical	25	44
13	high vine	horizontal	0	0
14	mid-vine	horizontal	0	20
15	mid-vine	horizontal	0	0
16	ground	horizontal	17	58
Check			0	0
Check			0	0
Station South				
1	high open	horizontal	100	100
2	low open ho	horizontal	39	86
3	mid-vine	horizontal	0	11
4	ground vine	horizontal	0	0
5	high open	horizontal	100	100
6	low open	horizontal	68	84
7	mid-vine	horizontal	18	27
8	ground vine	horizontal	6	24
9	high open	vertical	94	100
10	low open	vertical	67	89
11	mid-vine	vertical	0	6
12	low vine	vertical	0	5
13	high open	vertical	100	100
14	low open	vertical	81	95
15	mid-vine	vertical	10	29
16	low vine	vertical	0	0
Check			0	0
Check			0	0

AERIAL APPLICATION OF FLIT MLO® TO IRRIGATED PASTURES: FACTORS CONTRIBUTING TO PERFORMANCE VARIABILITY

G. V. Chambers[1], P. A. Gillies[2] and M. R. Pittman[3]

INTRODUCTION.– A high degree of variability in the performance of aerially applied FLIT MLO® against *Aedes nigromaculis* in irrigated pastures was reported in 1972 from several California Mosquito Control Agencies. A cooperative field and laboratory investigation involving the Turlock Mosquito Abatement District, the California Department of Health, Esso Research and Engineering Company, and Exxon Company, USA, was undertaken in 1973 to elicit potential factors contributing to such variability. These factors were considered to fall into one of three categories: product, aircraft operations, or physical and biological conditions existing in the irrigated pastures (Table 1). The first two categories were evaluated for their contribution to performance variability.

Three factors listed in Table 1 have been previously investigated and all three are known to contribute to variability. Contamination of FLIT MLO with water prior to spraying reduces larvicidal activity (Chambers 1972). The use of too wide a swath contributes to skips in the application resulting in uneven coverage. Quantitative recovery measurements of aerial applications of FLIT MLO have shown that swaths wider than 50 feet result in deficient deposit between adjacent passes, flying 40 foot swaths eliminates the deficiency, or "skip" (Chambers 1972). FLIT MLO, unlike conventional organophosphorous larvicides, is more effective against the later stages of larval development (Micks et al., 1968 and Gillies et al., 1971). Comparison of the results obtained from treating a pasture with second instar larvae to these obtained in a pasture with fourth instar larvae may provide a misleading conclusion as to the variability in larvicidal activity.

Table 1. Potential factors contributing to performance variability.

- PRODUCT - Storage stability under desert conditions
- AIRCRAFT OPERATIONS
  - Type of aircraft ground assistance
    - + Flagging vs. free flying
  - Time of Day
    - + Temperature
    - + Wind
  - Influence of potential contaminants on larvicidal activity
    - + Water (previously reported)
    - + Baygon
    - + Pyrethrum
    - + Swath width (previously reported)
  - Edge effect
- CONDITIONS OF IRRIGATED PASTURE
  - Species of Aedes involved
  - Water surface conditions
  - Stage of development of immatures (previously reported)
  - Time of year
    - + Water temperature

General Experimental Procedures--The effect of ground assistance by flagging vs free flying was evaluated in the field by comparing the decrease in larval population following the two treatments. FLIT MLO was applied at 2 gallons/acre to irrigated pastures by a Pawnee 260 flying a 50 foot swath. The treatment was delayed until the immature population was predominantly fourth instar larvae, with a few thirds and pupae. Larval populations in the experimental fields were quite uniform except for the field designated "F", which contained more third instars (Table 2). Greater uniformity in experimental fields was achieved by dividing a larger pasture into two portions. In these instances, half of the pasture was treated in the early morning and the other half was treated in late morning in an attempt to define the effect of time of day on treatment. The initial plan was to continue treating pastures for several consecutive days in this manner; however, the supply of experimental fields was exhausted by the third day.

Product storage stability and influence of potential contaminants on larvicidal activity were evaluated in the laboratory.

Product Storage Stability--The larvicidal activity of FLIT MLO did not change with storage under conditions of high summer temperatures. Samples were removed from large size storage tankage at both Fresno and Turlock following

Table 2.– Irrigated pastures treated*.

Treatment Date Aug., 1973	Application Time, Early or Late Morning	General Description of Stage of Development	
		Flagged	Free Fly
14	E	(A)ø 4ths, few p. (B) 4ths, few p.	(C) 4th, few 3rds
	L	(D)ø 4ths	(E) 4ths
15	E	(G)ø 4ths, few 3rds**	(F) 4ths, some 3rds, few 2nds øø
	L	(H)ø 4ths, few 3rds	
16	-	(I) 4ths, few 3rds, few p. (J) 4ths	

* Pawnee 260; 2 gallons/acre; 50 ft. swath width

** 98% 4ths.

ø Split pasture.

øø 65% 4ths.

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[2] California Department of Health, Vector Control Section, 5545 East Shields Avenue, Fresno, California 93727.

[3] Turlock Mosquito Abatement District, Post Office Box 1629, Turlock, California 95380.

various intervals of storage time through the summer of 1973 and over the winter of 1972-1973. These samples were evaluated for larvicidal activity by WHO-M-18 (Anonymous, 1967). The larvicidal activity of these samples compared favorably with the activity of a reference standard of a known composition run at the same time.

Percent Decrease in Larval Populations--After establishing sampling stations and pretreatment population levels in the test fields, larval populations were sampled for several days after treatment. Table 3 shows the decrease in immatures at 24 hours and 48 hours as reflected by dip counts. These changes are reported as percentage changes; the percent mortality levels are much greater because the water surface area and the volume change substantially with time in an irrigated pasture, affecting the density of the survivors. The increase in mortality beyond 24 hours also can be masked by this concentration factor.

Ground Assistance for Precise Aircraft Application--Better control was achieved in the flagged fields, based on the percent decrease in larval density at the sampling stations (Table 3). Since Field F contained more thirds, it was nec- to make a comparison on fourths. Comparison of Field F with Field G applied with the same load of FLIT MLO (Table 4) shows fewer fourth instars per dip at pretreatment in Field F and a higher number of pupae per dip 24 hours post treatment, indicating less control.

The swath interval of 50 feet was selected as a compromise between an ideal swath width of 33 to 40 feet with a Pawnee 260 and the greater swath widths utilized in larviciding with organophosphorous compounds. It is known from previous spray deposit recovery measurements that a narrow, insufficiently treated strip occurs in the distribution pattern at the areas of overlap with a flagged 55 foot swath. Thus, slight deviation in the line of flight in free flying with a 50 foot swath could produce skips in the distribution pattern and cause the marked difference in control noted in the comparison of flagged and unflagged fields. A more uniform distribution pattern and better control would be expected with a 40 foot swath interval.

Contamination--Samples of FLIT MLO were taken from the aircraft distribution system before application of each load. These samples were evaluated in the laboratory and it was found that the larvicidal activity was substantially decreased when the load followed a Baygon water application (Table 5). These samples of FLIT MLO with a lower larvicidal activity were "wet" as reflected by the more than a 10 fold increase in water content. Contamination by water prior to spraying changes the surface chemistry thereby apparently changing either the rate or degree of penetration of the larvicide into the larvae. Subsequent laboratory comparison of the larvicidal activity of FLIT MLO with Baygon or with pyrethrum showed that the water, not the other potential contaminants, decreased the larvicidal activity of FLIT MLO.

The amount of water in the FLIT MLO and the accompanying decrease in larvicidal activity may not be as great for the load as the samples indicated. These samples, except for the FLIT MLO-pyrethrum loads, were removed from the aircraft pump following loading and may not be representative of the contents of the aircraft tank. A further study is being undertaken with sampling from both the pump and the tank.

Table 3.–Percent decrease in immatures per dip at 24 hours (at 48 hours).

Type of Material in Previous Load	Flagged	Free Fly
FLIT MLO	A 80 (dry)	C 48 (63)
	B 96 (dry)	
Baygon-water	D 86 (dry)	E unknown
Baygon-water	G 95 (94)	F 39 (47)
FLIT MLO-pyrethrum	H 99 (99+)	
Baygon-water	I 98 (96)	
FLIT MLO	J 93 (94)	

Table 4.–Comparison of field F and G based on 4th instar larvae.

		(G) Flagged	(F) Free Fly
Pre:	4ths/dip	11.2	4.0
Post:	Pupae/dip	0.06	0.18
Post:	Stations with pupae, %	33	50

Table 5.–Water content and larvicidal activity of FLIT MLO samples removed from aircraft..

Sampling Date	Description of Sample	Prior Load	Water Content, ppm	Larvicidal Activity*
14	1st FLIT MLO load	FLIT MLO	165	100
	2nd FLIT MLO load	Baygon-water	2735	42
15	1st FLIT MLO load	Baygon-water	2163	43
	FLIT MLO-Pyrethrum	FLIT MLO	190	100
	2nd FLIT MLO load	FLIT MLO-pyrethrum	-	-
16	FLIT MLO	Baygon-water	2165	37
	FLIT MLO-Pyrethrum	FLIT MLO	133	100

* $\frac{(\%\text{ Mortality at 48 hours with Sample})}{(\%\text{ Mortality at 48 hours with Reference Standard})} \times 100$

Evaluated at 4 μℓ with WHO-M-18 procedure, Culex p. quinquefasciatus.

Time of Day--An evaluation of the influence of time of day of treating on performance was discontinued when it was discovered that the larvicidal activity of the FLIT MLO may not be the same for the early and late morning flights because of contamination with water.

Edge Effect--An examination of the percent decrease in the larval population was made for each of the sampling stations. Two examples are shown in Figure 1. These samples illustrate a reduction in the control near the edges of treated fields where obstacles, such as power lines, trees, houses, etc., interfere with maintaining a low altitude of the aircraft.

Implications of Findings on Operational Practices--Surface treatment with FLIT MLO requires a greater precision in aerial application than volumetric treatments with organophosphorous larvicides. The factors which affect precision of application contribute to performance variability. Examples are optimum swath width and stage of larval development treated. The low material cost of organophosphorous larvicides permitted a substantially higher dosage rate over the susceptible level, thereby minimizing the necessity for precision application. Further, the organophosphorous larvicides were effective against adults in the field and the frequency of application did permit the control of adults which resulted from skips in previous applications. In contrast, the application of FLIT MLO for larval control is a surface treatment with a recommended dosage rate only slightly higher than the susceptible level for the 3rd instar larval through pupal stages. The larvicide does not possess sufficient adulticiding activity for controlling adult mosquitoes. Treatment of the 3rd instar through pupal stages minimizes the dosage rate required, but delaying the application until the 3rd larval stage appears usually means that the water surface in the pasture has become less continuous, thus requiring greater precision than the past practice of treating the larger flooded areas containing the earlier instars. This need for greater precision in aerial application is not unique to FLIT MLO; various mosquito control agents (including FLIT MLO) are generally more specific to a particular life stage and the recommended dosage rate of the newer agents is close to the minimum level because of economic and/or environmental considerations.

Many of the factors contributing to performance variability with aerial application are controllable. Adequate coverage with minimum skips can be achieved by using the optimum swath width for the type of aircraft. Where the use of flagmen is impractical, the employment of field markers as a visual aid to the pilot can improve precision. Dress off procedures and the use of an extra swath on the upwind edge of a pasture are essential. Contamination of FLIT MLO by water before application can be eliminated by utilizing equipment for storage and aircraft loading for FLIT MLO use only and by draining the aircraft tank and spray system before loading.

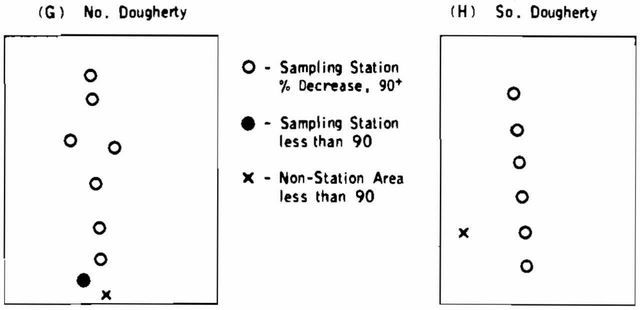

Figure 1.--Examples of edge effect on degree of control.

Other factors contributing to performance variability can be minimized. Employing the concept of "wait-a-day" can reduce variability due to first and second instar larvae. The operational program should be geared to treating thirds and fourths and a few pupae. Other factors, such as water surface conditions (excessive vegetation, surface scum, etc) are considered uncontrollable.

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SURVEILLANCE FOR ARTHROPOD-BORNE VIRUSES AND DISEASE BY THE CALIFORNIA STATE DEPARTMENT OF HEALTH, 1973[1]

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Surveillance for mosquito-borne encephalitis during the 1973 season again confirmed the persistence of western encephalitis (WEE) and St. Louis encephalitis (SLE) viruses in their natural cycles in many areas of the State. However, vector control and equine immunization programs kept human and equine cases of disease at the low levels characteristic of the past two decades. As usual, surveillance activities were carried out in collaboration with many other local, State, and Federal agencies and personnel.

At least 1,037 persons suspected of having an arbovirus infection during the year were screened serologically by the State Viral and Rickettsial Disease Laboratory or County Health Department laboratories (Table 1). No human cases of WEE were detected, and there was no evidence of Venezuelan equine encephalitis (VEE) in California, nor any resurgence of this disease in the Central and Northern areas of Mexico which were affected in previous years. However, 5 cases of SLE were confirmed, as shown in Table 2: (1) a 62 year old woman from Riverside County, onset July 19; (2) a 17 year old girl from Kern County, onset August 12; (3) a 29 year old woman from San Joaquin County, onset August 24, who visited in Butte County shortly before her illness, but whose mosquito exposure was most likely in her home environment; and two cases in San Diego County, detected by the local Public Health Laboratory and confirmed by the State Viral and Rickettsial Disease Laboratory--(4) a 12 year old boy with onset September 5 and exposure most likely in a San Diego suburban area; and (5) a 31 year old man presumably exposed in Imperial County, onset October 10. Neutralization, hemagglutination-inhibition, complement-fixation, and indirect fluorescent antibody methods were all used to confirm these diagnoses. The patients all recovered without sequelae. No fatality from WEE or SLE has been recorded in California since 1962.

Fifty-six clinically suspect equine cases were reported to the Department, but only 2 cases of WEE could actually be confirmed by serologic tests: a 1 year old unvaccinated horse from Yolo County, with onset August 12; and a 4 month old unvaccinated colt from Shasta County, with onset September 4 (Table 3).

The mosquito testing program was carried out as usual by the State Vector Control Section, local mosquito abatement Districts, and the State Viral and Rickettsial Disease Laboratory. Of 4,838 mosquito pools tested in suckling mice, the yield was 281 virus isolates, the highest recovery rate in recent years (Tables 4-7). There were 97 isolates of WEE virus and 75 of SLE virus, mostly from Imperial County where mosquito collection was emphasized because of concern that VEE might move northward from Mexico. In addition, 74 strains of Turlock virus, 26 of Hart Park virus, 3 of California encephalitis virus, 2 of Main Drain virus, 3 of Bunyamwera group virus, and 1 as yet unidentified virus were isolated.

Despite the frequency of Turlock and Hart Park virus isolations from *Culex tarsalis* (the common and efficient vector for WEE and SLE viruses), little or no evidence of human or equine illness or even of infection with these viruses has been obtained in California. And despite the recent occurrence of California encephalitis virus in mosquitoes after many years apparent absence from the State, no human cases of disease have been detected.

The only other arbovirus disease of significance during 1973 was Colorado tick fever. A total of 25 cases occurred, the largest number since recordkeeping began in 1954, bringing the total proven cases in California to 162.

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

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[2] Viral and Rickettsial Disease Laboratory and Infectious Disease Section.

[3] Vector Control Section.

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Table 1.–Humans tested serologically for mosquito-borne arboviruses by the Viral and Rickettsial Disease Laboratory, California State Department of Public Health and by county health department laboratories, by county of residence and month of illness onset, California, 1973.

COUNTY	TOTAL	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	UNR
California														
Alameda	18	-	-	1	1	2	2	5	2	1	1	-	-	3
Butte	15	-	1	1	-	3	-	1	3	4	1	-	-	1
Colusa	2	-	-	-	-	-	-	-	1	-	1	-	-	-
Contra Costa	33	-	-	-	1	4	8	4	5	-	7	1	-	3
Fresno*	268	16	-	-	-	26	28	56	64	18	30	12	16	2
Glenn	3	-	-	-	-	-	-	-	1	2	-	-	-	-
Imperial	3	-	-	-	-	-	1	-	-	1	-	-	-	1
Inyo	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Kern	48	-	-	1	-	3	7	8	6	3	6	2	-	12
Kings	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Lake	5	-	-	-	-	-	-	-	-	3	-	-	-	2
Lassen	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Los Angeles**	52	-	-	1	5	11	11	5	4	12	1	2	-	-
Marin	14	-	-	-	-	2	3	2	3	1	-	1	-	2
Mendocino	4	-	-	-	-	-	-	2	2	-	-	-	-	-
Merced	1	-	-	-	-	-	1	-	-	-	-	-	-	-
Modoc	1	-	-	-	-	-	-	-	-	1	-	-	-	-
Monterey	6	-	-	1	-	2	-	-	-	1	-	-	-	2
Nevada	6	-	-	-	-	-	-	1	4	-	1	-	-	-
Placer	4	-	-	-	-	-	-	1	1	-	-	-	-	2
Riverside	13	-	-	-	-	-	5	5	1	1	-	-	-	1
Sacramento***	79	-	-	-	-	1	23	16	13	15	4	7	-	-
San Bernardino	17	-	-	-	-	2	3	4	3	2	1	1	-	1
San Diego****	75	-	-	-	2	13	12	10	13	13	6	4	-	2
San Francisco	92	-	-	1	1	12	11	19	12	12	8	6	-	10
San Joaquin	19	-	-	-	-	-	2	3	5	2	4	-	-	3
San Luis Obispo	3	-	-	-	-	-	1	-	-	1	-	1	-	-
San Mateo	47	-	1	-	-	4	4	5	11	7	5	8	-	2
Santa Barbara	21	-	-	-	-	3	2	5	5	-	1	-	-	5
Santa Clara	92	-	1	1	2	9	5	17	21	14	10	3	-	9
Santa Cruz	12	-	-	-	-	2	3	1	5	-	-	-	-	1
Shasta	6	-	-	-	-	-	-	2	1	3	-	-	-	-
Siskiyou	2	-	-	-	-	-	-	2	-	-	-	-	-	-
Solano	11	-	-	-	1	-	1	1	1	4	1	1	-	1
Sonoma	13	-	-	-	-	2	2	1	2	1	2	1	-	2
Stanislaus	12	-	-	-	-	-	2	6	-	3	-	-	-	1
Sutter	2	-	1	-	-	-	-	-	-	-	1	-	-	-
Tehama	8	-	-	-	-	-	1	3	-	2	2	-	-	-
Tulare	5	-	-	-	-	-	-	1	3	-	1	-	-	-
Tuolumne	1	-	-	-	-	-	-	1	-	-	-	-	-	-
Ventura	5	-	-	-	-	-	-	1	-	2	1	1	-	-
Yolo	16	-	-	-	3	3	1	3	1	2	1	1	-	1
Total	1,037	16	4	7	16	104	139	193	193	132	96	52	16	69

* Tested by County Health Department Laboratory (includes 9 patients tested by State VRDL)
** Tested by County Health Department Laboratory (includes 12 patients tested by State VRDL)
*** Tested by County Health Department Laboratory (includes 10 patients tested by State VRDL)
**** Tested by County Health Department Laboratory (includes 4 patients tested by State VRDL)

Table 2.–Human cases of arbovirus encephalitis in California, 1973.

Patient identification and exposure	Clinical Course	Serologic test	Antibody Titer Acute	 Convalescent		Remarks
1. L.O., 62, female Blythe, Riverside County; no specific exposure stated.	Onset July 19, 1973; fever, lethargy, semi-stupor; CSF-57 mononuclear cells; 58 mgm % protein; hospitalized; good recovery.		July/23/73	Aug/20/73	Sept/5/73	CF tests for WEE, VEE, polio 1,2 & 3 negative; mumps and herpes low stationary; HAI for 9 other arboviruses negative.
		SLE-CF	1:4	1:8	-	
		SLE-CF	1:8	-	1:16	
		SLE-HAI	1:40	1:80	1:20	
		SLE-IFA	1:512	1:512	-	
		SLE-IFA	1:256	1:256	1:256	
		SLE-NI	3.3	3.4	-	
		SLE-NI	3.9	-	4.9	
2. A.A., 17, female, Bakersfield, Kern County; probable mosquito exposure at Hart Park.	Onset August 12, 1973; fever, headache, mild nuchal rigidity; CSF-74 WBC; hospitalized, good recovery		Aug/17/73	(sent)Sept/1/73	Sept/11/73	CF tests for WEE, VEE, herpes, negative; mumps 1:16 stationary; HAI tests for WEE, Turlock, negative.
		SLE-CF	<1:4	1:8	1:8	
		SLE-HAI	1:160	1:80	1:160	
		SLE-IFA	1:64	1:128	1:128	
		SLE-NT	1.9	3.0	4.5	
3. L.H., 29, female, Stockton, San Joaquin County; possible mosquito exposure while water skiing in Delta; August 12 and 16; also visited Chico August 18-19.	Onset August 24, 1973, severe headache, backache, malaise, fever, stiff neck, vomiting; rapid recovery.		Sept/3/73	Sept/13/73	Oct/1/73	CF tests for WEE, VEE, herpes, negative, mumps 1:8 stationary; HAI tests for 9 other arboviruses negative.
		SLE-CF	1:32	1:64	-	
		SLE-CF	-	1:32	1:16	
		SLE-HAI	1:40	≥1:160	≥1:160	
		SLE-IFA	1:128	1:256	1:256	
		SLE-NI	6.2	6.3	6.4	
4. K.B., 12, male, LaMesa, San Diego County; probable mosquito exposure at Santee, near his home; SLE virus isolated from C. <u>pipiens</u> and SLE antibody found in pheasants and chickens in area.	Onset September 5, 1973; high fever, headache, stiff neck, vomiting, confusion, loss of memory, hallucinations; CSF - 150 WBC (80% polys)→67 WBC (85% lymphs); hospitalized; good recovery.		Sept/8/73	Sept/25/73		CF test for WEE negative; no antibody rise for mumps or herpes; HAI tests for WEE, Turlock, negative.
		SLE-CF	<1:8	1:32		
		SLE-HAI	1:160	≥1:640		
		SLE-IFA	1:64	1:256		
		SLE-NI	2.2	QNS		
5. E.S., 31, male Spring Valley, San Diego County; probable mosquito exposure near Picacho, Colorado River, September 29-30; possible exposure on job as mechanic in Santee Lakes area.	Onset October 10, 1973; headache, high fever, dizzy, vomiting, delirium; hospitalized; good recovery.		Oct/14/73	Oct/29/73	Nov/28/73	HAI tests for 8 other arboviruses negative (<1:10); Rio Bravo HAI 1:10 - 1:20 - 1:10
		SLE-CF	<1:8	1:16	1:16	
		SLE-HAI	1:20	≥1:160	1:80	
		SLE-IFA	1:32	1:128	1:256	
		SLE-NI	-	-	-	

Table 3.–Suspected clinical cases of arbovirus encephalitis in equines, by county and month, for California 1973.

COUNTY	MONTH OF ONSET													
	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Undetermined* or not tested	Totals
Totals	1	1	2	7	9	3	5	18	6	3	1	1	6	63
Butte				1				1						2
Contra Costa								1					1	2
Glenn								1						1
Humboldt								1						1
Imperial	1			1			1	1						4
Kern		1						1	1				1	4
Lake				1				1						2
Los Angeles								2		1				3
Marin				1										1
Mariposa							1							1
Mendocino					2				1					3
Placer													1	1
Riverside					2	1	1							4
Sacramento					1									1
San Bernardino				1										1
San Diego				2				2					1	5
San Joaquin					1	1		1		1	1			5
Santa Barbara							1	1						2
Santa Clara			2				1					1		4
Shasta					2				2**					4
Sonoma								1						1
Tehama						1		1						2
Trinity										1				1
Tuolumne					1			1	1				1	4
Yolo								1**	1				1	3
Yuba								1						1

* Inadequate serum specimens available for testing. Complement fixation tests performed by the Viral and Rickettsial Disease Laboratory, California State Department of Health.

** Only two cases confirmed serologically for WEE: a 1-year old horse with onset in August from Yolo County with no history of vaccination; rising antibody titers of 1:8 to 1:32 taken two weeks apart; and a 4-month old colt from Shasta County with onset in September, no history of vaccination, the antibody titer rising from 1:64 to 1:256 on samples taken two weeks apart.

Table 4.—Numbers of mosquitoes and other Diptera tested, by county and month, California, 1973, by the Viral and Rickettsial Disease Laboratory.*

County	January	February	March	April	May	June	July	August	September	October	November	Total
Colusa					167 (6)	250 (7)	1,001 (21)	2,326 (47)		519 (11)		4,263 (92)
Fresno					39 (6)	83 (6)	156 (5)	71 (8)	107 (8)	32 (3)		488 (36)
Imperial	2,023 (96)	2,198 (91)	4,396 (149)	8,268 (229)	20,062 (444)	11,215 (304)	6,324 (167)	14,351 (408)	4,581 (168)			73,418 (2,056)
Kern						29 (7)	1,103 (31)	452 (15)	2,479 (53)	27 (1)		4,090 (107)
Kings					722 (19)	126 (9)	96 (7)	97 (6)	404 (12)			1,445 (53)
Lassen								500 (10)				500 (10)
Los Angeles								1,469 (34)		17 (9)		1,486 (43)
Madera					51 (7)	21 (5)	250 (7)	435 (12)	218 (7)			975 (38)
Merced						519 (15)	525 (16)	800 (22)	488 (17)			2,332 (70)
Modoc						600 (12)						600 (12)
Orange									1,122 (23)			1,122 (23)
Placer					117 (4)	459 (11)	441 (10)	63 (6)	33 (3)			1,113 (34)
Riverside							1,114 (35)	8,100 (219)	1,933 (64)	58 (5)		11,205 (323)
Sacramento					22 (1)	74 (2)	173 (4)		32 (2)			301 (9)
San Bernardino							38 (6)	1,453 (72)		544 (44)		2,035 (122)
San Diego	156 (15)	2,345 (91)	1,929 (155)	5,300 (167)	1,257 (43)	5,409 (154)	2,273 (56)	1,279 (37)	1,178 (70)	2,335 (136)	1,498 (69)	24,959 (993)
San Joaquin						25 (1)	802 (18)		32 (1)			859 (20)
Shasta						231 (6)	333 (8)	1,177 (27)	591 (14)			2,332 (55)
Siskiyou							578 (13)					578 (13)
Stanislaus						362 (18)	1,278 (32)	195 (7)	669 (19)			2,504 (76)
Sutter					105 (3)	426 (11)	1,125 (23)	1,838 (39)		129 (4)		3,623 (80)
Tehama						416 (9)	557 (16)	48 (3)				1,021 (28)
Tulare						13 (4)	29 (2)	53 (6)	135 (4)			230 (16)
Ventura							557 (15)					557 (15)
Yolo									19 (1)			19 (1)
Yuba							46 (1)					46 (1)
Yuma,Arizona	33 (6)	203 (12)	827 (23)	1,903 (42)	8,723 (184)	2,119 (46)	267 (9)	5,042 (115)	2,343 (55)			21,460 (492)
Mojave, Arizona								714 (20)				714 (20)
Total	2,212 (117)	4,746 (194)	7,152 (327)	15,471 (438)	31,265 (717)	22,377 (627)	19,066 (502)	40,463 (1,113)	16,364 (521)	3,661 (213)	1,498 (69)	164,275 (4,838)

* Total mosquitoes (pools) tested.

Table 5.–Number of mosquitoes and other Diptera tested, by species and month, California 1973, by the Viral and Rickettsial Disease Laboratory.*

Species	January	February	March	April	May	June	July	August	September	October	November	Total
Culex												
tarsalis	1,249 (49)	1,449 (56)	907 (70)	5,860 (183)	14,299 (336)	17,266 (422)	14,239 (362)	15,062 (436)	10,430 (279)	1,179 (58)	15 (7)	81,955 (2,258)
erythrothorax	769 (25)	2,641 (72)	3,588 (107)	8,662 (187)	6,705 (147)	1,763 (88)	766 (17)	6,170 (143)	2,413 (64)	1,748 (53)	1,395 (41)	36,620 (944)
peus		3 (3)	29 (15)			474 (14)	832 (26)	216 (8)	182 (10)	125 (19)	1 (1)	1,862 (96)
pipiens	33 (5)	7 (4)	21 (4)	35 (1)	311 (8)	117 (10)	54 (7)	133 (11)	415 (28)	83 (19)	4 (1)	1,213 (98)
restuans								4 (1)				4 (1)
Aedes												
melanimon					50 (1)	93 (5)	50 (1)	48 (2)		150 (3)		391 (12)
vexans			26 (4)	559 (15)	9,769 (201)	1,873 (44)		4,085 (94)	80 (9)	61 (5)		16,453 (372)
dorsalis			28 (10)	61 (3)	20 (2)	349 (9)		197 (21)	18 (5)	16 (3)		689 (53)
sierrensis								1 (1)				1 (1)
increpitus			2 (1)									2 (1)
squamiger			10 (4)	50 (3)							2 (1)	62 (8)
taeniorhynchus							1 (1)	2 (2)	4 (1)			7 (4)
Anopheles												
freeborni	2 (1)	2 (1)	5 (2)	26 (2)		262 (11)	82 (4)	53 (7)	65 (5)	25 (10)		522 (43)
franciscanus		3 (1)	23 (8)		12 (1)	57 (4)		142 (19)	89 (14)	45 (9)	57 (9)	428 (65)
punctipennis								18 (1)				18 (1)
Culiseta												
incidens	9 (3)	20 (5)	42 (22)	40 (2)		36 (3)		5 (2)	7 (7)	7 (6)	5 (2)	171 (52)
inornata	150 (34)	221 (37)	388 (54)	178 (42)	94 (20)	21 (6)	3 (2)		60 (14)	212 (25)	17 (6)	1,344 (240)
particeps								9 (1)		10 (3)	2 (1)	21 (5)
Psorophora												
confinnis					5 (1)	66 (11)	3,039 (82)	14,296 (355)	2,401 (83)			19,807 (532)
signipennis								22 (9)				22 (9)
Culicoides												
variipennis		326 (11)	1,895 (17)						200 (2)			2,421 (30)
freeborni		53 (1)										53 (1)
mixed species		5 (2)	144 (6)									149 (8)
Simulium griseum		16 (1)	44 (3)									60 (4)
Total	2,212 (117)	4,746 (194)	7,152 (327)	15,471 (438)	31,265 (717)	22,377 (627)	19,066 (502)	40,463 (1,113)	16,364 (521)	3,661 (213)	1,498 (69)	164,275 (4,838)

* Total mosquitoes (pools) tested.

Table 6.–Viruses isolated from mosquitoes by the Viral and Rickettsial Disease Laboratory, California State Department of Health, by county and month of collection, 1973.

County	April	May	June	July	August	September	October	Total
Colusa				Hart Park(1)	Turlock(1)		WEE(1)	Turlock(1) WEE(1) Hart Park(1)
Imperial	SLE(1) Turlock(1)	SLE(1) Turlock(12) Main Drain(1)	SLE(12) Turlock(15) WEE(36)	SLE(18) Turlock(4) WEE(32) Unident.(1)	SLE(7) (Turlock(1) Main Drain(1) WEE(4)	SLE(9) Turlock(2)		SLE(48) WEE(72) Turlock(35) Main Drain(2) Unidentified(1)
Kern					Turlock(1)	SLE(2) CEV(1)		SLE(2) CEV(1) Turlock(1)
Kings		Hart Park(2)	Hart Park(2)					Hart Park(4)
Lassen					SLE(1) Turlock(1)			SLE(1) Turlock(1)
Madera				Hart Park(1)	Hart Park(1) Turlock(1)			Turlock(1) Hart Park(2)
Merced				Hart Park(1) Turlock(1)				Turlock(1) Hart Park(1)
Placer				Turlock(1)				Turlock(1)
Riverside				SLE(2) WEE(4) Turlock(1)				SLE(2) WEE(4) Turlock(1)
San Bernardino					WEE(2) SLE(3)		CEV(1)	SLE(3) CEV(1) WEE(2)
San Diego			Turlock(3) Hart Park(1)	Turlock(4)	Turlock(4)	Turlock(1) SLE(4) Hart Park(3)	Turlock(1) SLE(3) Bunyamwera Group(1) Main Drain(1)	SLE(7) Hart Park(4) Turlock(13) Bunyamwera Group(1) Main Drain(1)
San Joaquin				Hart Park(3) Turlock(1)				Turlock(1) Hart Park(3)
Shasta				Hart Park(1)	Hart Park(1) Turlock(1)			Turlock(1) Hart Park(2)
Stanislaus			Hart Park(1)	Hart Park(2)		WEE(1)		Hart Park(3) WEE(1)
Sutter			Hart Park(1)	Hart Park(4) Turlock(2)	WEE(1) CEV(1) Turlock(1)			Turlock(3) WEE(1) Hart Park(5) CEV(1)
Tehama				Hart Park(1)				Hart Park(1)
Tulare					Turlock(1)	SLE(1)		SLE(1) Turlock(1)
Ventura				Turlock(1)				Turlock(1)
Mojave, Arizona					SLE(3) WEE(4)			SLE(3) WEE(4)
Yuma, Arizona		Turlock(4)	SLE(4) Turlock(7) WEE(10)	SLE(1) Turlock(1) WEE(2)	SLE(3)	Bunyamwera Group(1)		SLE(8) WEE(12) Turlock(12) Bunyamwera Group(1)
TOTALS	SLE(1) Turlock(1)	SLE(1) Turlock(16) Hart Park(2) Main Drain(1)	SLE(16) Turlock(25) WEE(46) Hart Park(5)	SLE(21) Turlock(16) WEE(38) Hart Park(14) Unident.(1)	SLE(17) Turlock(12) WEE(11) Hart Park(2) Main Drain(1) CEV(1)	SLE(16) Turlock(3) WEE(1) Hart Park(3) CEV(1) Bunyamwera Group(1)	SLE(3) Turlock(1) WEE(1) Main Drain(1) CEV(1) Bunyamwera Group(1)	SLE(75) Turlock(74) WEE(97) Hart Park(26) Main Drain(3) CEV(3) Bunyamwera Group(2) Unidentified(1)
	2	20	92	89	44	25	8	281 isolates

Table 7.- Viral isolates from mosquito pools, by the Viral and Rickettsial Disease Laboratory, California State Department of Health, 1973

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V5-3133	Imperial	Seeley	4/4	Culex tarsalis	50	SLE
V5-3255	Imperial	Winterhaven	4/5	C. tarsalis	50	Turlock
V5-3408	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3420	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3446	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3455	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3466	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3478	Imperial	Calexico	5/1	C. tarsalis	50	Turlock
V5-3500	Imperial	Westmoreland	5/1	C. tarsalis	50	Turlock
V5-3535	Imperial	Niland	5/2	C. tarsalis	50	Turlock
V5-3573	Imperial	Winterhaven	5/3	C. tarsalis	51	Turlock
V5-3694	Imperial	Winterhaven	5/3	C. tarsalis	50	Turlock
V5-3723	Imperial	Winterhaven	5/3	C. tarsalis	27	Turlock
V5-3755	Imperial	Winterhaven	5/3	C. tarsalis	54	Turlock
V5-3779	Imperial	Winterhaven	5/3	A. vexans	50	SLE&Main Drain
V5-3974	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V5-3989	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V5-4018	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V5-4021	Yuma, Arizona	Yuma	5/4	C. tarsalis	50	Turlock
V4-1241	Kings	Lemoore	5/29	C. tarsalis	50	Hart Park
V4-1253	Kings	Lemoore	5/29	C. tarsalis	50	Hart Park
V5-4031	Imperial	Holtville	6/5	C. tarsalis	47	Turlock
V5-4040	Imperial	Calexico	6/5	C. tarsalis	50	SLE
V5-4082	Imperial	Calexico	6/5	C. tarsalis	50	WEE
V2-2008	Stanislaus	Newman	6/6	C. tarsalis	17	Hart Park
V5-4094	Imperial	Calipatria	6/6	C. tarsalis	50	Turlock
V5-4098	Imperial	Calipatria	6/6	C. tarsalis	50	Turlock
V5-4102	Imperial	Seeley	6/6	C. tarsalis	50	Turlock
V5-4103	Imperial	Seeley	6/6	C. tarsalis	50	Turlock
V5-4104	Imperial	Seeley	6/6	C. tarsalis	48	WEE
V5-4119	Imperial	Brawley	6/6	C. tarsalis	50	SLE
V5-4120	Imperial	Brawley	6/6	C. tarsalis	50	WEE
V5-4121	Imperial	Brawley	6/6	C. tarsalis	50	SLE
V5-4123	Imperial	Brawley	6/6	C. tarsalis	50	SLE
V5-4125	Imperial	Brawley	6/6	C. tarsalis	54	Turlock
V5-4140	Imperial	Niland	6/6	C. tarsalis	50	WEE
V5-4142	Imperial	Niland	6/6	C. erythrothorax	50	WEE
V5-4149	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4152	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4155	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4162	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4163	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4165	Imperial	Westmoreland	6/6	C. tarsalis	50	WEE
V5-4176	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4177	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4178	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4179	Imperial	Andrade	6/7	C. tarsalis	12	SLE
V5-4187	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4192	Imperial	Andrade	6/7	C. tarsalis	50	WEE
V5-4201	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4206	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4234	Imperial	Winterhaven	6/7	C. tarsalis	45	WEE
V5-4236	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4242	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4244	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4247	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4251	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4255	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4256	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	SLE & Turlock
V5-4257	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	Turlock
V5-4258	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4260	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	SLE & Turlock
V5-4261	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4262	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4263	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4264	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	WEE
V5-4265	Yuma, Arizona	Yuma	6/7	C. tarsalis	50	Turlock
V5-4269	Imperial	Winterhaven	6/7	C. tarsalis	51	Turlock
V5-4273	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4274	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE

Table 7.-(continued)

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V5-4275	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4277	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4278	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4281	Imperial	Winterhaven	6/7	C. tarsalis	50	SLE
V5-4282	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4285	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4286	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4287	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4293	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4294	Imperial	Winterhaven	6/7	C. tarsalis	50	Turlock
V5-4300	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4301	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4302	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V5-4306	Imperial	Winterhaven	6/7	C. tarsalis	50	WEE
V6-1092	Imperial	Winterhaven	6/8	C. tarsalis	17	WEE
V6-1093	Imperial	Winterhaven	6/8	C. tarsalis	50	SLE
V6-1094	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1095	Imperial	Winterhaven	6/8	C. tarsalis	50	SLE
V6-1096	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1097	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1107	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1112	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	Turlock
V6-1114	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	Turlock
V6-1115	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1119	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	Turlock
V6-1120	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	SLE
V6-1123	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1131	Imperial	Winterhaven	6/8	C. tarsalis	50	Turlock
V6-1132	Imperial	Winterhaven	6/8	C. tarsalis	50	WEE
V6-1133	Imperial	Winterhaven	6/8	C. tarsalis	43	Turlock
V6-1148	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1149	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V6-1152	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	SLE
V6-1154	Yuma, Arizona	Yuma	6/8	C. tarsalis	50	WEE
V4-1275	Kings	Lemoore	6/13	C. tarsalis	11	Hart Park
V4-1277	Kings	Lemoore	6/13	C. tarsalis	40	Hart Park
V2-1515	Sutter	Pleasant Grove	6/14	C. tarsalis	50	Hart Park
V5-6151	San Diego	Escondido	6/28	C. tarsalis	27	Turlock
V5-6121	San Diego	Chula Vista	6/29	C. tarsalis	50	Turlock
V5-6123	San Diego	Chula Vista	6/29	C. tarsalis	51	Turlock
V5-6128	San Diego	San Ysidro	6/29	C. tarsalis	35	Hart Park
V5-4417	Imperial	Calexico	7/3	C. tarsalis	9	SLE
V5-4421	Imperial	Calexico	7/3	C. tarsalis	7	SLE
V5-4427	Imperial	Holtville	7/3	C. tarsalis	50	WEE
V5-4428	Imperial	Holtville	7/3	C. tarsalis	50	WEE
V5-4430	Imperial	Holtville	7/3	C. tarsalis	43	SLE
V5-4432	Imperial	Seeley	7/3	C. tarsalis	50	SLE
V5-4435	Imperial	Holtville	7/3	C. tarsalis	19	SLE
V5-4440	Imperial	Holtville	7/3	P. confinnis	50	WEE
V5-4443	Imperial	Calexico	7/3	C. tarsalis	50	Turlock
V5-4444	Imperial	Calexico	7/3	C. tarsalis	34	WEE
V5-4446	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4449	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4450	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4451	Imperial	Calexico	7/3	C. tarsalis	50	SLE
V5-4452	Imperial	Calexico	7/3	C. tarsalis	50	WEE
V5-4456	Imperial	Calexico	7/3	C. tarsalis	36	WEE
V5-4458	Imperial	Brawley	7/4	C. tarsalis	61	SLE
V5-4468	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4469	Imperial	Seeley	7/4	C. tarsalis	50	SLE
V5-4475	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4476	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4477	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4480	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4481	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4482	Imperial	Westmoreland	7/4	C. tarsalis	50	WEE
V5-4484	Imperial	Westmoreland	7/4	C. tarsalis	56	WEE
V5-4489	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4494	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE
V5-4495	Imperial	Westmoreland	7/4	C. tarsalis	50	SLE

Table 7.-(continued)

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V5-4497	Imperial	Westmoreland	7/4	C. tarsalis	33	WEE
V5-4498	Imperial	Niland	7/4	C. tarsalis	50	SLE
V5-4500	Imperial	Seeley	7/4	C. tarsalis	46	SLE
V5-4504	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4505	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4506	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4509	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4510	Imperial	Seeley	7/4	C. tarsalis	50	Turlock
V5-4511	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4512	Imperial	Seeley	7/4	C. tarsalis	50	WEE
V5-4514	Imperial	Seeley	7/4	C. tarsalis	47	WEE
V4-1315	Merced	Merced	7/5	C. tarsalis	50	Hart Park
V5-4313	Imperial	Winterhaven	7/5	C. tarsalis	31	WEE
V5-4315	Imperial	Winterhaven	7/5	C. tarsalis	41	WEE
V5-4316	Imperial	Winterhaven	7/5	C. tarsalis	4	Turlock
V5-4324	Imperial	Winterhaven	7/5	C. tarsalis	48	WEE
V5-4326	Imperial	Winterhaven	7/5	C. tarsalis	15	WEE
V5-4329	Yuma, Arizona	Yuma	7/5	C. tarsalis	46	SLE
V5-4330	Imperial	Winterhaven	7/5	C. tarsalis	11	WEE
V5-4332	Yuma	Yuma	7/5	C. tarsalis	17	WEE
V5-4333	Yuma, Arizona	Yuma	7/5	C. tarsalis	50	WEE
V5-4335	Imperial	Winterhaven	7/5	C. tarsalis	50	WEE
V5-4339	Yuma, Arizona	Yuma	7/5	C. tarsalis	40	WEE
V5-4340	Yuma, Arizona	Yuma	7/5	C. tarsalis	24	Turlock
V2-1559	Sutter	Pleasant Grove	7/6	C. tarsalis	41	Hart Park
V2-1562	Placer	Lincoln	7/6	C. tarsalis	50	Turlock
V5-4341	Riverside	Blythe	7/6	C. tarsalis	7	SLE
V5-4351	Imperial	Palo Verde	7/6	C. tarsalis	19	Turlock
V5-4353	Riverside	Blythe	7/6	C. tarsalis	17	WEE
V5-4354	Riverside	Blythe	7/6	C. tarsalis	28	WEE
V5-4360	Riverside	Blythe	7/6	C. tarsalis	4	WEE
V5-4365	Riverside	Ripley	7/6	C. tarsalis	22	WEE
V5-4372	Riverside	Blythe	7/6	C. tarsalis	38	SLE
V5-4374	Imperial	Palo Verde	7/6	C. tarsalis	20	WEE
V5-4381	Imperial	Palo Verde	7/6	C. tarsalis	50	WEE
V5-4383	Imperial	Palo Verde	7/6	C. tarsalis	50	WEE
V5-4384	Imperial	Palo Verde	7/6	C. tarsalis	50	WEE
V5-4387	Imperial	Palo Verde	7/6	C. tarsalis	53	Unknown
V5-4390	Imperial	Palo Verde	7/6	C. tarsalis	31	WEE
V1-1034	Tehama	Gerber	7/10	C. tarsalis	50	Hart Park
V2-1574	Colusa	Princeton	7/12	C. tarsalis	21	Hart Park
V2-2038	San Joaquin	Escalon	7/12	C. tarsalis	50	Hart Park
V2-2039	San Joaquin	Escalon	7/12	C. tarsalis	50	Turlock
V2-2044	San Joaquin	Escalon	7/12	C. tarsalis	50	Hart Park
V2-2049	San Joaquin	Escalon	7/12	C. tarsalis	50	Hart Park
V1-1040	Shasta	Palo Cedro	7/13	C. tarsalis	32	Hart Park
V5-8504	Ventura	Thousand Oaks	7/14	C. peus	50	Turlock
V2-2058	Stanislaus	Newman	7/17	C. tarsalis	52	Hart Park
V2-2063	Stanislaus	Mountain View	7/17	C. tarsalis	44	Hart Park
V5-8001	Riverside	Riverside	7/19	C. peus	12	Turlock
V4-1332	Madera	Madera	7/24	C. tarsalis	41	Hart Park
V4-1346	Merced	Merced	7/25	C. tarsalis	19	Turlock
V2-1587	Sutter	Sutter City	7/26	C. tarsalis	50	Turlock
V2-1588	Sutter	Sutter City	7/26	C. tarsalis	50	Turlock & Hart Park
V2-1589	Sutter	Sutter City	7/26	C. tarsalis	50	Hart Park
V2-1590	Sutter	Sutter City	7/26	C. tarsalis	50	Hart Park
V5-6179	San Diego	San Ysidro	7/31	C. peus	11	Turlock
V5-6192	San Diego	San Ysidro	7/31	C. tarsalis	38	Turlock
V5-6208	San Diego	Santee	7/31	C. tarsalis	50	Turlock
V5-6209	San Diego	Santee	7/31	C. tarsalis	50	Turlock
V5-6236	San Diego	Rancho Santa Fe	8/1	C. tarsalis	31	Turlock
V5-6238	San Diego	San Diego	8/1	C. tarsalis	50	Turlock
V5-6240	San Diego	Rancho Santa Fe	8/1	C. tarsalis	55	Turlock
V5-6261	San Diego	Rancho Santa Fe	8/1	C. tarsalis	56	Turlock
V1-1066	Lassen	Wendel	8/2	C. tarsalis	50	SLE
V1-1074	Lassen	Wendel	8/2	C. tarsalis	50	Turlock
V5-4527	Imperial	Holtville	8/2	C. tarsalis	15	WEE
V5-4531	Imperial	Calexico	8/2	C. tarsalis	35	WEE
V5-4537	Imperial	Calexico	8/2	C. tarsalis	50	WEE
V5-4588	Imperial	Niland	8/3	C. tarsalis	47	SLE

Table 7.-(continued)

Identifying Number	County	Place	Date Collected	Species	Number in pool	Isolate
V2-1684	Sutter	Sutter	8/3	A. melanimon	24	CEV
V2-1687	Sutter	Sutter	8/3	C. tarsalis	50	WEE
V5-4609	Imperial	Winterhaven	8/4	C. tarsalis	21	Main Drain
V5-4635	Imperial	Winterhaven	8/4	C. tarsalis	39	SLE
V5-4664	Yuma, Arizona	Yuma	8/4	C. tarsalis	50	SLE
V5-4682	Yuma, Arizona	Yuma	8/4	C. erythrothorax	50	SLE
V4-1380	Madera	Madera	8/7	C. tarsalis	42	Turlock & Hart Park
V4-1383	Tulare	Woodville	8/8	C. tarsalis	8	Turlock
V1-1081	Shasta	Palo Cedro	8/10	C. tarsalis	50	Turlock & Hart Park
V2-1642	Sutter	Meridian	8/12	C. tarsalis	50	Turlock
V2-1648	Colusa	Moon Bend	8/12	C. tarsalis	50	Turlock
V4-1409	Kern	Maricopa	8/15	C. tarsalis	36	Turlock
V5-9205	San Bernardino	Needles	8/17	C. tarsalis	47	WEE & SLE
V5-9212	Mojave	Bermuda	8/17	C. tarsalis	50	WEE
V5-9214	Mojave	Bermuda	8/17	C. tarsalis	50	WEE & SLE
V5-9215	Mojave	Bermuda	8/17	C. tarsalis	50	SLE
V5-9216	Mojave	Bermuda	8/17	C. tarsalis	50	WEE
V5-9217	Mojave	Bermuda	8/17	C. tarsalis	50	WEE
V5-9218	Mojave	Bermuda	8/17	C. tarsalis	53	SLE
V5-9228	San Bernardino	Needles	8/17	C. tarsalis	33	WEE
V5-9242	San Bernardino	Needles	8/17	C. tarsalis	50	SLE
V5-9243	San Bernardino	Needles	8/17	C. tarsalis	58	SLE
V5-5099	Imperial	Seeley	8/28	C. tarsalis	30	SLE
V5-5118	Imperial	Calexico	8/28	C. tarsalis	27	SLE
V5-5126	Imperial	Calexico	8/28	C. tarsalis	36	SLE
V5-5148	Imperial	Seeley	8/29	C. tarsalis	50	Turlock
V5-5181	Imperial	Niland	8/29	C. tarsalis	50	SLE
V5-4952	Imperial	Winterhaven	8/30	C. tarsalis	48	SLE
V5-5078	Yuma, Arizona	Yuma	8/30	C. erythrothorax	50	SLE
V5-5086	Imperial	Winterhaven	8/30	C. tarsalis	50	WEE
V5-6277	San Diego	Santee	9/5	C. pipiens	10	SLE
V5-6291	San Diego	Chula Vista	9/5	C. tarsalis	17	Hart Park
V5-6321	San Diego	Rancho Santa Fe	9/6	C. tarsalis	49	Hart Park
V5-6332	San Diego	Rancho Santa Fe	9/6	C. tarsalis	50	SLE
V5-6333	San Diego	Rancho Santa Fe	9/6	C. tarsalis	50	SLE
V5-6334	San Diego	Rancho Santa Fe	9/6	C. tarsalis	28	SLE & Hart Park
V5-6338	San Diego	Rancho Santa Fe	9/6	C. tarsalis	31	Turlock
V4-1481	Kern	Bakersfield	9/12	C. tarsalis	51	SLE
V4-1494	Kern	Maricopa	9/12	C. tarsalis	50	SLE
V4-1499	Kern	Maricopa	9/12	C. tarsalis	50	CEV
V2-2083	Stanislaus	Newman	9/13	C. tarsalis	45	WEE
V4-1520	Tulare	Woodville	9/18	C. tarsalis	50	SLE
V5-5297	Imperial	Calexico	9/25	C. tarsalis	57	SLE
V5-5300	Imperial	Calexico	9/25	P. confinnis	39	SLE
V5-5317	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5320	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5325	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5330	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5332	Imperial	Calexico	9/25	C. tarsalis	50	Turlock & SLE
V5-5333	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5336	Imperial	Calexico	9/25	C. tarsalis	50	SLE
V5-5347	Imperial	Seeley	9/26	C. tarsalis	50	Turlock
V5-5455	Yuma, Arizona	Yuma	9/27	C. inornata	3	Bunyamwera group
V2-1704	Colusa	Maxwell	10/3	C. tarsalis	27	WEE
V5-6378	San Diego	Rancho Santa Fe	10/4	C. tarsalis	38	Turlock
V5-6406	San Diego	Rancho Santa Fe	10/4	C. tarsalis	50	SLE
V5-6430	San Diego	Santee	10/19	A. franciscanus	4	Main Drain
V5-6460	San Diego	Lakeside	10/19	C. erythrothorax	21	SLE
V5-6465	San Diego	Santee	10/19	C. tarsalis	18	SLE
V5-6480	San Diego	Santee	10/19	C. inornata	25	Bunyamwera group
V5-9253	San Bernardino	Parker Dam	10/25	C. inornata	38	CEV