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Biology and control of Aedes aegypti.

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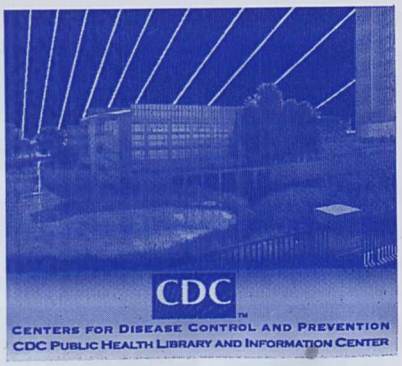

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VECTOR TOPICS

NO. 4

Biology and Control of *Aedes aegypti*

SEPTEMBER 1979

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
CENTER FOR DISEASE CONTROL
BUREAU OF TROPICAL DISEASES
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PUBLIC HEALTH IMPORTANCE OF *AEDES AEGYPTI*

Introduction

Aedes aegypti is a highly domestic mosquito, characteristically breeding in artificial containers in and around human habitation. The species is widely distributed around the world, generally within the limits of 45° north and 35° south latitude. While it has been known to thrive outside these limits, such populations are probably introduced during the warm seasons but do not survive the winters. *Aedes aegypti* is thought to be a tropical old-world species which was introduced into the Western Hemisphere during the early European explorations and colonizations. In the Western Hemisphere, *Ae. aegypti* is currently known to exist or to have existed in all countries and territories except Canada. In the United States, the species is widely distributed throughout the Southeast, being found in at least 10 states and in Puerto Rico and the U.S. Virgin Islands.

The role of *Ae. aegypti* as a vector of human disease was first demonstrated in 1900-1901, when studies in Cuba definitely incriminated the species as a vector of yellow fever. In 1906 in Australia, *Ae. aegypti* was suggested as a vector of dengue, another important viral disease. It was proven to transmit dengue during an epidemic in that country in 1916. The species has also been implicated in the transmission of several additional viral infections (e.g., Chikungunya and Zika viruses) and has been demonstrated as an important vector of the dog heartworm, *Dirofilaria immitis*.

Because of its habit of breeding and feeding in and around human habitations, populations of *Ae. aegypti* may at times reach proportions sufficient to become a serious pest mosquito in some U.S. mainland areas; however, only in the U.S. territories in the Caribbean has the species been involved in the transmission of human disease during the past several decades. Because of the recent dengue outbreaks in Puerto Rico and the Virgin Islands, active *Ae. aegypti* surveillance and control programs exist in these areas. However, in the southeastern United States such programs are relatively rare, existing in only a few urban situations with active mosquito abatement programs which include specific efforts directed at the control of this potential vector.

The control of *Ae. aegypti* has been of importance in the Western Hemisphere since it was demonstrated that the species was the principal vector of yellow fever. At that time, considerable enthusiasm was generated in favor of the concept that yellow fever could be eradicated by vector control. This concept was developed on limited knowledge of the natural history of yellow fever and a conviction that the control of *Ae. aegypti* would prevent the maintenance of the virus in nature. Later information showed that the natural cycle of yellow fever virus involved monkeys and mosquito species other than *Ae. aegypti* in a jungle environment and, therefore, the natural cycle would remain unaffected by efforts to eradicate yellow fever from the urban areas.

In the early 1920's some antimosquito programs in South America succeeded in eliminating *Ae. aegypti* from defined areas. Such success was often cited in support of a growing concept of species eradication for the control of yellow fever. In 1947, the member nations of the Pan American Health Organization (PAHO) resolved to eradicate *Ae. aegypti* from the Western Hemisphere. Nearly all of the involved countries and smaller political entities subscribed to this concept, and during the last three decades, at one time or another, have planned or conducted a program with *Ae. aegypti* eradication as the objective. Based on standard criteria developed by PAHO, by

1965 17 countries had eradication confirmed. In 1977 only seven countries and the Panama Canal Zone were shown by PAHO to have completed eradication and were under a surveillance program. Many countries have now experienced the discouragement of administrative, technical, or financial problems, and regardless of the level of control effort, few areas, if any, have remained permanently free of resurgent *Ae. aegypti* populations.

Yellow Fever

Yellow fever is a severe, mosquito-borne hemorrhagic viral disease of the tropics characterized by high fever, generalized pain, hemorrhage, jaundice and prostration. Inapparent, subclinical yellow fever infections do occur and many overt cases are mild, but the usual clinical course is the sudden onset of fever (often greater than 40°C), headache, and generalized pain. Other early signs are conjunctivitis, photophobia, low white blood cell count, and low platelet count. Early involvement of the liver results in an enlarged liver and jaundice. The heart and kidneys may be involved and renal shutdown may occur. Hemorrhagic manifestations follow which may vary from bleeding gums to massive vomiting of blood, the so-called "black vomit." Prostration, coma, and death may ensue. Although in some epidemics more than half of those with yellow fever have died, the mortality rate is usually 5 to 10%.

There is no specific treatment for yellow fever. Supportive measures such as maintenance of proper hydration and procedures to decrease the high fever are all that are available. Infection elicits an immune response that protects an individual for life. Reinfection is not known to occur.

Diagnosis: Yellow fever should be suspected in any patient exposed in an area where yellow fever transmission is occurring, who has high fever and prostration, followed by heptomegaly, jaundice, hemorrhagic manifestations and renal problems. Laboratory confirmation should be immediately sought. Inapparent and mild cases do occur.

Serum samples collected acutely and then 10-14 days later will show a rise in titer by neutralization (N), hemagglutination-inhibition (HI) or complement fixation (CF) tests. Because the yellow fever virus is a flavivirus (group B arbovirus), serologic cross reactions occur to related viruses such as dengue, Japanese B encephalitis, and St. Louis encephalitis. Therefore, for the diagnosis to be established the virus must be isolated, or serologic titers and neutralization indices must be greater for yellow fever than for the other flaviviruses.

Yellow fever virus can be isolated from blood collected 2-3 days after onset of illness and inoculated into suckling mice or mosquito cell culture. The virus can also be isolated from liver material obtained at postmortem examination.

Yellow fever virus may be isolated from *Ae. aegypti* mosquitoes or from monkeys, but such isolation does not help in the clinical diagnosis of a patient since it merely confirms the presence of virus activity in the area.

Epidemiology: *Aedes aegypti* is the major vector of urban yellow fever and *Haemogogus* or *Sabethes* spp. are the major vectors responsible for sylvatic transmission in the New World.

The mosquito becomes infectious 1-2 weeks (depending on the ambient temperature) after biting an infected person. At that time, spherical viral particles can be found in the vector's salivary gland. The mosquito remains infectious for life and infects the primate host during the ingestion of blood. Clinical symptoms

appear within 3-6 days, and the virus is present in the blood for at least 3 days after the onset of symptoms. Although severity of the disease may vary according to age, in a nonimmune population all age groups are involved.

Human infection with yellow fever results from two different cycles of virus transmission, urban and sylvatic. The urban cycle is the simple transmission from person to person by *Ae. aegypti*, usually occurring in epidemic form. The sylvatic or jungle yellow fever cycle may vary according to the ecologic situation, available primate reservoirs, and vector populations. Some species of monkeys have a high case fatality rate, but others rarely succumb to sylvatic yellow fever. The principal sylvatic primate hosts include several species of monkeys; in the Western Hemisphere the virus is transmitted among these hosts by mosquitoes of the genera *Haemogogus* and *Sabethes*, which typically inhabit the treetops or forest canopy. Humans become involved in the sylvatic cycle incidentally in two ways: either human invasion of the forest or human-directed change in the terrain brings people into contact with the otherwise remote treetop mosquitoes of the same areas, or infected primates approach a semi-sylvan village, are bitten by peridomestic mosquitoes (such as *Ae. simpsoni* in Africa), and then these mosquitoes transmit the disease to humans. The initiation of an urban epidemic outbreak may then ensue, providing an urban vector such as *Ae. aegypti* is present.

For centuries, yellow fever was a serious scourge in the tropical Americas and Africa, extending to temperate areas in violent epidemics during the summers, chiefly in seaport and river cities. In the United States, devastating epidemics occurred during the period from 1668 (New York) to 1905 (New Orleans), striking cities from Texas to New England. Philadelphia suffered 20 epidemics, New York 15, Boston 8, and Baltimore 7. The 1793 Philadelphia epidemic was most severe, with 4,041 deaths from August to November in a city of only 40,000. The explosive nature of the outbreaks is illustrated by the 1878 epidemic in Memphis, Tenn., where approximately 4,000 people died, and by the 1898 epidemic in New Orleans, La., which produced 13,817 cases and 3,894 deaths. The last epidemic in the United States (1905), with 8,399 cases and 908 deaths, struck most heavily in New Orleans which reported 3,384 cases and 443 deaths. The fact that the 1905 epidemic was much less extensive in New Orleans than that of 1898 was attributed largely to a concerted drive against *Ae. aegypti*, the sole urban vector of yellow fever.

Unlike the United States, the countries of Central and South America continued to experience urban epidemics of yellow fever. In 1928 and 1929, the disease reappeared in Rio de Janeiro, Brazil, after an absence of 20 years, with 435 recorded deaths. During the years 1932 to 1954, urban yellow fever occurred in one or more municipalities in Bolivia, Brazil, Colombia, Paraguay, and Trinidad. In addition, outbreaks of jungle yellow fever have continued to occur in all of the Central and South American countries with the exception of El Salvador, Uruguay, and Chile.

In areas of South America where the mosquito-nonhuman primate cycle continues the natural maintenance of yellow fever transmission, hundreds of cases of human sylvatic yellow fever have been reported. Cases and deaths were reported from regions of Paraguay, Brazil, and Argentina in 1966. Fatal cases are reported almost annually from Peru, Colombia, and Venezuela. A resurgence of sylvatic yellow fever in Ecuador was noted in 1975 due to the opening of the eastern regions of the country to oil exploration, and has periodically occurred in Bolivia and Venezuela. In 1948 there was an outbreak of sylvatic yellow fever in Panama, and during the next decade it appeared to spread northward through Central America as far as southern Mexico. Sylvatic yellow fever reappeared in eastern Panama in 1974, but no northward spread was apparent following this outbreak. In 1978-1979 sylvatic yellow fever was reported from Trinidad. Despite the relatively frequent occurrence of human cases of yellow fever acquired in the sylvatic cycle, no significant epidemics of urban yellow fever have been reported in the Americas since 1954.

Sylvatic yellow fever is common in recognized areas of sub-Sahara Africa and has given rise to epidemics in Eastern Nigeria, the Sudan, Ethiopia, and Senegal. The most recent epidemics have been reported from the Gambia in 1978-1979.

Yellow fever has never invaded Asia despite the widespread distribution of man-biting *Ae. aegypti*. The reason is unknown.

In theory, the possibility of the recurrence of epidemics of yellow fever persists wherever there are populations of *Ae. aegypti*. However, epidemics have not occurred within the United States since 1905, and such epidemics in North America or in the Antilles would seem unlikely to occur in the future, even with the presence of *Ae. aegypti* populations, because of the absence of nearby jungle areas where the sylvan cycle of yellow fever persists. Current opinion is that, should a yellow fever outbreak occur in this area, it could be quickly brought under control with the use of vaccines and modern mosquito control methods.

Prevention and control: Effective vaccines are available which offer protection against yellow fever. No case of yellow fever has been reported in a properly vaccinated individual. Toxic reactions are minimal; however, persons with known allergies to eggs or egg products should be vaccinated with caution. Current recommendations suggest revaccination at 10-year intervals.

Epidemic yellow fever can be prevented by control of the vector, *Ae. aegypti*. Elimination of sylvatic yellow fever in the jungles of Africa and South America is presently inconceivable because of the multiplicity of mosquito vectors, the unknown range and identity of vertebrate reservoirs, and the vast areas involved.

In the Americas, the principal vectors are members of the genera *Haemagogus, Aedes, and Sabethes*. In Africa, jungle yellow fever is essentially a disease of monkeys. *Aedes africanus* and *Ae. luteocephalus* appear to be the active vectors of the monkey population, whereas *Ae. simpsoni*, a semidomestic mosquito, transmits the virus from monkey to man and, at least in some areas, joins *Ae. aegypti* in transmission from person to person.

Dengue

Dengue is a mosquito-borne viral infection of the tropics and subtropics, classically characterized by fever and severe eye, joint, muscle, and bone pain—hence the name "breakbone fever." Skin rashes may occur. Though generally considered an acute, nonfatal disease, in some areas of the world dengue infection commonly results in severe, frequently fatal diseases known as "dengue hemorrhagic fever" and "dengue shock syndrome." *Aedes aegypti* is the most important vector.

Infection with any of the 4 known dengue serotypes may be asymptomatic, may cause nonspecific febrile or respiratory illness, or may cause "classical" dengue fever. In some patients a reticular, morbilliform, or macular rash develops on the 2nd to 5th day, occurring first on the trunk but later spreading to the arms, legs, and face. Itching of the palms and soles and generalized lymphadenopathy may also occur. A later petechial rash on the extremities, axillae, or in mucuous membranes may occur on the last day of fever in 20-70% of patients. The uncomplicated case is rarely, if ever, fatal but the patient may suffer from continued fatigue and weakness for several weeks.

The syndromes "dengue hemorrhagic fever" (DHF) and "dengue shock syndrome" (DSS), occur primarily in children 2-6 days after the onset of fever and are manifest by vomiting, shortness of breath, enlarged liver, and bleeding in the skin, in the intestines, and from the gums. These hemorrhagic manifestations may progress to shock and death. It is likely that the DHF and DSS are related, with DSS representing the severe consequences of the hemorrhagic disease. The cause of these more

severe manifestations of dengue is unclear. The viral agents appear to be the same as those responsible for the classical nonfatal disease; however, further studies are required.

Diagnosis: The dengue viruses are classified as flaviviruses (group B arboviruses) and demonstrate considerable serologic cross-reactivity with other flaviviruses such as yellow fever, Japanese B encephalitis, and St. Louis encephalitis. There are 4 distinct serologic types of dengue virus (dengue 1, 2, 3, and 4). Infection with one serotype does not induce lasting immunity to others.

In individuals without prior flavivirus experience, the diagnosis of dengue can be established by the demonstration of seroconversion or a 4-fold rise in titer between acute and convalescent serum by hemagglutination inhibition (HI), complement fixation (CF), or plaque reduction neutralization tests. If 2 different serologic tests show a primary antibody response to a single dengue serotype, that serotype is probably the cause of the disease.

Subsequent infections with flaviviruses result in broadly cross-reactive results and are more difficult to interpret. Strict proof of causation demands virus isolation.

Epidemiology: Dengue virus has been reported most frequently between latitudes 25°N and 25°S. The disease is transmitted by mosquitoes of the subgenus *Stegomyia* with *Ae. aegypti* being the principal species involved in transmission. There is no significant reservoir amplifying bird or animal host although several species of monkeys are known to be susceptible to dengue. The endemic/epidemic cycle is human-mosquito-human.

After a person is bitten by an infected mosquito, the virus can be found in the blood (viremia) after about 5-6 days (prepatent period) at about the same time that the initial symptoms of the disease develop (incubation period). At this time, and for the next 4-5 days, the person is infective for the vector mosquitoes which may take a blood meal. After an incubation period of 2-15 days, generally 8-11 days, the female *Ae. aegypti* which acquires dengue virus by feeding on a viremic human is infective for life with the potential of transmitting the infection each time she feeds on a new human subject.

Historically, dengue has been seen in sweeping epidemics in many areas of the world. One of the earliest accounts of such an epidemic was that of Dr. Benjamin Rush who described a severe outbreak in Philadelphia during the summer and fall of 1780. Since the 18th century, numerous epidemics have occurred in tropical and neotropical areas throughout the world. Notable among these have been epidemics in the southern United States in 1922, with perhaps as many as 2 million cases, and in Greece in 1927-1928 with approximately 1 million cases. Although the last continental U.S. epidemic occurred in Louisiana in 1945, there have been epidemics in Puerto Rico in 1963, 1969, 1975, 1977, and 1978. Dengue has persisted as an endemic disease in Puerto Rico and other areas in and adjacent to the Caribbean, with occasional epidemics occurring throughout this region. The largest recent epidemic occurred in Colombia in 1972, with an estimated half-million cases. In Puerto Rico there have been estimates as high as 224,000 clinical cases (1,358 confirmed) in 1977 and 450,000 clinical cases (2,602 confirmed) in 1978.

Dengue continues to be endemic and epidemic in wide areas of south and southeast Asia and in the southern and western Pacific. In these regions all four serotypes are found and hemorrhagic manifestations of the disease are relatively common.

Until 1977, only dengue virus types 2 and 3 had been isolated in the Western Hemisphere, although there had been serologic evidence of dengue type 1 in older

individuals. However, since 1977 epidemics of dengue type 1 have been reported from Puerto Rico, Jamaica, Virgin Islands, Belize, Guatemala, El Salvador, Honduras, Colombia, and Mexico. Of concern was the report in Puerto Rico in late 1975 of several cases with more severe hemorrhagic manifestations than usual for this area and which were possibly definable as dengue hemorrhagic fever (DHF), the severe form of the disease which had not been previously reported from this area. Although borderline cases of DHF have been observed in Curacao, other cases have not been found in the Western Hemisphere.

Dengue is an ever present threat in any area within the range of the vector. The potential for the establishment of an epidemic from an imported case is obvious given the 5- to 6-day incubation period during which time an infected person may be completely asymptomatic, the rapid air transport now available, and the wide distribution of the principal vector, *Ae. aegypti*. The recent rapid spread and propagation of dengue 1 epidemics in the Caribbean is testimony to the epidemic potential existing with the combination of a nonimmune population, a receptive vector, and imported cases. In 1978 alone, 89 confirmed cases of dengue were diagnosed in persons arriving in the United States from the Caribbean area. In spite of this importation of cases, no introduction of transmission resulting in secondary cases was seen. Nevertheless, with the probable continuing epidemic outbreaks in nearby areas, the risk of introduction of dengue into the southeastern United States where *Ae. aegypti* populations are widespread, remains a significant potential health problem. Dengue also continues to be endemic and epidemic in wide areas of south and southeast Asia and in the southern and western Pacific. In these areas *Ae. aegypti* is joined by several other *Aedes* species which may serve as efficient vectors. While importation of cases into the United States from these areas seems less likely, there were at least six confirmed cases imported into Guam from Vietnam in 1975 during the admission of refugees from that country; there was no transmission of the infection reported in Guam as a result of the case importation. However, it does seem likely that the occasional dengue epidemics in island situations in the southern Pacific area result from the importation of a case or cases during the incubation period and subsequent infection of local mosquitoes.

Prevention and control: Because no practical immunization or therapeutic measures exist for preventing or treating dengue, control of this disease relies entirely on the control of the mosquito vector, on the early identification of cases and, if possible, on the isolation of cases from contact with vector mosquitoes. At present there is no vaccine available for dengue, although experimental attempts to develop a vaccine are being carried out.

While *Ae. aegypti* is recognized as the vector of dengue in the Western Hemisphere, in other areas other mosquito species can transmit dengue virus. *Aedes albopictus* can be an efficient vector of dengue in all areas where both the mosquito and the disease appear together. This species breeds commonly in tree and rock holes as well as artificial containers and is widely distributed in south and southeast Asia and in many Pacific islands.

Aedes scutellaris consists of a complex of about 17 distinct but very closely related species known as the *scutellaris* group. Because *scutellaris* appears in large numbers and attacks humans readily and because other vectors are absent, it is regarded as a vector of dengue in New Hebrides and northern New Guinea. Members of the complex will breed in almost any small, well-shaded collection of clear rainwater, both in ground pools and artificial containers.

BIOLOGY AND HABITS OF *AEDES AEGYPTI*

Aedes aegypti is a highly domestic mosquito. In the Western Hemisphere the species is closely associated with humans. Artificial containers, so abundantly provided by modern industrial society, are by far its most important breeding place and are essential to the production and maintenance of large populations of *Ae. aegypti*. Although treeholes and possibly other naturally occurring containers produce *Ae. aegypti*, the overwhelming majority come from auto tires, buckets, pet watering pans, tin cans, vases, jars, clogged roof gutters, and, in fact, from almost any man-made object that retains water and has walls other than soil.

Some containers are more attractive to the mosquitoes than others. Female *Ae. aegypti* are attracted to dark-colored containers with wide openings, especially when they are located in shaded areas. Dark-colored water and the presence of decaying leaves stimulate oviposition but odorous and highly polluted receptacles will be avoided.

Oviposition occurs mainly in the afternoon. If the walls of the container are very smooth (e.g., glass), the eggs may be scattered on the water surface but usually they are attached to the sides of the container at or near the water line. The eggs are less than 1 mm in length and are white at first, but within 2 hours they darken to an almost black color. At oviposition the embryos within the eggs are not yet ready to hatch. A period of 2-3 days at the high humidity near the water line is necessary for their full development to the larval stage. Should the eggs become dry during this developmental period, they will collapse and the embryos will die. By the time larvae are fully formed the eggs are resistant to dessication and can survive for periods of several months to more than a year. Under dry conditions, the dormant larvae within the eggs remain capable of hatching whenever the eggs are submerged by a rising water level and the consequent decreased oxygen supply furnishes the necessary hatch stimulus. Not all eggs hatch the first time they are flooded.

The larva which emerges from the ruptured egg shell is the first of 4 larval stages, each larger than the one preceding (Appendix I illustrates the characters used in identification of *Ae. aegypti* larvae and includes a pictorial larval key). During the course of its development the larva increases from about 1 mm to 6 or 7 mm in length. Passage from one larval stage (instar) to the next is achieved by the process of molting, during which the insect sheds its old exoskeleton (body covering). At molting, a fluid is secreted that allows separation of the exoskeleton from the newly developed body covering underneath. The head capsule and thorax of the exoskeleton split open and the larva emerges with a complete new body covering which allows for increase in size.

The larva spends most of its time feeding, using its fan-like mouth brushes to sweep microorganisms and particulate matter out of the water, browsing on submerged objects and on any organic material which accumulates on the sides and bottom of the container. Larvae of *Ae. aegypti* can be recognized by their characteristic sinuous swimming movements, avoidance of light, and the relatively blunt air tube which is their connection with the atmosphere.

Larval development normally requires 5-7 days and ends when the fourth stage larva molts to the nonfeeding pupal stage. Adverse conditions can greatly extend this time. Inadequate food supply also increases development time and results in undersized pupae and adults. Overcrowding of larvae, if severe, has a similar effect. Transformation from the larval to the adult form is completed during the 2- to 3-day pupal period.

The adult that emerges from the pupal skin is a dark mosquito distinctly marked with a silver-white, lyre-shaped design on the thorax and white rings on the legs (Fig. 1) (see Appendix I for detailed information on mosquito identification). Males and females are similarly marked, but the males are less robust than the females and are easily identified by their antennae, which resemble miniature bottle-brushes. The female's antennae are much more slender and less brushlike. Males and females alike take nectar and sugary fluids from whatever sources are available but only the females feed on blood. Though they can survive using sugar as the only source of food, the females require blood in order to develop eggs. The adults do not disperse widely and seldom range more than a few hundred meters from the place where they emerged. This is especially true of males and their presence is a dependable indicator of a nearby breeding place.

Mating normally takes place within a few hours after emergence. Once inseminated, a female may produce several batches of fertile eggs, provided that she obtains a blood meal each time. The females are strongly attracted to humans (although they will feed on other animals) and will bite throughout the day. Biting sometimes occurs at night too, especially in lighted rooms. The behavior of an *Ae. aegypti* female seeking a blood meal has been described as "cunning and cautious." She usually approaches from the shady side and from downwind and frequently bites around the ankles. For some reason, the male behaves much like the female, hovering and darting about and even following a person from room to room. This can be annoying but males seldom alight on the skin and never attempt to probe. Following a blood meal, 2-3 days is usually enough time for development of her eggs and the female is ready to seek an oviposition site, thus completing the cycle from the eggs of one generation to those of the next.

Influence of Climate on Life Cycle and Distribution of Populations

Aedes aegypti is primarily a tropical or subtropical mosquito. Due to its inability to tolerate areas where severe winters occur, the distribution of this mosquito is limited by latitude. Generally, it rarely occurs beyond latitudes of 45°N and 35°S. These latitudinal limits appear to be directly related to temperature. For example, in the Northern Hemisphere (North America, Europe, Asia) the isothermic range is 1.8-10.0°C in January to 23.9-26.7°C in July. However, the seasonal isothermic ranges are reversed in the Southern Hemisphere (South America, Africa, Australia) with January isothermic values ranging from 21.8-26.7°C and the July isothermic range 10.0-15.6°C.

Experimental data indicate that specific temperatures limit the growth of *Ae. aegypti* larvae, and temperature as well as humidity is critical to the egg and adult stages. Temperatures ranging from 8°C to 41.4°C tend to be the limits for the larval stage, with 8°C and 41.4°C both being lethal if exposure is prolonged.

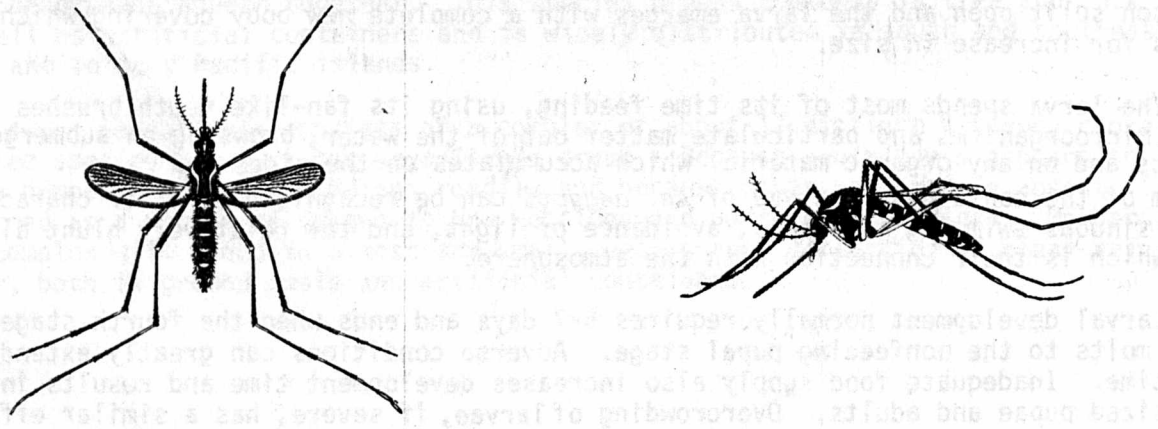

Figure 1. Adult *Aedes aegypti* mosquito.

The egg stage varies extensively in its ability to withstand a wide range of temperatures. *Aedes aegypti* eggs have survived winter temperatures as low as -8°C (recorded in tires in which eggs were held from November to May). The critical period in the egg stage is during the first 48 hours where temperature and moisture are crucial to survival. The eggs are very resistant to drying and can be kept up to 12 months if this initial 48-hour moisture level is maintained.

The adults are not cold-hardy; a temperature of 6°C for 24 hours is lethal. Temperatures greater than 42°C for 5 minutes are also destructive. However, adult longevity varies according to temperature, humidity, and nutrition. For example, adults provided with food and held at 10°C and 100% relative humidity (RH) lived for 30 days, but unfed adults held at 23°C and 70% RH lived for only 4 days.

The seasonal distribution of *Ae. aegypti* in tropical environments tends to follow rainfall patterns. The recent work of Moore et al. (1978) in Puerto Rico, shows that additional rainfall increases the number of larval habitats, thus increasing adult densities. In temperate regions where larval sites exist, the factors limiting densities are temperature, frequency of rainfall, and the duration and severity of winter conditions. Where winter temperatures are moderate, eggs survive in a variety of containers. However, during severe winters only well-protected containers are able to provide adequate conditions for survival, and these may provide foci for population expansion during summer months. Where winter conditions are too severe for survival of eggs, temporary summer populations may develop as a result of reintroduction each year. Such reintroduction can easily occur by way of eggs in flower vases or other containers that are transported with people moving from infested areas. Adult mosquitoes could also be transported with travelers from infested areas.

Other Organisms Inhabiting Containers

During larval and adult surveys, careful sampling of sites likely to be inhabited by mosquitoes is one of the most important aspects of a control program. The ability to distinguish *Ae. aegypti* from other species of mosquitoes and other arthropods that may inhabit the same environment is also critical. Many arthropods can easily be misidentified as mosquitoes (both adults and larvae) if they are examined hastily or the identifier is not familiar with these other animals. A key to the mosquitoes associated with *Ae. aegypti* is presented in Appendix I.

A diverse fauna of other insects has been associated with container mosquitoes and these forms are found on the walls of the container, on the water surface, within the water, and within the bottom debris. Also, along with those forms which live within the container environment, others are found periodically invading the containers. Some of the more common insects occasionally found in or around both natural and domestic containers include ants, beetles, springtails, and various species of flies (Appendix II). Besides insects, several other arthropod forms are common in and around containers. These include spiders, mites, pseudoscorpions and phalangids. Other fauna can include annelid and nematode worms, as well as tadpoles or adult frogs.

Moore, C.G., B.L. Cline, E. Ruiz-Tiben, D. Lee, H. Romney-Joseph, and E. Rivera-Correa. 1978. *Aedes aegypti* in Puerto Rico: Environmental determinants of larval abundance and relation to dengue virus transmission. Am. J. Trop. Med. Hyg., 27(6):1225-1231.

Although a great many natural enemies of mosquitoes have been recorded in the literature, not many relate to *Ae. aegypti* since the immature habitat is to a large extent restricted to domestic containers and the adult is protected by its indoor habits. Below is a list of enemies that have actually been recorded or observed attacking *Ae. aegypti* either experimentally or naturally.

Natural Enemies of the Eggs

Arthropods
Mites, psocids, ants

Natural Enemies of the Aquatic Stages

Lower invertebrates and plants
Hydra, Vorticella, Planaria

Mosquito Larvae
Toxorhynchites, Lutzia mucidus

Other Arthropods
Tipulidae, Ceratopogonidae, aquatic hemiptera

Vertebrates
Frogs, turtles

Natural Enemies of the Adult

Arthropods
Spiders, mites, dragonflies

Vertebrates
Frogs, turtles, fish

Some of the parasites found in conjunction with *Ae. aegypti* experimentally and naturally are bacteria, rickettsia, yeasts, molds, fungi, spirochaetes, gregarines, flagellates, ciliates, trematodes, and nematodes. However, comparatively few parasites have been recorded from *Ae. aegypti*, which again stems from the nature of its usual habitat which presents less exposure than anophelines and many culicines that are found in natural waters.

Although the list of parasites and predators of *Ae. aegypti* spans many categories of both the animal and plant kingdoms, little information is available regarding the impact of specific predators and parasites on populations of *Ae. aegypti*. However, in recent years a few of these natural enemies of *Ae. aegypti* have been explored for their possible implementation as biological control instruments. Researchers are exploring the use of predaceous mosquito larvae such as *Toxorhynchites* species, and parasites such as *Lankesteria, Coelomomyces* and *Nosema* species that could possibly be used alone or in conjunction with other control procedures.

SURVEILLANCE OF *AEDES AEGYPTI* POPULATIONS

Purposes of Surveillance

Before considering methods of control for use against *Aedes aegypti* and *Ae. aegypti*-borne diseases, one needs to know whether or not the species is present in a particular area and, if so, its relative abundance in that area as compared to other areas. Also needed are methods that will allow evaluation of the relative effectiveness of control measures and the influence of climatic conditions on populations.

A single survey, made during the portion of the year when rainfall is frequent and abundant and when temperatures are adequate for development of larvae, can demonstrate the presence of *Ae. aegypti* and provide some idea of its relative abundance in the areas surveyed. However, if disease transmission is likely to occur and/or control of *Ae. aegypti* is planned, a great deal more information is needed. To provide this information, a formal surveillance system should be employed. A variety of sampling measures for *Ae. aegypti* are available and an effective surveillance system will include several different methods, the results of which complement each other. Whatever methods are employed, it is important to apply them consistently from place to place and throughout the period in which surveillance is used. Accumulation of surveillance records for several years for a given area greatly simplifies planning of control efforts by making it possible to project probable mosquito abundance throughout the season.

Described below are sampling methods for *Ae. aegypti* that have been proven successful in field use for each of the life stages.

Sampling Methods

Egg sampling: *Aedes aegypti* is one of relatively few mosquitoes whose habits make sampling of the egg stage easy and practical. Sampling is done by collecting the eggs in oviposition traps or "ovitraps" as they are usually called. An ovitrap is a wide-mouth, pint-sized, black jar containing a narrow paddle (3/4 in. x 5 in.). A number of absorbent materials such as wood and heavy paper will serve for paddles, but a nontempered, dark colored hard board is recommended. It is clipped vertically to the inside of the jar with its back (rough) side facing the center and its lower end standing in at least an inch of water. As it absorbs water, the paddle becomes an attractive surface on which the mosquitoes deposit their eggs. The trap works by taking advantage of the natural responses of the gravid mosquito which include attraction to dark objects, a preference for water that appears dark, and a rough substrate for egg laying.

Proper placement of the ovitrap in the field is crucial to its success and requires that certain other aspects of the mosquito's oviposition behavior be kept in mind. Adherence to the guidelines listed below will help realize the ovitrap's full potential as a sampling tool.

1. The female normally flies near the ground, so the trap must be placed at or near ground level.

2. The mosquito's responses are in part visual, so the trap must be visible to a female flying over it.

3. The trap should not receive excess water from such sources as garden

sprinklers or runoff from eaves or broadleaf plants.

4. Adult mosquito resting places such as shrubbery and junk piles are good trap locations.

5. Ovitraps should be placed in partial or total shade. Avoid direct afternoon sunlight and fully exposed paved areas.

6. Ovitraps should not be located in tire yards or near piles of tires. Tires are highly attractive to female *Ae. aegypti* seeking oviposition sites, and their presence will reduce the effectiveness of the ovitraps.

All mosquito eggs found on ovitrap paddles are not necessarily those of *Ae. aegypti*. Other mosquitoes which breed in water-holding receptacles may also deposit their eggs in ovitraps. The eggs of *Ae. triseriatus* are most frequently encountered with those of *Ae. aegypti* on ovitrap paddles in the continental United States. Other species whose eggs may on occasion occur with *Ae. aegypti* include *Ae. atropalpus* and *Ae. zoosophus*. In Hawaii, *Ae. albopictus* is found with *Ae. aegypti*, and in Puerto Rico and other Caribbean islands *Ae. mediovitattus* oviposit in artificial containers. The eggs of only a few species in a given region will occur with those of *Ae. aegypti* and familiarity with them is quickly achieved. Eggs that appear to be different can be hatched and the larvae identified to be certain they are not *Ae. aegypti*.

Ovitraps provide an efficient and economical method for monitoring changes in the *Ae. aegypti* population of an area. They are particularly useful during dry periods when the lack of rain minimizes competition from other containers. Though their primary use relates to long-term population changes, they can serve in situations where assessment of short-term changes is required, for example, evaluation of adulticide applications, provided that the traps are serviced daily and enough are available to compensate for short-term trap-to-trap variation.

Ordinarily, ovitraps are serviced on a weekly schedule. They are cleaned of debris, the water level is adjusted, and the paddles are replaced with new ones. Clean jars should be used to replace those that cannot be easily cleaned in the field. After paddles are removed from the traps, they should be kept from contact with each other, thus preventing the accidental transfer of eggs. Accurate interpretation of ovitrap data requires that all eggs on the paddles be counted under a dissecting microscope and that all records show, in addition, the location of all ovitraps and their condition (flooded, dry, broken, upset, moved, missing, etc.) each time the paddles are collected.

Larval sampling: Sampling for *Ae. aegypti* in the larval stage requires a thorough inspection of premises to locate all water-holding containers. It is essential to proceed carefully in searching for larvae because disturbing the water, jarring the container, or even casting a shadow will cause them to dive to the bottom where they may escape detection. When the inspector finds a container with water, he observes the surface of the water carefully, looking for mosquito larvae that may be either resting quietly or moving in their characteristic fashion. If no larvae or pupae are seen at the surface, he taps the container gently and watches for motion.

When larvae and/or pupae are found, a sample is collected and placed in a vial of water or alcohol for species identification with a microscope in the laboratory; a label specifying date, location, and type of container sampled, should be placed in or on the sample vial. If the larvae can be examined on the day of collection, water is normally used; but if a delay is anticipated, they should be preserved in alcohol. For best results 95% alcohol should be used; lower concentrations may be used but are less desirable.

Since larvae occur in a wide variety of receptacles ranging in size from wading pools and boats to tin cans and fence pipes, a variety of collecting devices is necessary for taking samples. A dipper, preferably the white enamel type, is frequently used in sampling although strainers or nets of cloth or wire mesh fabric are more efficient for sampling large containers. Since larvae are most easily seen against a white background, it is often worthwhile to pour the contents of small containers such as cans and jars into the dipper or a white plastic or enamel pan for examination.

An ordinary squeeze-bulb syringe of the type used for servicing auto batteries or for basting food is well suited for removal of water from narrow-mouth receptacles or those too small for a dipper. It is particularly useful for taking samples from treeholes. For especially deep holes, a length of rubber or plastic tubing may be added to the syringe. Other items of equipment which will prove valuable in the course of larval collecting include a flashlight, a tea strainer (which is used for transferring specimens from debris-laden or dark-colored water to clean water), a white plastic or enamel tray for examining material from the dipper, a syringe or a medicine dropper for moving individual larvae into collection vials.

The larvae of several other *Aedes* species and of certain other genera frequently inhabit the same containers as *Ae. aegypti*. The pictorial key in Appendix I provides a means of distinguishing *Ae. aegypti* larvae from those most likely to be found with them in the continental United States.

Larval sampling is most effective during periods of high rainfall and intensive mosquito breeding when sampling can provide a quick answer to the question of whether or not a particular urban or suburban area is infested. Generally, four indices have been used to determine incidence of *Ae. aegypti* based on whether larvae are present or absent. The House (Premise) Index has been used for many years and is probably the most widely employed index; it is calculated by the percent of houses examined that have *Ae. aegypti*. Another index widely employed is the Container Index which one derives from the percentage of water-holding containers that have larvae of *Ae. aegypti*. The Breteau Index which is calculated from the total number of containers with larvae of *Ae. aegypti* per 100 houses also has been widely employed. Another index that has occasionally been used is the Larval Density Index which is the mean number of *Ae. aegypti* larvae per house and is obtained by counting all larvae in the containers.

Adult sampling: Sampling the adult population of *Ae. aegypti* is far more difficult than sampling the larval stages, since the adults are not restricted to a small area as are the larval stages, and the sampling techniques for adult populations are less efficient.

One method of collecting is to search for resting adult mosquitoes in houses, garages, outbuildings, sheds, and similar adult resting places. Since *Ae. aegypti* is, in general, active throughout the day, resting specimens found during the day will usually be those which have recently fed and are quiescent during the period of blood meal digestion and egg development. Resting individuals will be found most often in dark corners, under tables and desks, and in similar places where light intensities are low, so a flashlight, in addition to an aspirator, will be required for their capture.

Another method widely employed for adult surveys is a landing-biting count. Both male and female *Ae. aegypti* are attracted to humans and frequently they may be collected on or near the collector before resting mosquitoes are seen. The mosquitoes may be captured individually as they approach or land on the person making the collection. In practice it may be desirable to combine resting collections with landing-biting collections and express the results as a house index (the percentage

of positive houses).

Numbers of adults collected in the same location will vary with the time of day and with changes in climatic conditions. Realizing that these variables not only occur but change quickly with time, it is essential that sampling methods and time of day used are as consistent as possible.

Standard light traps are not effective for sampling adult *Ae. aegypti* populations. However, a modified trapping technique for capture of adult *Ae. aegypti* has recently been demonstrated in Puerto Rico. Standard New Jersey light traps were painted black and modified to serve as suction traps by removal of the light and cover. Placed in protected situations in buildings where the mosquitoes seek shelter, the traps attracted and captured large numbers of *Ae. aegypti* of both sexes when operated at ground level during daylight hours (D. Eliason, Center for Disease Control, personal communication).

In areas with low level infestations, collection of adults may be the most efficient and economic procedure. On occasion, well-hidden containers may be the source of considerable numbers of resting adult mosquitoes. The presence of adults reveals breeding in the immediate vicinity and may help in location of the source.

Organization and Management of Surveillance Systems

The development of an *Aedes aegypti* surveillance system requires much planning and research. Factors to be considered include (1) the presence or likelihood of introduction of dengue or yellow fever viruses; (2) methods of control that may be considered; (3) availability of sampling equipment and personnel to collect samples and identify specimens collected; (4) variation in habitats and climate within the areas to be sampled; and (5) the number of sites needed to represent distinct geographic or political subdivisions.

Personnel for collection of samples in the field need little formal education, but they must be reliable in following explicit instructions and in reporting unusual problems encountered. Sampling schedules and methods, once established, should be rigidly followed so that population trends are detected as they occur. Supervision should be close enough to ensure that samples are taken as scheduled and that sampling methodology does not vary. Forms and sample labels should provide all essential information and should be filled out at the sampling site when the sample is taken. Samples should be delivered to the laboratory for processing without delay and appropriate forms and labels should accompany or be attached to the samples.

Laboratory personnel should have sufficient training and competence to process and identify the samples submitted. This usually requires some formal training beyond completion of high school. One or two years of college with special training in mosquito taxonomy would be ideal. The competence of laboratory personnel will dictate the amount of supervision and quality control required but regardless of their competence, periodic independent checks should be made to ensure accuracy of identification and counting of specimens. Storage of samples for a period of time after they are identified by laboratory personnel will facilitate such independent checks.

Included in an effective surveillance system will be provisions for organization and appropriate presentation of data collected into tables, charts, or graphs that clearly summarize the data in such a way that data can be used for planning mosquito or disease control programs.

CONTROL OF *AEDES AEGYPTI*

Approaches to Control

Because of the habitats of *Ae. aegypti*, methods for control of this mosquito vary from those used against other mosquitoes, especially in the pre-adult stages. Since most breeding occurs near or inside human dwellings, conducting effective control measures is both difficult and expensive and for these reasons there must be very strong justification before any control measures are begun. Such justification may be based on reduction of nuisance where *Ae. aegypti* are overly abundant, prevention of disease, or control of disease outbreaks.

Nuisance reduction: In most areas of its distribution, *Ae. aegypti* is not considered to be a major pest mosquito. However, where rainfall is frequent and artificial containers are abundant, populations occasionally become large enough to require local temporary control measures. Standard mosquito adulticiding methods such as use of thermal fogs or ultra-low volume (ULV) aerosols may be sufficient. Occasionally, it may be necessary to apply larvicides to breeding containers, especially to large accumulations such as discarded tires at service stations or tire-recapping plants. Education of property owners and/or enforcement of sanitation laws can be used effectively to eliminate any large accumulation of breeding containers. In residential areas where excessive numbers of containers are found on many of the premises, it may be necessary to conduct neighborhood source reduction campaigns.

Prevention of disease transmission: When there is reason to believe that dengue or yellow fever viruses are likely to be introduced via travelers from areas where outbreaks are occurring, efforts should be made to reduce *Ae. aegypti* populations to levels that are unlikely to support disease transmission. A premises infestation rate of 5% (larval infestation) has been said to be adequate to support transmission. However, factors such as quality of housing, number of residents, longevity of adult mosquitoes, and distance between houses could influence the likelihood of an area to support transmission if dengue or yellow fever were introduced. To have a margin of protection, larval infestations of the premises in a given area should be reduced to considerably less than 5%.

Education of the public in source reduction through the news media, civic organizations, and public schools may be adequate to achieve safe *Ae. aegypti* infestation levels in some areas. In others it may be necessary to implement active source reduction or larviciding programs.

During peak periods of *Ae. aegypti* production in the summer and fall months or until other control efforts have reduced larval infestations to safe levels, it may be necessary to use adulticiding methods to temporarily reduce the adult mosquito populations.

Control during disease outbreaks: Once the virus of dengue or yellow fever has been introduced and disease transmission is occurring, control efforts must be concentrated on adulticiding. With dengue, if cases are not numerous, spraying small areas surrounding each case using truck-mounted equipment may be sufficient. If cases are too numerous for localized treatment, use of aerial spray applications may be necessary to bring the outbreak under control. Should urban yellow fever occur, the entire urban area should be sprayed at least once every 3-4 days until vaccination against the virus is accomplished or until cases are no longer reported.

Larviciding and source reduction efforts should also be considered during disease outbreaks, especially where an extended period of high *Ae. aegypti* production is anticipated, but should not be carried out at the expense of adulticiding efforts.

Methods of Control

Source reduction: The objectives of an *Ae. aegypti* source reduction program are to eliminate as many water-holding containers from an area as possible and to provide sufficient motivation and education to the public to reduce the reaccumulation of new containers. Elimination of breeding places is by far the most desirable method of *Ae. aegypti* control, but source reduction programs are expensive and require consistent and continuing efforts over long periods of time. If successful, source reduction not only greatly reduces the likelihood of disease transmission; but, because of smaller mosquito populations and lower potential for production of mosquitoes, emergency control is easier should a virus be introduced. Source reduction also improves the general level of sanitation by eliminating harborage for rodents and other pests.

The need for source reduction in some residential areas may be a direct result of inadequate solid waste or garbage removal efforts. The combination of inadequate solid waste removal and deteriorating quality of housing and neighborhood pride are almost certain to result in accumulation of sufficient numbers of water-holding containers to support large populations of *Ae. aegypti*. Successful source reduction programs attempt to deal with those contributing factors simultaneously.

Existing garbage or solid waste removal agencies should be encouraged to improve both the frequency and quality of their pick-up service. It may be possible to work out agreements to utilize their resources in special clean-up campaigns but if not, it is important to coordinate closely with them. Matters such as consent forms to remove junk automobiles and clearance to remove abandoned automobiles should be worked out prior to implementation of source reduction efforts.

Community involvement is a basic part of source reduction efforts and much can be done through civic organizations, commercial establishments, and schools in the area. Actual removal of containers from private property must be carried out by the property owner or resident of the premises, and motivation of these people requires considerable effort through a variety of methods. Good quality film strips, posters, brochures, and other motivational aids should be provided to groups that are cooperating in the clean-up efforts.

Personnel needed in source reduction include: health educator aides to make direct contact with cooperating groups and especially with individual homeowners or residents; inspectors to perform surveys before and after clean-up campaigns; laborers to load containers and other materials placed at curbside by the residents; truck drivers and special equipment operators to drive the trucks and special loading equipment; and, supervisory, clerical, and other administrative and support personnel. In some instances the same individuals may work on surveys, contact homeowners, and serve as laborers.

Detailed maps are needed for planning and for recording the status of clean-up activities. Records must be kept of all activities, especially the dates and results of clean-up campaigns. The amount of containers and other materials removed are usually recorded as truck loads or cubic measure removed, but a more important measure is the comparison of the number of various types of containers remaining after clean-up drives with what was found before the campaign.

Implementation of source reduction requires close coordination of all cooperating groups or agencies, and all concerned need to understand both immediate and long-term objectives of the program. Results will not be apparent for some time and

chemical control measures may be needed in the interim.

Clean-up drives in relatively small areas (usually less than 100 blocks) are probably the most efficient means of source reduction. All cooperating groups and program personnel can focus on this area and schedule a single date for pick up of discarded containers and other solid waste. Planning and motivational efforts can go on simultaneously in several areas with staggered dates for pick-up. Available equipment can then be concentrated in one area at a time to ensure that everything put out by the residents of the area is picked up on schedule. Failure to pick up on the date or dates scheduled will discourage future cooperation. Where clean-up drives fail to reduce the number of containers to acceptable levels, follow-up campaigns and other special efforts may be needed.

Chemical control: Chemical control methods for *Ae. aegypti* basically involve the application of a larvicide to water-holding containers or an adulticide to infested areas. Both methods require compliance with the manufacturer's label for the material, the formulation, and consideration of the potential effects on the environment and nontarget animals. Selection of safe and effective pesticides and application techniques are crucial.

(1) Larviciding. Chemicals are used for *Ae. aegypti* larval control in situations where source reduction would be impractical or only partially effective. Larvicides currently registered by the U.S. Environmental Protection Agency are listed in Appendix III.

Larviciding can be accomplished by either hand-held application equipment or by power spraying. Typically, the hand-held applicators are used in domestic situations with relatively few containers, while the power sprayers are used in commercial and industrial areas, with numerous oviposition sites.

Liquid formulations are common, but certain dusts, pellets, and granules may also be indicated. The approach to larviciding premises involves the selective treatment of all actual or potential water-holding containers. The treatment should slightly exceed the minimal level for larval destruction. The amount sprayed in each container to accomplish this is a judgment of the sprayman.

Although hand-spray applications are seen most frequently, hand dusting with either a plastic squeeze bottle or a bulb duster might be considered. An inspector or a member of a source reduction evaluation crew, who must record field observations, and who is also required to make spot treatments in the course of his duties, would be unnecessarily restricted by the size and weight of a liquid sprayer. However, he could carry a small duster for use as needed. Hand dusting of plants rooted in water is an effective method of control for *Ae. aegypti* larvae, provided the plants are not damaged by the dusts.

Power-spray equipment is used for the treatment of large and more difficult areas. For example, power sprayers are used to treat aggregations of used automotive tires, as might be found adjacent to tire recapping companies.

Choice of insecticides and formulations are especially important for use in *Ae. aegypti* breeding sites because of the presence of children and pets in residential areas. Excessive applications are to be avoided, and spraymen will need supervision and frequent reminders of the importance of judicious application techniques. Spraying of potable water should be avoided as there are currently no approved insecticides for such use. Treatment of animal watering places and fish ponds should also be avoided wherever possible. Residents of the dwelling should be informed of the need for frequent cleaning of these sources.

(2) Adulticiding. Applications of space sprays are an important means of reducing or managing *Ae. aegypti* populations. Space sprays include ultra-low volume (ULV) aerosols, thermal fogs, or dusts, using either truck-mounted equipment or aircraft. Adulticiding is a temporary measure because adult mosquitoes from unsprayed areas can migrate rapidly into those areas which have been sprayed, or newly emerged adults can replace those killed by the space spray. Adulticiding applications exert little or no effect on the aquatic stages of *Ae. aegypti*, and adults will continue to emerge after spraying.

Space spraying has the greatest effect when performed during cooler parts of the day (< 80°F) and when winds are not excessive (< 10 mph). If applications are made during the middle of a hot day, the small droplets of an aerosol or thermal fog tend to be dispersed rapidly by thermals, the currents of warm air rising from the ground. If wind velocity is greater than 8-10 mph, the small droplets of the space spray are blown away too quickly and the chance of the spray contacting many adult mosquitoes is lowered.

Adulticides approved by the U.S. Environmental Protection Agency are found in Appendix III.

Applications with ground (truck-mounted) equipment. Ultra-low volume aerosols, dusts, and mists applied by ground equipment have been successful in controlling adult *Ae. aegypti*. Under ordinary operating conditions, ground applications work well as part of a control program. However, the effectiveness of space sprays may be limited by conditions peculiar to a given area. For example, access roads may be inadequate to accommodate spray trucks, or dwellings may be constructed very close together and serve as a barrier to the spray, preventing it from reaching backyard areas. For adequate performance, the spray equipment should be of heavy duty construction in order to withstand the stresses of constant operation. ULV machines must consistently produce the size of spray droplets required by the insecticide label.

Tests of ULV aerosols and thermal fogs show similarities in effectiveness. The ULV method has a number of advantages: ULV aerosol generators use technical grade material (undiluted or concentrated), usually without a diluent or carrier, resulting in significant savings in fuel costs and loading time; ULV does not generate a dense fog which reduces visibility and creates traffic hazards. The ULV spray machine is light weight and does not require a large fuel oil reservoir like the thermal fog generator, and thus can be mounted on a small pickup truck. ULV performance requirements are found in Appendix IV.

Application by aircraft. The aerial application of insecticide has been successful in controlling *Ae. aegypti* populations during recent epidemics of dengue fever in Puerto Rico (Chiriboga et al. 1979). In *Ae. aegypti* control programs, the only practical use of aircraft is the spraying of ULV adulticides in order to limit transmission of disease. Aerial spraying is considered in time of epidemics when it appears that ground equipment will not cover the area in sufficient time. Two insecticides are currently registered by the U.S. Environmental Protection Agency for adult mosquito control by ULV application from aircraft: malathion at 3.0 fluid ounces per acre, and naled at 0.5 to 1.0 fluid ounce per acre.

Chiriboga, J., D. A. Eliason, C. G. Moore, S. G. Breeland, E. Ruiz-Tiben, A. Casta-Velez, and S. D. Von Allmen. 1979. Dengue control during the 1977 epidemic in Puerto Rico. In Dengue Control in the Caribbean, 1977. PAHO Sci. Publ. No. 375, pp. 101-106.

Whereas, ULV ground equipment is manufactured by several companies, ULV equipment for aircraft must be custom fabricated by the owner for an individual plane. The equipment includes special insecticide tanks, electrically-driven pumps, spray booms, and small orifice nozzles.

Aerial spraying is contracted with private companies. There are relatively few contractors due to the limited demand for this service, and all are not equally capable of applying ULV spray effectively. When writing a contract for these services, performance requirements concerning the application technique (insecticide label and area coverage specifications) should be included. Some contractors do not have the equipment to apply all adulticides, with naled being an example of one requiring corrosion-resistant equipment. Performance requirements are found in Appendix IV.

Aerial ULV has been used on several occasions for adult *Ae. aegypti* control during outbreaks of dengue, but because of the emergency nature of the operations and the employment of multiple control measures, there has been little opportunity to fully evaluate its effectiveness in disease control. However, experimental trials in Florida demonstrated significant control of *Ae. aegypti* populations using malathion at 3.0 fluid ounces per acre on a weekly or twice-weekly schedule, and ULV applications have been shown to be effective in reducing transmission of malaria in Haiti and populations of the vectors of St. Louis encephalitis during an epidemic in Dallas, Texas.

(3) Resistance. Insecticide resistance is defined as the ability of an insect population to withstand a poison which was generally lethal to earlier populations. Most populations of insects contain individuals that vary widely in their susceptibility to insecticides. As a result, putting selective pressure on a population with a toxic chemical leads to survival of those individuals tolerant to the chemical. Continued selective pressure causes a shift toward a population having mostly tolerant individuals and such populations are said to be resistant to that chemical.

As insecticide resistance becomes more widespread, through heavy agricultural spraying of the same or related compounds as used in vector control or by improper use of vector control pesticides, an understanding of resistance and its problems becomes more essential to effective vector control. It is important to remember that not every pesticide failure is caused by pest resistance. Failure to achieve satisfactory control may also be caused by choice of an inappropriate insecticide or formulation or by poor timing or techniques of application, and these should be ruled out before resistance is considered.

In general, two types of resistance occur in insects: physiological resistance and behavioral resistance.

Physiological resistance is the ability through physiological processes to withstand a toxicant by differences in (1) the permeability of the insect exoskeleton to insecticides; (2) the detoxification of insecticides into less harmful compounds; (3) the storage of insecticides in less metabolically accessible body tissues such as fat; or (4) the excretion of insecticides. Some biochemical mechanisms for the development of resistance are so general that cross-resistance develops between similar or virtually unrelated pesticides. Insects with cross-resistance to many types of pesticides may be termed multiresistant. In theory, resistance based on the genetic enhancement of fundamental enzyme systems, such as the microsomal oxidases or esterases, will be directed toward any pesticide chemicals sensitive to degradation by these enhanced enzyme systems.

Behavioral resistance is the ability to avoid lethal contact with a toxicant through protective habits or behavior, such as anopheline mosquitoes resting outdoors rather than on treated interior wall surfaces. Such resistance is also believed to

be genetic and the behavioral traits conferring resistance are selected for in the same way.

Resistance to insecticides has been reported in many species of insects. Among arthropods of public health importance, this phenomenon was first noted in Italy in 1946 and 1947 in culicine mosquitoes. By 1958, 35 species of insects of public health importance were reported to be resistant to organochlorines and 4 were resistant to organophosphates. By 1971, there were reported to be 104 species resistant to organochlorines and 18 resistant to organophosphates. Since that time, resistance has continued to appear. In some areas, such as parts of California, because of widespread resistance to multiple compounds, chemical control of some species of mosquitoes is no longer possible except through the use of larvicidal oils.

Resistance of *Ae. aegypti* to organochlorines is general in tropical America and southeast Asia and is rapidly increasing in Africa and the Pacific Islands. Organophosphate resistance has been recorded in the field in a number of places in tropical America and in South Vietnam, but multiple resistance was not detected and the resistant strains could not be colonized. The reports of organophosphate resistance in New Caledonia, Malaysia, Congo, and Thailand have not yet been confirmed. Resistance to bioresmethrin, a pyrethroid compound, has been reported in Bangkok, Thailand, after a short period of use; however, this needs confirmation. Another dengue vector, *Ae. albopictus*, is now resistant to DDT and dieldrin in several countries of southeast Asia and the western Pacific. It is also resistant to malathion in South Vietnam and to fenitrothion in Madagascar (WHO, 1976).

The potential impact of resistance on *Ae. aegypti* control programs involves important factors such as the increased cost of the replacement insecticide, increased operational costs due to strengthened surveillance for resistance, safe use of replacement insecticides which are generally more toxic than those formerly used, and general financial difficulties resulting from control program failures.

There are two regions where the problem of *Ae. aegypti* resistance and the need for alternative insecticides are of special interest. In the Americas, there is almost universal resistance to organochlorine pesticides and, in a number of places, moderate levels of organophosphate resistance. In these areas, control is still possible with organophosphates, though at higher dosage levels. In southeast Asia throughout the past decade there has been a continuing threat of hemorrhagic dengue, which is largely transmitted by *Ae. aegypti*. In view of widespread organochlorine resistance, the use of these compounds for control of *Ae. aegypti* would be out of the question. Control has been achieved in several parts of these regions by the use of various organophosphate compounds as larvicides or as ultra-low volume (ULV) applications. It remains to be seen how long such treatments will be able to achieve control with the potential of further development of organophosphate resistance.

Many genetic, biological, and operational factors lead to development of (or loss of) resistance in a population. Recently, Georghiou and Taylor (1977a and b) at

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World Health Organization, 1976. Resistance of vectors and reservoirs of disease to pesticides. Twenty-second Report of the WHO Expert Committee on Insecticides. Tech. Rep. Ser. 585, WHO, Geneva, Switzerland.

Georghiou, G. P. and C. E. Taylor, 1977a. Genetic and biological influences in the evolution of insecticide resistance. J. Econ. Ent. 70(3):319-323.

Georghiou, G. P. and C. E. Taylor, 1977b. Operational influences in the evolution of insecticide resistance. J. Econ. Ent. 70(5):653-658.

the University of California, Riverside, have produced analyses of these factors obtained through computer simulation. Studies of this type and field studies of vector ecology based on their results may offer the greatest hope for solution of resistance problems. Factors influencing resistance may be genetic, biological/ecological, or operational. It is in the careful attention to operational factors in the development of resistance that control personnel can do the most to avoid problems. Use of pesticides at widely spaced intervals and avoidance of persistent pesticides help reduce the selective pressure placed on a population of *Ae. aegypti*. In many cases, insecticidal pressure on the larval stage is more likely to cause resistance than is pressure on the adult stage, probably because of longer larval exposure to the toxicant and simultaneous selection of males and females. However, the effect of pressure on larvae resulting in resistance in the adult stage and vice-versa, may vary with the pesticide, formulation, and resistance mechanism.

Residual applications generally result in greater selection pressure than applications with short-term effect. However, if nonresidual applications such as space sprays are used at too frequent intervals, they may have the same effect as residual applications. Previous exposure to insecticide pressure that has produced some resistance may facilitate the development of a new type of resistance, even if no clear cross-resistance is apparent. Susceptibility of *Ae. aegypti* in areas under chemical control should be periodically tested. It is particularly important to have an early baseline level of susceptibility for comparison with later measurements, so that a shift toward resistance can be demonstrated.

Field bioassay (discussed in a later section), is a practical means for assessing insecticide susceptibility, although laboratory testing is needed to provide precise measurements of susceptibility levels. As a word of caution, variables such as season of the year, condition of nutrition, period since a blood meal, stage of egg development, and age all may affect response to an insecticide test.

To counteract resistance problems, an *Ae. aegypti* control operation can reduce the intensity of chemical selection by reducing the frequency of applications and the coverage of pesticide application to the minimum area needed for disease control and by supplementing chemical control by alternative methods, such as source reduction, whenever feasible.

Health hazards and safety considerations: The use of pesticides for *Ae. aegypti* control requires considerable caution to assure the safety of the public and the spray team. The greatest potential exposure of the public is encountered when larvicides are used. In an *Ae. aegypti* control program, the direct application of larvicide to domestic breeding habitats, frequently on premises where there are children and pets, requires the utmost caution. In adulticide applications the public experiences only temporary exposure to airborne droplets; experience to date indicates no adverse health effects following ultra-low volume aerial applications for mosquito control over an extensive area. In one study of people working in an urban setting during a large-scale emergency control operation, risks to human health were determined to be negligible. As a precaution, in case of an accidental spill of the concentrated insecticide, the local public health medical director should be aware of the type of insecticides being used in the vector control efforts. Through the medical director, the local poison control center and the emergency rooms of hospitals could be notified in case of an accident; then these facilities would be better prepared to respond to cases of suspected pesticide poisoning.

The spray crew comes into contact with technical grade insecticides regularly and may experience skin contamination as well as exposure to aerosols. At high risk are the laborers who fill the ULV machines, the men operating the machines, and the field mean who perform larviciding. If organophosphates are used in the control program, those persons associated closely with the insecticides should have

cholinesterase levels checked at the suggested frequency. (See Appendix V for information on cholinesterase testing.) To avoid skin contamination proper protective gloves, aprons, and boots should be worn by those handling the technical grade materials. Chlorpyrifos has been shown to depress cholinesterase activity in spraymen having high exposure to dilute sprays, and other organophosphates are capable of the same effects. The dangers of accidentally ingesting the insecticide through eating and drinking practices associated with working conditions should be stressed in training programs and must also be part of the supervisory check on performance of personnel at risk of exposure.

Adulticiding operations, especially by aircraft, can present a hazard to certain nontarget animal species. Honeybees are especially susceptible to some insecticides applied when bees are active. Most aerial ultra-low volume sprays for public health purposes have not resulted in serious harm to bee colonies because of the low dosages used. It is important to notify beekeepers of a planned application; they may protect their hives by moving them, closing them, or by turning on water sprinklers over the hives before daylight (when early morning spraying occurs) to keep the bees inside during the time of the spray application. The beekeepers' association and the state experiment station should be contacted for advice regarding methods of protecting bees. During the last 10 years, a few fish kills have occurred in shallow, warm water where there appeared to have been other environmental stresses on the fish prior to the insecticide application.

In planning control measures in areas where delicate ecosystems could be disrupted by mosquito control practices, assistance and cooperation should be sought from competent conservationists, fish and game specialists, and biologists.

EVALUATION OF CONTROL MEASURES

Regardless of the type of control measure used, evaluation is needed to determine its effectiveness and to provide information for making decisions on methodology, insecticides, quality and quantity of work, timing of spray cycles, and other aspects of control. Evaluation can provide assurance of effectiveness or can help demonstrate reasons for failure. When failures do occur, the use of carefully planned and executed evaluation measures will provide data for selecting and planning alternative measures.

Evaluation of Source Reduction Activities

Source reduction programs that utilize small area cleanup campaigns, usually of less than 100 blocks, are simpler to evaluate than those covering large areas where efforts may be extended over a period of weeks or months. Use of small area campaigns (with only 1 or 2 days for pickup of discarded containers) should be encouraged for this reason.

A basic method used in evaluation of source reduction is that of detailed pre- and post-cleanup inspections of premises for all potential breeding containers for *Aedes aegypti* larvae. This is best done by a separate team with special training in inspection techniques and field recognition of *Ae. aegypti*. Personnel used in the source reduction activities may be used in comprehensive inspections of all premises, but the information gathered on such inspections is much less detailed. An evaluation team can concentrate on detailed inspections of a sample of perhaps 10% of the premises in the area of the cleanup campaign. Comparison of data from inspections made before and after cleanup demonstrates the immediate results of the effort. By counting the potential sources of larvae before and after cleanup, a measure of the effectiveness of the effort is provided and the number of potential sources remaining in the area is known. Differentiation of various types of containers in the inspection allows an assessment of the relative effect on each type of container. Should large numbers of a certain type of container be found after cleanup, greater efforts should be made to motivate people to eliminate that particular type of container in future cleanup efforts. Inclusion of data on whether or not water and larvae are found in the containers (during the rainy season) allows decisions on the relative importance of a particular type of container for accumulating water and for producing *Ae. aegypti*.

Sampling adult *Ae. aegypti* before and after a cleanup campaign can provide some indication of the impact of source reduction on productivity of the area if the work has been done during the appropriate season and if similar sampling has been carried out in nearby areas at the same time and where no source reduction was accomplished. Because of the difficulties involved, however, it would probably be best to concentrate on measuring changes in the numbers of containers and the amount of larval production.

Evaluation of Chemical Control

Evaluation of chemical control measures requires a variety of methods including population sampling, bioassaying caged mosquitoes, and sampling spray aerosols or the deposits left by spray applications. Each of these methods yields certain information about the chemical control measures employed and each has limitations. Results of population sampling may suggest that an insecticide application was effect-

ive but the same decline in numbers could also be caused by other factors such as climatic changes or sampling errors. The bioassay of caged mosquitoes may suggest that the spray application was effective but may only indicate the mortality of mosquitoes in cages in the microhabitats in which they were placed, whereas wild mosquitoes could avoid the spray by seeking more protected microhabitats. Samples of spray droplets or residues indicate the degree of coverage of an area sprayed but do not indicate whether or not mosquitoes would be killed by the spray.

Each of these methods are described below. All of them should be used in order to provide a more complete evaluation of the impact of chemical control measures on *Ae. aegypti*.

Population sampling: A common method of evaluating the effectiveness of chemical control is that of sampling mosquito populations before and after a spray application and comparing the results. (Detailed information on sampling *Ae. aegypti* populations is included in the section on surveillance.) Care should be taken to ensure that representatives of all segments of the population are sampled and that sampling procedures are consistent in different areas being compared and in pre- and posttreatment sampling.

In a standard type of survey for adult mosquitoes, the number of *Ae. aegypti* found in or outside houses or other shelters is recorded. The selection of houses to be sampled can either be systematic, e.g., every other house in every 4th block of houses (Tinker, 1964) or at random (Tinker and Hayes, 1959). Whatever method is used, samples should be collected from houses in different suburbs, areas of towns, different types of houses and from different socioeconomic groups in order to obtain samples without bias. Also, to provide comparable data, the same method employed for the pretreatment survey should be followed exactly for the posttreatment survey. The total number of samples collected depends on the degree of precision required. For example, a simple method of evaluating a larval control procedure would be to determine a Premises Index (% of premises with at least one positive container) from a portion of houses within the treatment area. From a survey of the same houses after application, a posttreatment Premises Index is derived. The difference between the two percentages provides an estimate of control effectiveness. A more precise method to evaluate a control procedure would be to determine the number of positive containers per 100 houses within the treatment area both before and after application. The latter procedure provides more detailed information on population size, but is more time consuming.

Evaluation of the impact of adulticide applications using population sampling is more difficult than evaluation of larviciding applications. Adult populations within a given shelter may vary considerably from day to day and for this reason it is important to take samples at the same time of day on each of several days preceding the application to determine expected daily variation in numbers collected. At the same time, similar samples should be taken in a comparable area that will not be sprayed, to serve as a check on the sprayed area. Sampling must be done immediately preceding and following spray applications so that any impact of the insecticide is not obscured by emergence or immigration.

Tinker, M.E., 1964. Larval habitat of *Aedes aegypti* in the United States. Mosq. News 24(4):426-432.

Tinker, M.E., and G.R. Hayes, Jr., 1959. The 1958 *Aedes aegypti* distribution in the United States. Mosq. News 19(2):73-78.

Bioassay: Bioassays using mosquito adults or larvae are useful tools for answering many specific questions about the effectiveness of spray applications. Placement of caged mosquitoes in a particular place at a specific time so that they are exposed to spray applications and then determining the mortality of the caged mosquitoes provides a way of estimating expected mortality of wild mosquitoes in similar exposures at the same time. By using control cages placed in unsprayed areas at the same time and by limited sampling of wild mosquitoes before and after the spray application, conclusions can be drawn on the impact of the spray application.

With adequate numbers of mosquitoes in each cage and sufficient numbers of cages in both the sprayed and an unsprayed control area, the mortalities obtained can provide an indication of susceptibility to the insecticide being used, the quality of spray produced by the spray machines, or penetration of the spray into particular microhabitats. Bioassays can also provide a means of assessing performance of spray crews in thoroughness of coverage of assigned areas.

In practice, it is best to use at least 30 adults per cage (100 would be better). The cages may be constructed entirely of galvanized screen wire or they can be made by placing fine mesh fabric such as nylon tulle over each end of a cardboard tube such as those used as frames for pouring concrete columns. Tubes 6 inches in diameter cut into 2-inch sections work well for this purpose and may be discarded after use. Cages are placed in the field just prior to the spray application; 1 hours after exposure they are returned to a holding area where they are held 24 hours before mortalities are determined.

Larvicide applications or residues can be bioassayed by placing larvae in baskets of fine mesh wire fabric. At least 30 larvae (preferably 100) should be placed in each basket which are then placed in the water of the larval habitat to be tested and left in placed for 24 hours before mortality is determined. This can be repeated in the same container over a period of days or weeks to determine the residual life of the insecticide in that container. Bioassay baskets should be used in a variety of container types in adequate numbers in both sprayed and unsprayed areas to properly assess the impact of larvicide applications. Another method of larval bioassay is the use of cardboard cups containing water and larvae which are used to assess uniformity of coverage of spray applications. The cups can be placed in larval habitats or beneath vegetation or other obstructions to assess penetration of the insecticide into the microhabitat in question. This method is especially useful where large accumulations of *Ae. aegypti* sources are found such as in junk yards or tire yards.

Sampling spray applications: The theory of adult mosquito control by ULV pesticide application is to flood a space with millions of droplets to ensure contact with each mosquito. If spray droplets are too small, they may be blown away by the wind before they contact the mosquitoes. Large droplets, on the other hand, do not penetrate through vegetation adequately and also may cause paint damage. ULV control of *Ae. aegypti* depends on correct droplet size as specified by the pesticide label. To evaluate droplet size, the droplets are measured and the mass median diameter (MMD) of the sample is calculated from measurement data.

The procedures for sampling ULV aerosol are provided in technical brochures provided by the insecticide supplier. The manager of ULV spray operations should have technical information at his disposal and be thoroughly familiar with the requirements for each insecticide in use. Briefly, the sampling procedure is accomplished

by collecting the aerosol droplets on microscope slides. The slides must first be coated with silicone (Dri-Film*) or Teflon** in order to retard the tendency of droplets to spread or lose their original shape.

Droplet samples of ground ULV sprays are collected by attaching the slide to a yardstick and swinging it rapidly through the spray so as to collect sufficient but not excessive numbers of droplets. For an accurate assessment, it is important to attempt to get a representative sample of all parts of the spray cloud.

The slide is then taken to the laboratory and viewed under the high power (400x) of a compound microscope. Using an ocular micrometer for droplet measurement, it is necessary to obtain the diameter of two hundred droplets on each slide. By utilizing methods in the manufacturer's manual, the MMD and percentage of droplets of different sizes can be calculated and compared with label requirements.

The result of the MMD computation is an indicator of the functional capacity of the equipment, and hence the spray program. If the MMD is incorrect, usually an adjustment to the machine or change of the nozzle will rectify the problem. Sampling is required periodically and when major repairs are made in order to ensure compliance with the label.

For sampling droplets of an aerial ULV application, coated microscope slides are placed horizontally in open areas so the spray can contact the slide. Dye cards are also used for evaluating the spray coverage, primarily to monitor thoroughness of spray in all areas.

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*Trademark - General Electric Company.
**Trademark - E. I. DuPont de Nemours Company, Inc.

CONTROL DURING DISEASE OUTBREAKS

When dengue or yellow fever cases are occurring in an area, the basis for planning control efforts will change. Different control measures are needed and there must be rapid response to changing conditions. Where source reduction efforts and perhaps larviciding of special sites such as accumulations of old auto tires, auto salvage yards, and cemeteries have been the only control efforts there will be a need for adulticiding operations in areas reporting cases, as well as nearby areas having *Aedes aegypti* populations but not yet reporting cases. Changing patterns of disease occurrence may require rapid changes in areas scheduled for spray in order to kill infected mosquitoes, as well as those that could become infected from viremic people being in the area. During periods of epidemic transmission, it is desirable to continue and even expand source reduction and larval control programs, but where resources are limited it may be necessary to discontinue them in favor of adulticiding efforts.

Because there are currently no vaccines for dengue, the only control measures are mosquito control, avoidance of mosquitoes, and exclusion of mosquitoes from contact with viremic individuals. Avoidance of mosquitoes and exclusion of mosquitoes from contact with viremic people can only be accomplished by a well informed public. For this reason, concerted efforts should be made to provide the news media with up-to-date information on the status of disease in the area and on methods people can employ to avoid *Ae. aegypti* and to eliminate breeding places inside and outside of their dwellings.

The availability of an effective vaccine for yellow fever makes possible the protection of the human population through mass immunization programs. Unfortunately, time is required to immunize a large number of people, and during this period adult mosquito control will be needed to prevent or reduce transmission of the virus. Because of the substantial mortality associated with the disease, mosquito adulticiding operations in the urban areas where yellow fever transmission is taking place is imperative.

During an epidemic there is great need to coordinate activities relating to surveillance or control of the disease and of vector populations. All such activities in a given city or region must share information; this might best be accomplished through a coordinating group consisting of key individuals from each agency or discipline involved in the epidemic with a single spokesman to keep the public informed. Some criteria should be adopted to give priority for spraying or other activities to areas of greatest need or having the greatest chance for success. Decisions will be needed on special problems such as whether or not to apply insecticides to areas in which cases are occurring that have some special vulnerability to insecticides such as honeybee populations.

One of the most difficult tasks during an outbreak of dengue or yellow fever is making decisions based on limited information. During the early stages of an outbreak, there usually are only clinically suspect cases and these are often small in number and perhaps from widely scattered areas. Physicians not familiar with these diseases may mistake them for a variety of other viral infections and fail to report them or to take blood specimens from the patients for diagnosis. If blood specimens are taken, it is frequently several weeks before laboratory results are available for both the acute and convalescent sera necessary to measure antibody responses to the infection. Once a few cases have been confirmed in the area, reported clinically

suspect cases will provide the most usable guidance to control efforts. Usually, data on confirmed cases will not be available until weeks after transmission has occurred, far too late to be useful in making decisions on control efforts. Numbers of suspect cases and *Ae. aegypti* surveillance reports will be the primary data guiding decisions on whether to spray a given area and with what frequency. Where relatively small numbers of cases are reported, every effort should be made to spray adulticides in the area within a radius of about 500 feet of the residence of each reported case. Where cases are too numerous to allow individual spraying, entire neighborhoods or communities should be sprayed. In either case, there should be at least two applications of adulticide to the area within a week of the case reports. Spraying should continue until cases are no longer reported or in the case of yellow fever, until immunization of individuals is virtually complete.

APPENDICES
I–VI

APPENDIX I: Keys to Mosquitoes Associated with *Aedes aegypti*

Fig. I. Diagram of *Aedes aegypti* larva

Fig. II. Diagram of adult *Aedes aegypti* mosquito

Fig. III. Head of *Anopheles* female

Fig. IV. Head of *Anopheles* male

Fig. V. Head of *Culex* female

Fig. VI. Head of *Culex* male

Fig. VII. Lateral view of mosquito thorax

1. Pictorial Key to Some Common Mosquito Larvae found in Artificial Containers
2. Key to Larval Mosquitoes found in Receptacles
3. Pictorial Key to Some Common Adult Mosquitoes Associated with *Aedes aegypti*
4. Key to Adults of Receptacle-Breeding Mosquitoes

Use of the Larval Keys

Figure I illustrates the characters used in identification of *Aedes aegypti* larvae. Study them before progressing to the pictorial larval key. The initial purpose is to learn the characters of a larva and compare the actual larva with the illustration.

Use the pictorial key (page 36) to learn more about the identifying characters and the use of identification keys. In this key, start at the top, making a choice among two or three important characters to see which description agrees with the specimen. Next proceed downward in the same manner until the specimen is identified. Any key is valid only for the species included and, if a species not represented on the key is identified, an incorrect determination will be made. Therefore, after the initial orientation period is finished, it is more satisfactory to use the couplet (*dichotomous*) key (pages 37-46) which includes additional mosquito species that may occur in receptacles.

Use of the Adult Keys

Figure II illustrates the anatomy of the adult mosquito, naming body parts used in adult identification. Figures III-VI illustrate the differences between the appendages of the head by which adult *Culex* males and females can be distinguished from *Anopheles* and compared to *Aedes aegypti*.

As experience is gained in mosquito identification, the presence of spiracular and postspiracular bristles, and occasionally the lower mesepimeral bristles, will be found very useful (Figure VII). On Mosquitoes that have lost part of their scales, these characters are often more easily found than body scales.

The pictorial key (page 47) is used in the same manner as the larval key in learning the use of keys. The *dichotomous* key (pages 48-54), which follows, should be

used after the identifier becomes familiar with the characters learned in the pictorial key. The illustrations may also be used in checking the determinations made. Should more advanced keys be desired, it may be well to obtain "Mosquitoes of North America," Carpenter and La Casse, or other volumes listed under the heading "Selected References." The use of different keys and different characters adds interest to the task of identifying mosquitoes and increases the competence of the public health worker.

Figure I. Diagram of *Aedes aegypti* larva

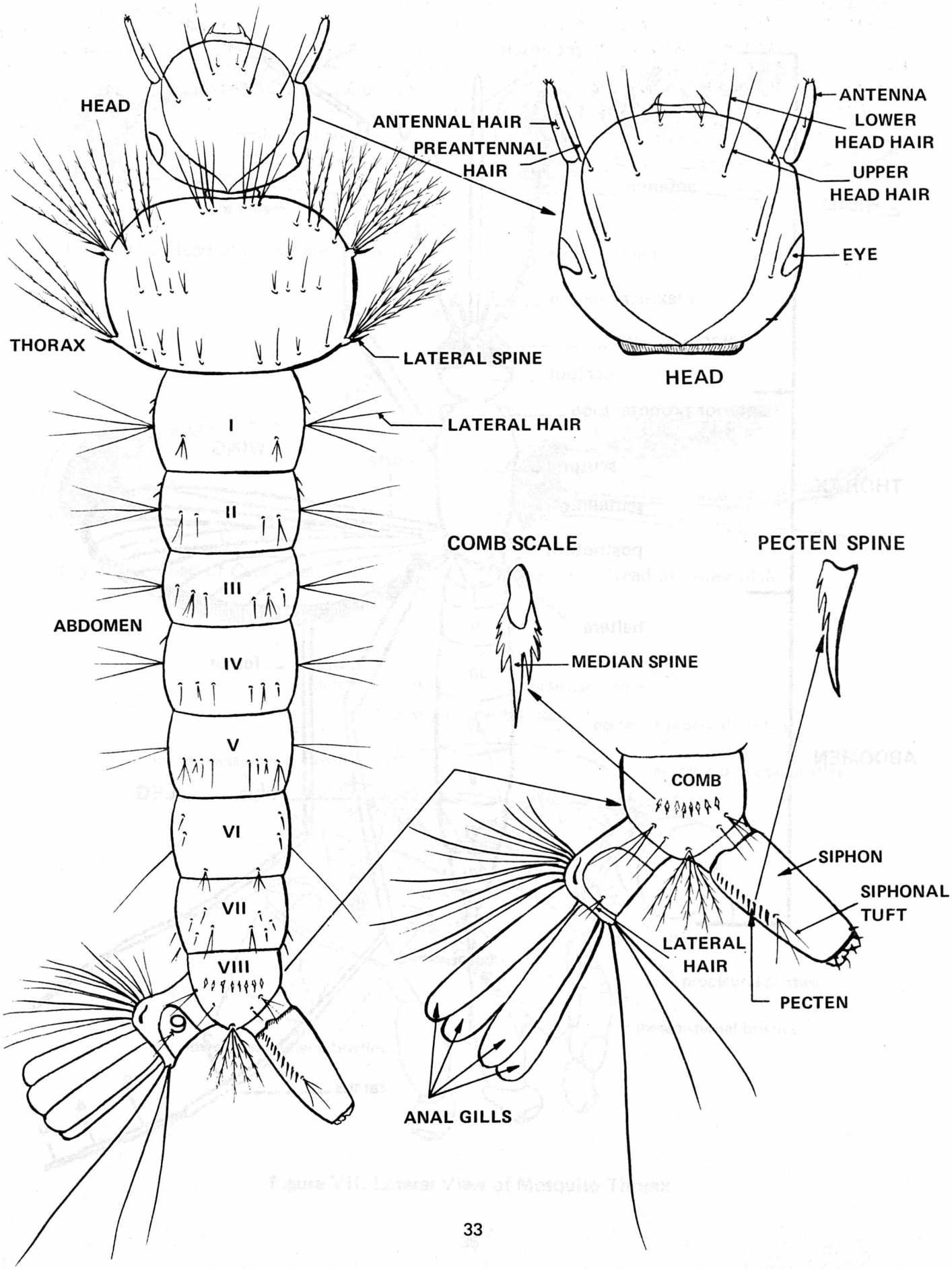
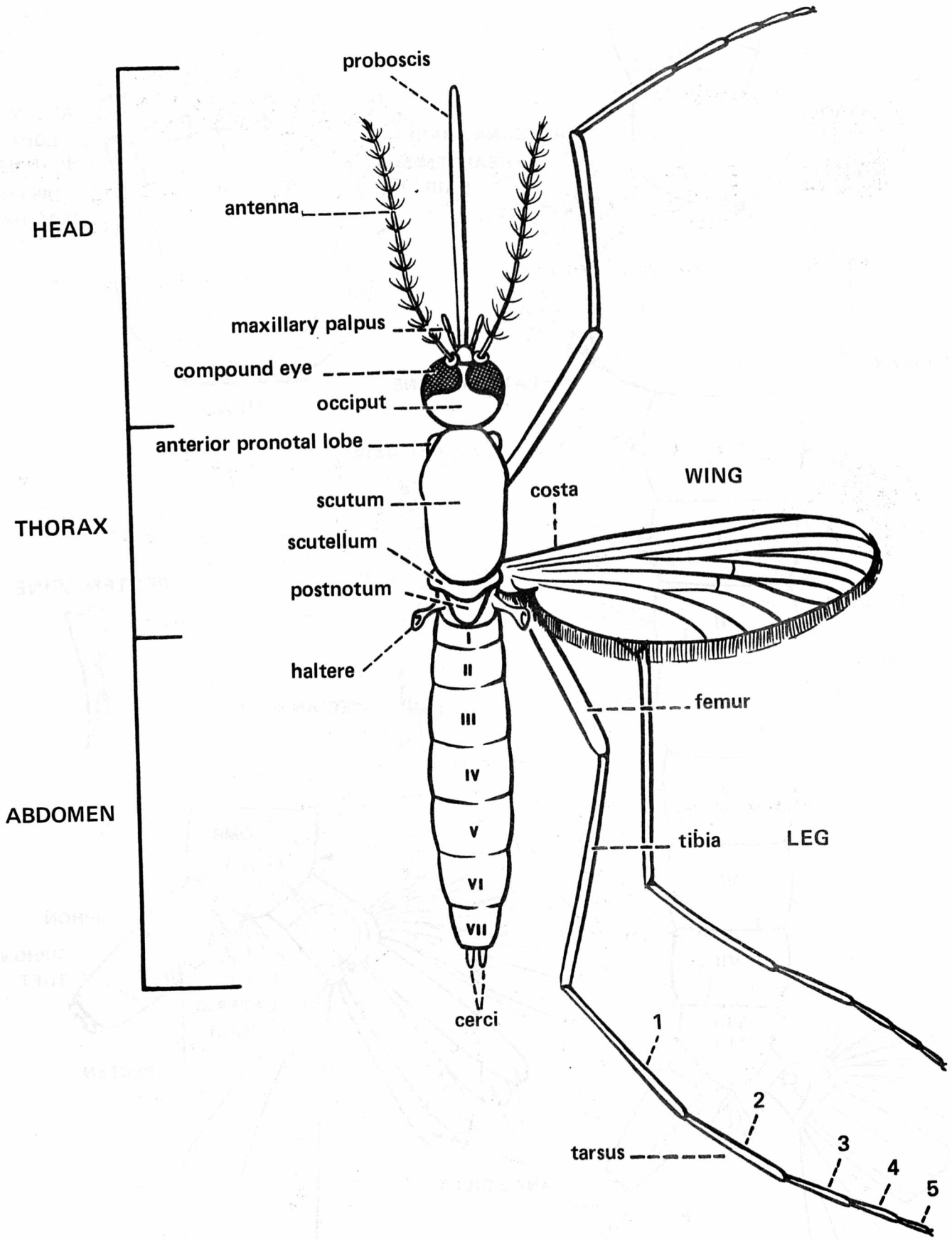


Figure II. Diagram of Adult *Aedes aegypti* Mosquito



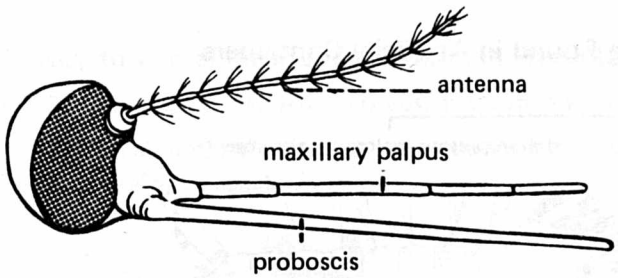


Figure III. Head of *Anopheles* Female

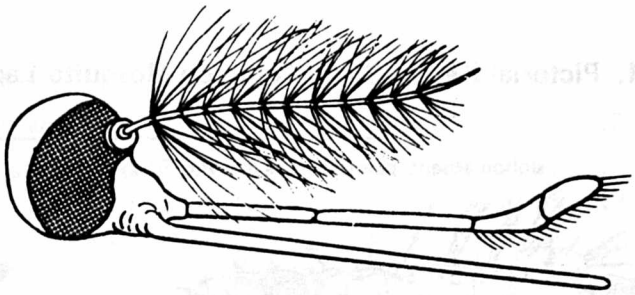


Figure IV. Head of *Anopheles* Male

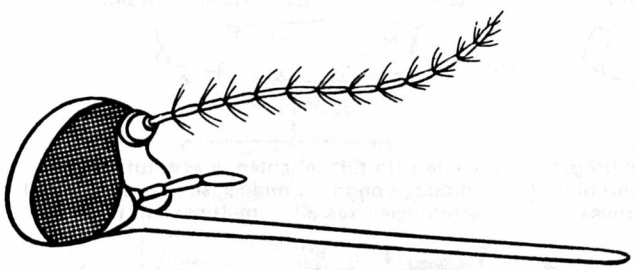


Figure V. Head of *Culex* Female

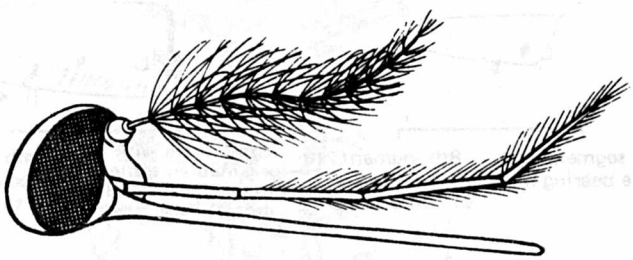


Figure VI. Head of *Culex* Male

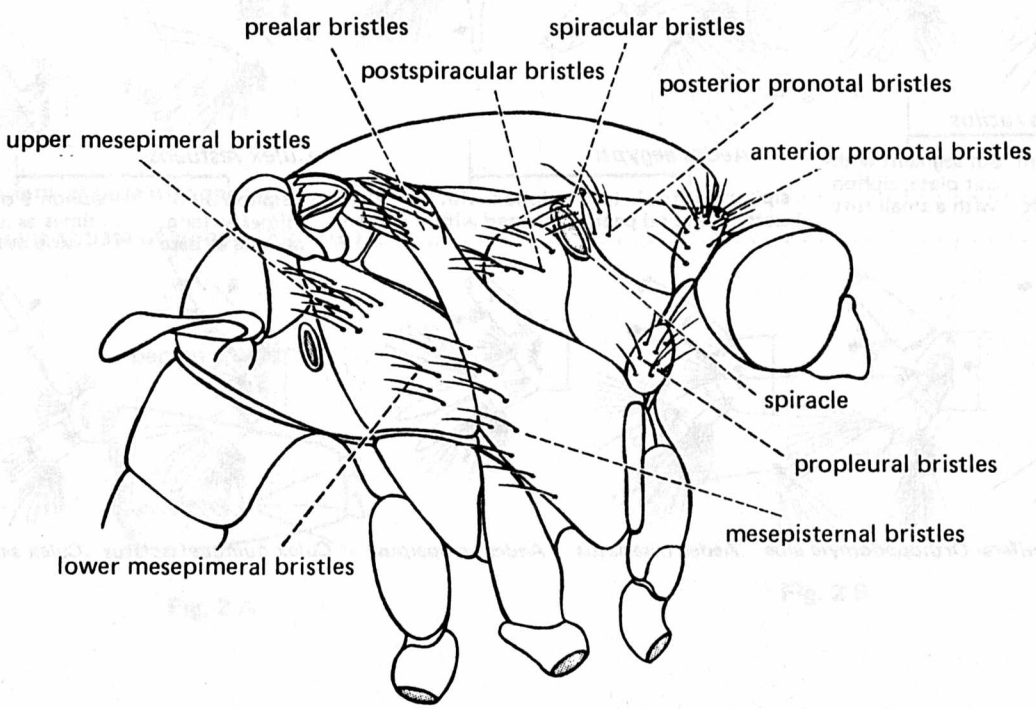
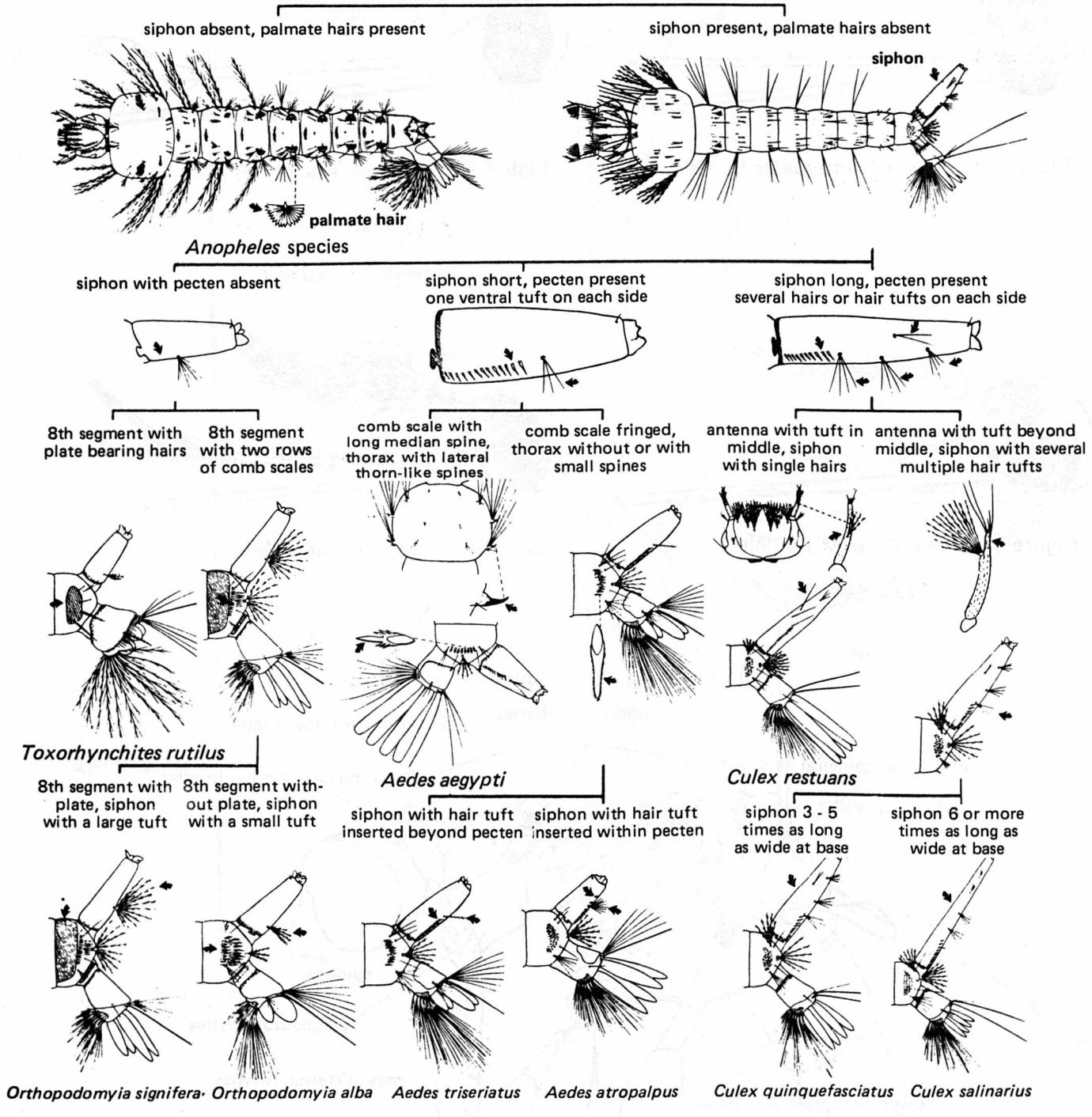


Figure VII. Lateral View of Mosquito Thorax

1. Pictorial Key to Some Common Mosquito Larvae Found in Artificial Containers



2. Key to Larval Mosquitoes Found in Receptacles

1. Siphon present; palmate hairs absent on abdominal segments (Fig. 1 A) 2

   Siphon absent; palmate hairs present on some abdominal segments (Fig. 1 B) . . . . . (Genus *Anopheles*) 23

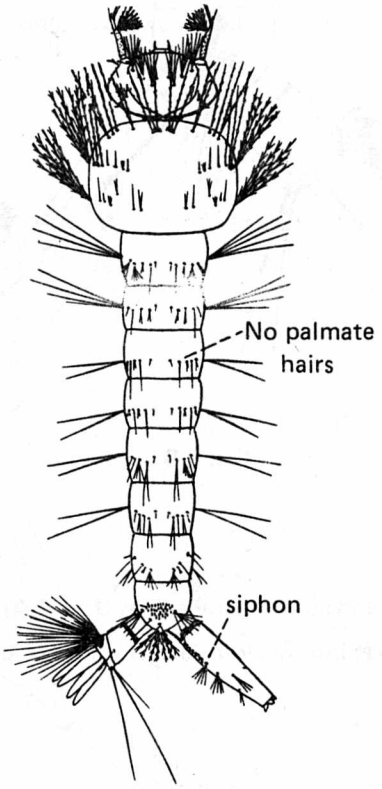


Fig. 1 A

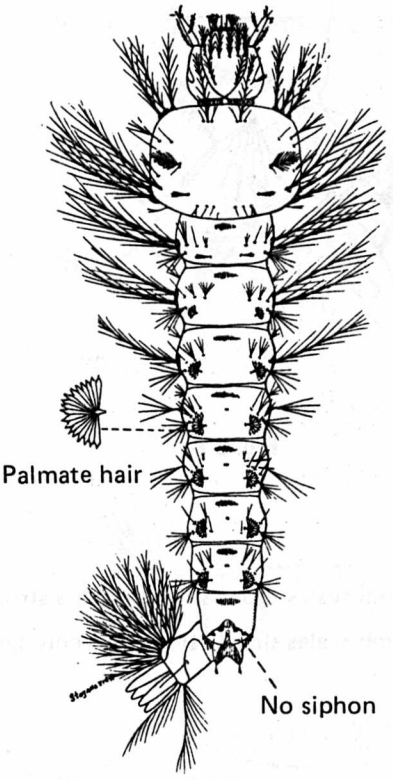


Fig. 1 B

2. Pecten present at base of siphon (Fig. 2 A) 3

   Pecten absent at base of siphon (Fig. 2 B) 21

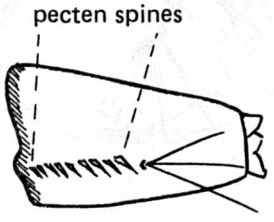


Fig. 2 A

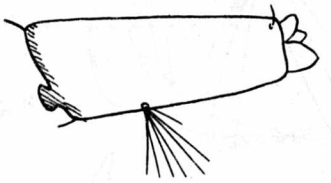


Fig. 2 B

3. Siphon with a single hair or tuft on each side; eighth abdominal segment with one to three rows of 6 to 21 comb scales (Fig. 3 A); (21 to 58 in *Aedes atropalpus*) 4

   Siphon with several hairs or tufts on each side; eighth abdominal segment with a triangular patch of many (30 to 60 or more) comb scales (Fig. 3 B) 13

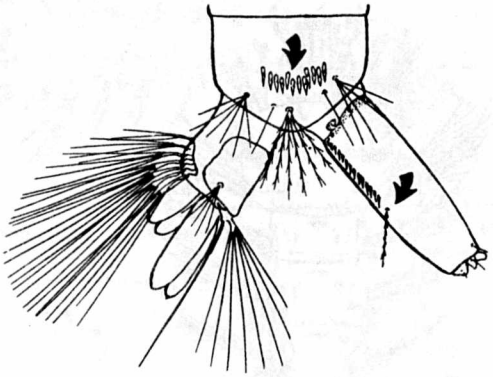


Fig. 3 A

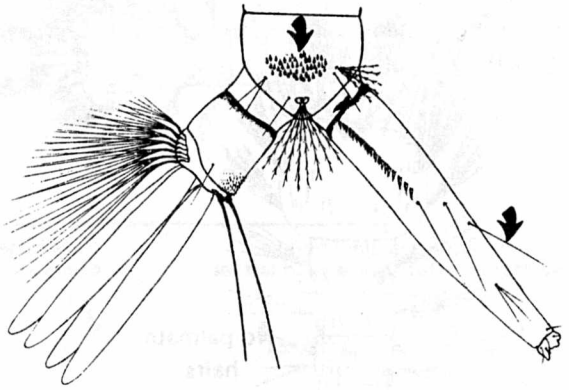


Fig. 3 B

4. Individual comb scales thorn-shaped with a strong median spine and stout lateral spines (Fig. 4 A) 5

   Individual comb scales slipper shaped, evenly tapered with a fringe but no stout lateral spines (Fig. 4 B) . . .7

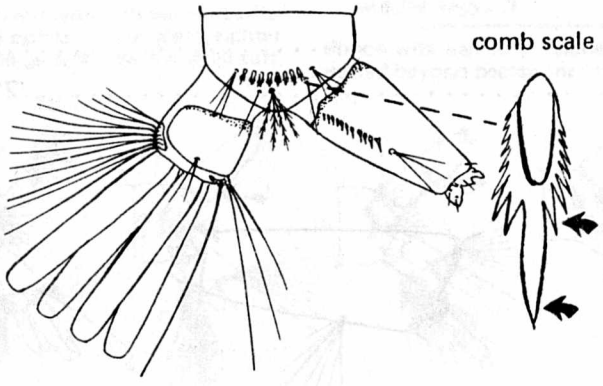


Fig. 4 A

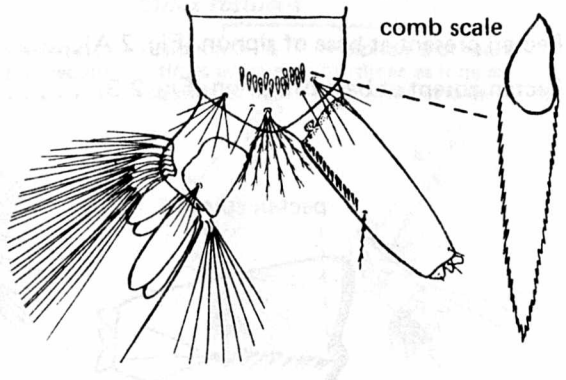


Fig. 4 B

5. Many pecten spines on siphon, anal segment not completely ringed by sclerotized plate (Fig. 5 A), air tube not inflated, preantennal hair single or double (Fig. 5 C) 6

   Few pecten spines on siphon, anal segment completely ringed by sclerotized plate (Fig. 5 D), air tube inflated, preantennal hair multiple (Fig. 5 F) . . . . . .*Psorophora confinnis**
   *Psorophora columbiae**

6. Tubercles of mesothoracic and metathoracic pleural groups with long sharp spine (Fig. 5 B), abdominal hairs not stellate, comb scale with strong median spine and several shorter stout spines (Fig. 5 G) . . *Aedes aegypti*

   Tubercles of mesothoracic and metathoracic pleural groups without long sharp spine (Fig. 5E), abdominal hairs strongly stellate, comb scale strongly sclerotized with sharp median spine and 1 or more smaller spines (Fig. 5H) . . . . . .*Aedes mediovittatus*

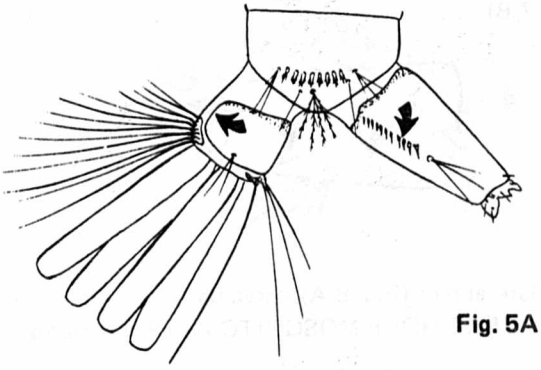


Fig. 5A

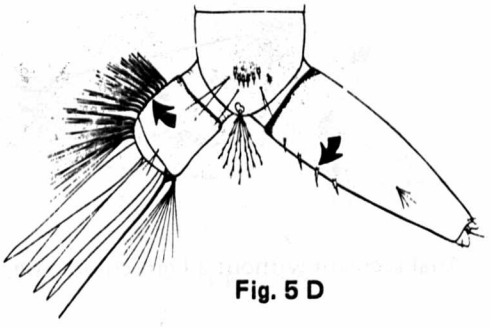
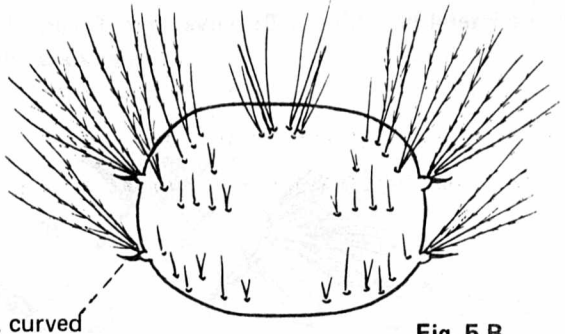
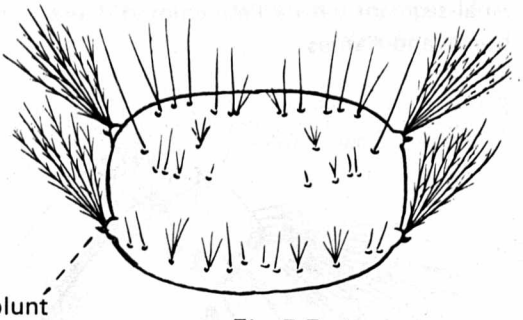


Fig. 5 D



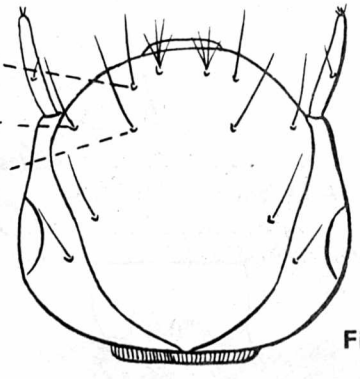
long, curved lateral spine

Fig. 5 B



short, blunt lateral spine

Fig. 5 E

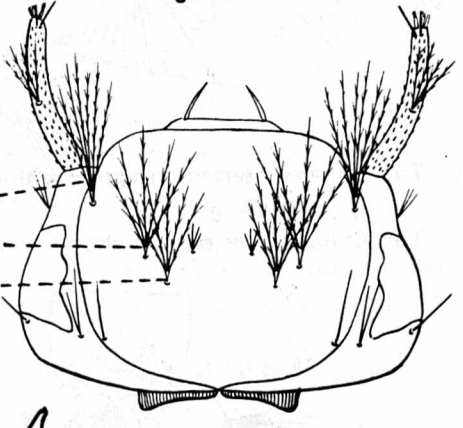


lower head hair

preantennal hair

upper head hair

Fig. 5 C



preantennal hair

lower head hair

upper head hair

Fig. 5 F

Fig. 5 G

Fig. 5 H

*Usually not found in receptacles

7. Anal segment not completely ringed by sclerotized plate (Fig. 6 A). 8

Anal segment completely ringed by sclerotized plate (Fig. 6 B) 11

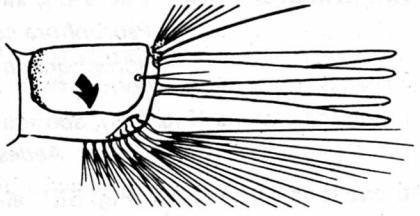


Fig. 6 A

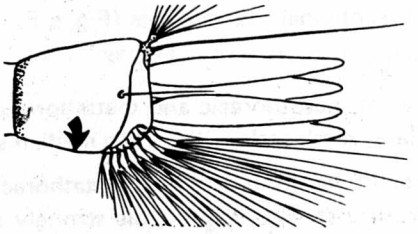


Fig. 6 B

8. Pecten with all spines evenly spaced (Fig. 7 A) 9

Pecten with one or more distal spines more widely spaced (Fig. 7 B) 10

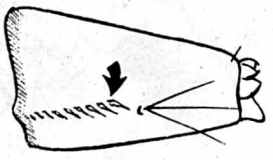


Fig. 7 A

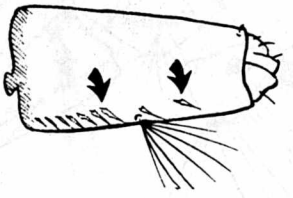


Fig. 7 B

9. Anal segment without a light-colored depression anterior to the lateral hair (Fig. 8 A); larva dark . . . . . TREE HOLE MOSQUITO *Aedes triseriatus*

Anal segment with a light-colored depression anterior to the lateral hair (Fig. 8 B); larva light. Texas, Oklahoma, and Kansas . . . . . *Aedes zoosophus*

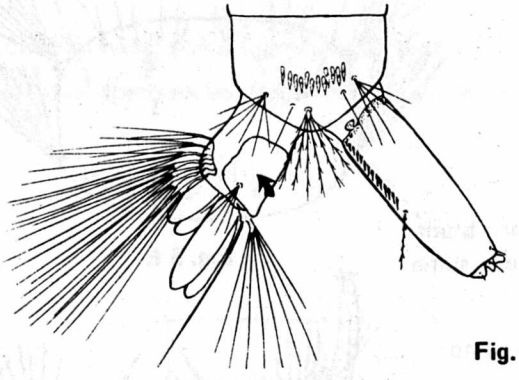


Fig. 8 A

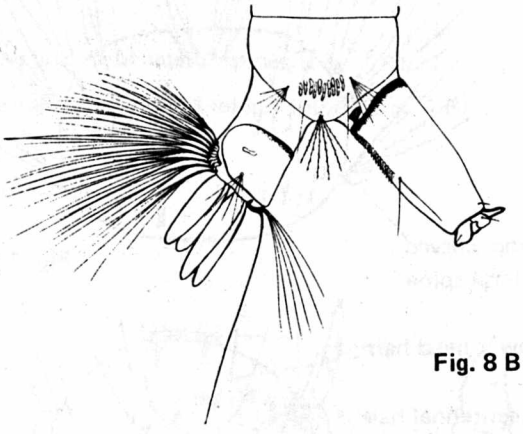


Fig. 8 B

10. Tuft of hairs inserted on siphon within the pecten (Fig. 9 A) . . . . . *Aedes atrophalpus**

Tuft of hairs inserted on siphon beyond the pecten (Fig. 9 B) . . . . . *Aedes vexans**

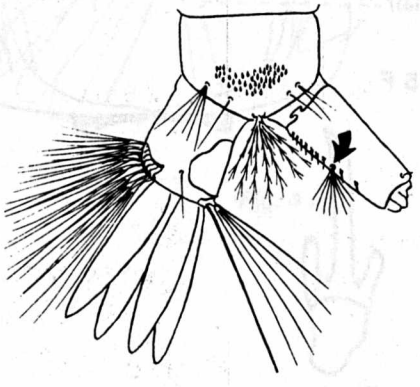


Fig. 9 A

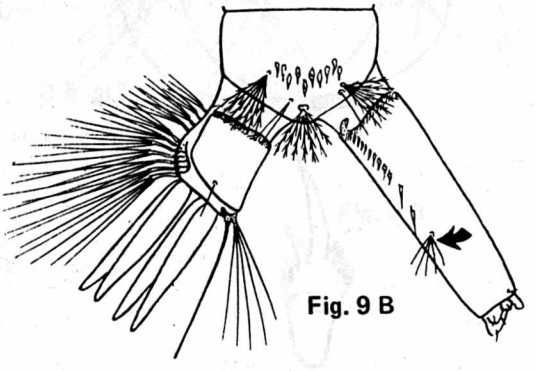


Fig. 9 B

*Usually not found in receptacles

11. Siphon average length, 3 to 3 1/2 times as long as basal width; pecten not quite reaching middle of the siphon (Fig. 10 A); upper head hair and lower head hair well barbed. . . . . *Aedes mitchellae**

    Siphon short, 2 to 2 1/2 times as long as basal width; pecten reaching middle of siphon or slightly beyond (Fig. 10 B); upper head hair and lower head hair smooth or finely barbed 12

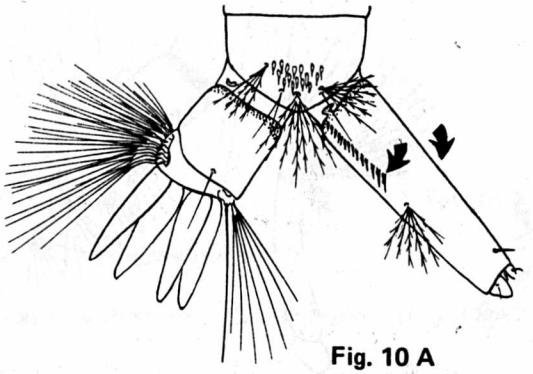


Fig. 10 A

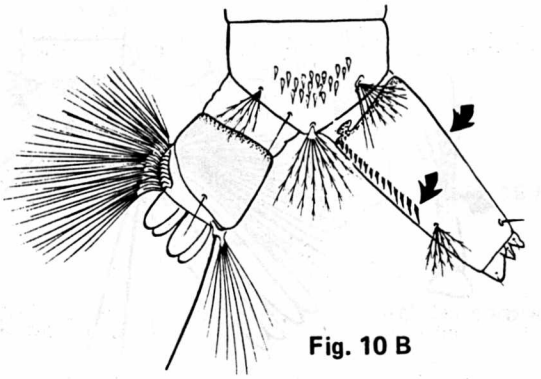


Fig. 10 B

12. Individual comb scale long and pointed apically (Fig. 11 A); lateral abdominal hairs double on segments 3 to 5 . . . . . *Aedes sollicitans**

    Individual comb scale short and rounded apically (Fig. 11 B); lateral abdominal hairs triple on segments 3 to 5 . . . . . *Aedes taeniorhynchus**

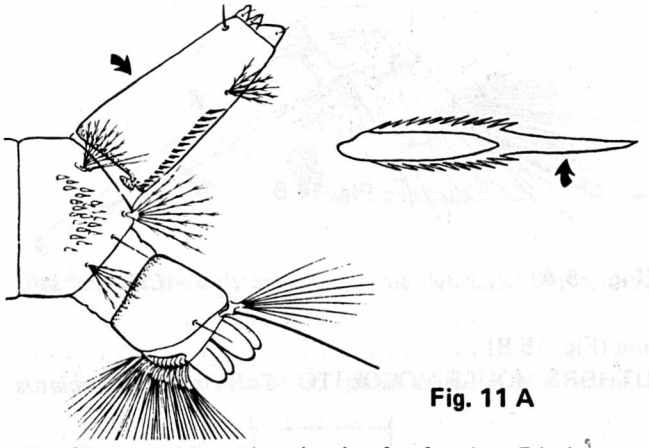


Fig. 11 A

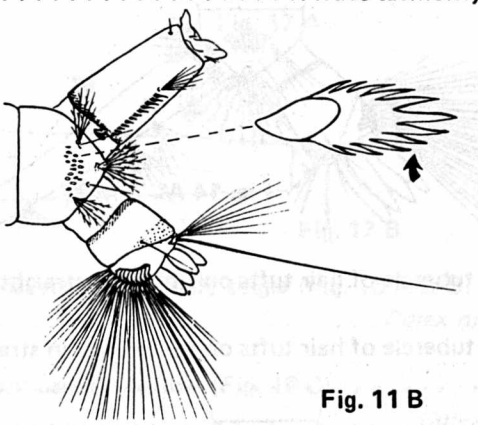


Fig. 11 B

13. Siphon without basal pair of tufts; 4 to 5 hairs or tufts of hairs beyond pecten (Fig. 12 A) . . . (Genus *Culex*) 14

    Siphon with a basal pair of hair tufts; many hairs or tufts of hairs beyond pecten (Fig. 12 B & C). . . . . (Genus *Culiseta*) 20

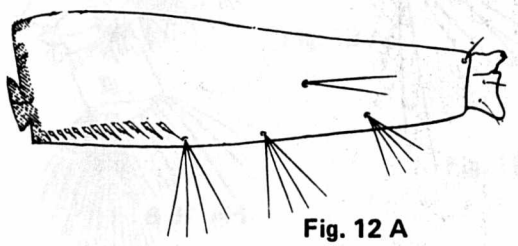


Fig. 12 A

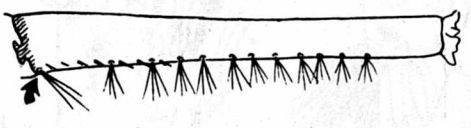


Fig. 12 B

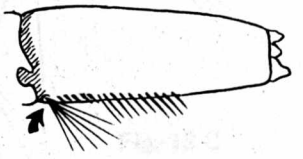


Fig. 12 C

*Usually not found in receptacles

14. Antenna with antennal tuft inserted at about middle of the shaft; siphon with a number of scattered single or double hairs (Fig. 13 A & B) . . . . . *Culex restuans*

Antenna with antennal tuft inserted beyond the middle of the shaft; siphon with most of the tufts with two or more branches (Fig. 13 C & D) 15

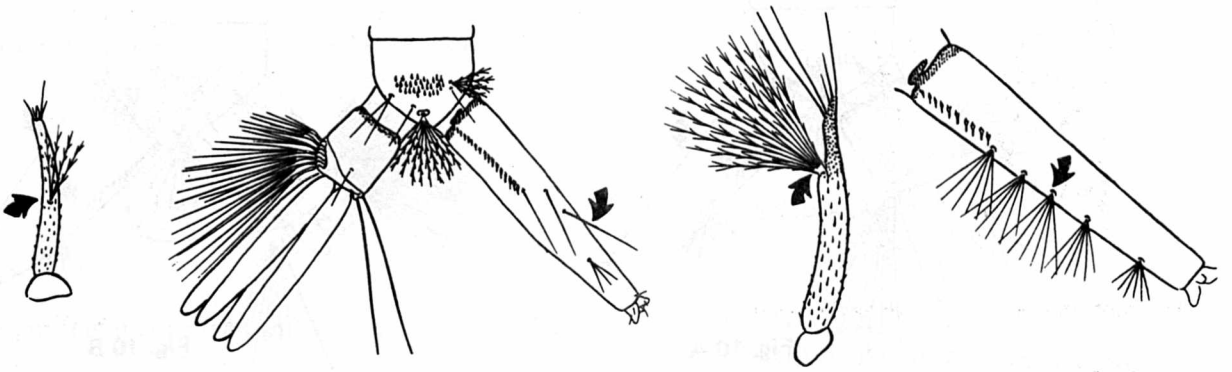


Fig. 13 A **Fig. 13 B** **Fig. 13 C** **Fig. 13 D**

15. Siphon moderately long, usually 4 to 6 times as long as basal width (Fig. 14 A). 16

Siphon very long, 6 to 10 times as long as basal width (Fig. 14 B). 17

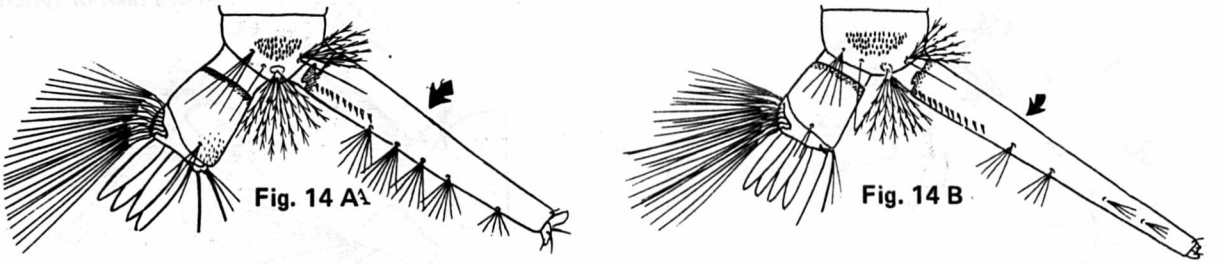


Fig. 14 A **Fig. 14 B**

16. Basal tubercle of hair tufts on siphon in straight line (Fig. 15 A). . . . . *Culex tarsalis*

Basal tubercle of hair tufts on siphon not in straight line (Fig. 15 B) . . . . . SOUTHERN HOUSE MOSQUITO *Culex quinquefasciatus*

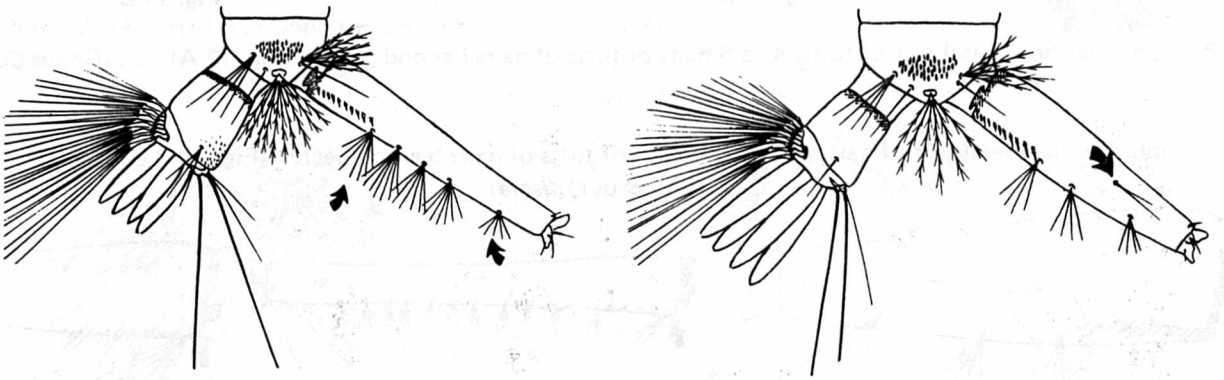


Fig. 15 A **Fig. 15 B**

17. Upper and lower head hairs single or double (Fig. 16 A) . . . . . *Culex territans**

Upper and lower head hairs with three to four branches (Fig. 16 B) 18

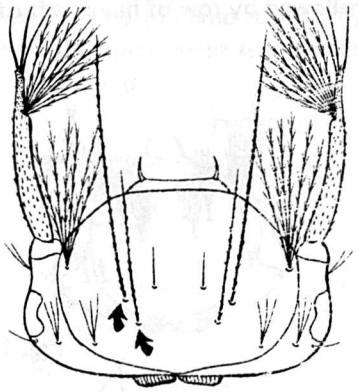


Fig. 16 A

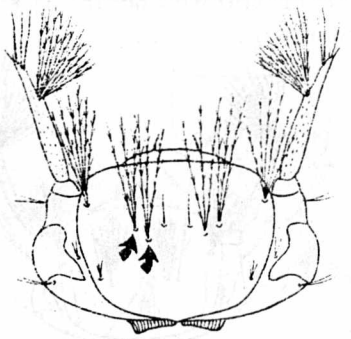


Fig. 16 B

18. Siphon with strong subapical spines (Fig. 17 A). Texas . . . . . *Culex coronator**

Siphon without strong subapical spines (Fig. 17 B) 19

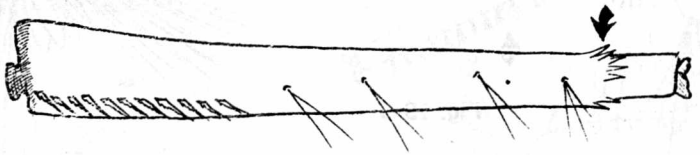


Fig. 17 A

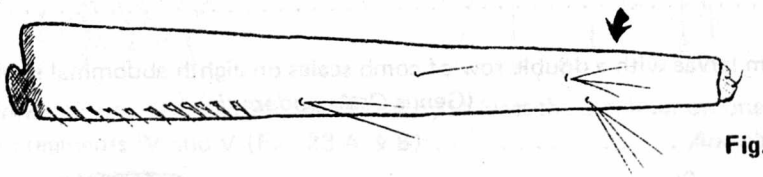


Fig. 17 B

19. Thorax densely spiculate (spicules dark); lateral hair of anal segment usually single (Fig. 18 A & B) . . . . . *Culex nigripalpus**

Thorax with few or no spicules; lateral hair of anal segment usually double (Fig. 18 C) . . . . . *Culex salinarius*

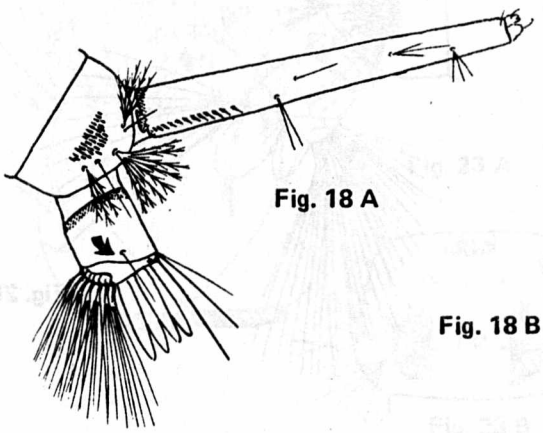


Fig. 18 A

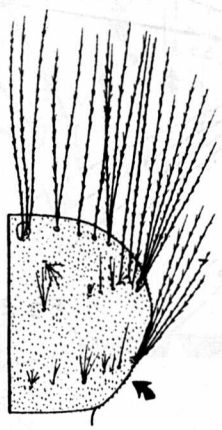


Fig. 18 B

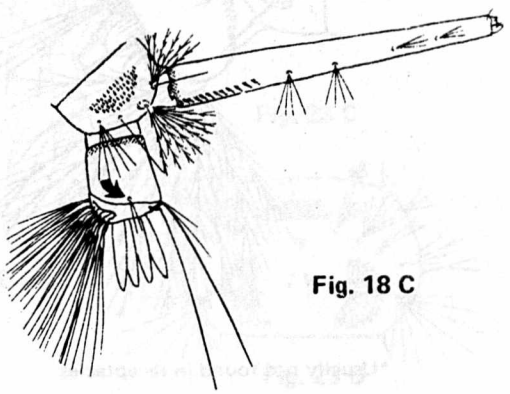


Fig. 18 C

*Usually not found in receptacles

20. Siphon long, 6 or more times as long as basal width, pecten followed by row of tufts; tuft of antenna inserted near end of shaft (Fig. 19 A & B) . . . . . *Culiseta melanura* *

Siphon average length, 2 to 4 times as long as basal width, pecten followed by row of hairs; tuft of antenna inserted at about middle of shaft (Fig. 19 C & D) . . . . . *Culiseta inornata* *

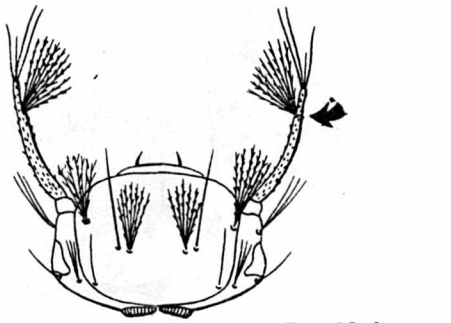


Fig. 19 A

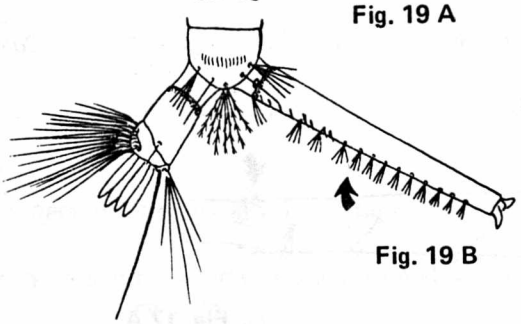


Fig. 19 B

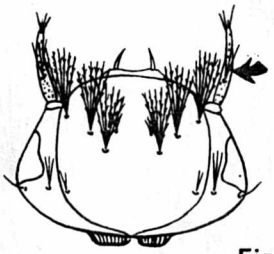


Fig. 19 C

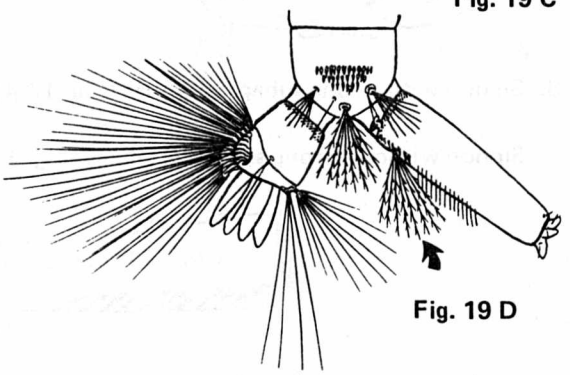


Fig. 19 D

21. Large larvae without comb scales on eighth abdominal segment, simply a plate bearing four hairs (Fig. 20 A) . . . . . *Toxorhynchites rutilus*

Small to medium larvae with a double row of comb scales on eighth abdominal segment (Fig. 20 B) . . . . . (Genus *Orthopodomyia*) 22

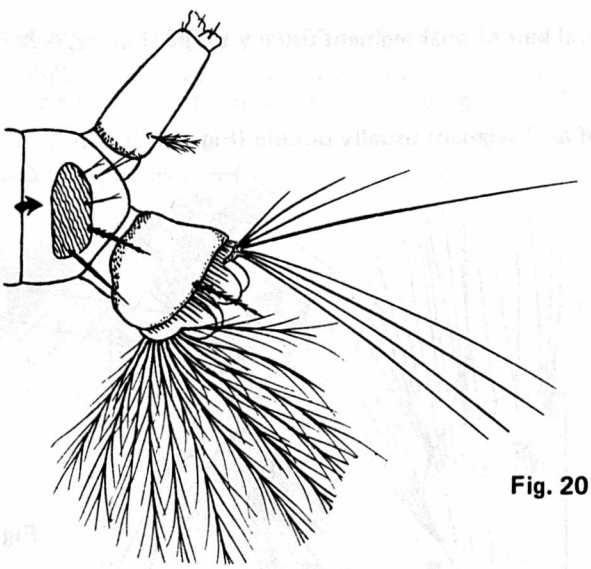


Fig. 20 A

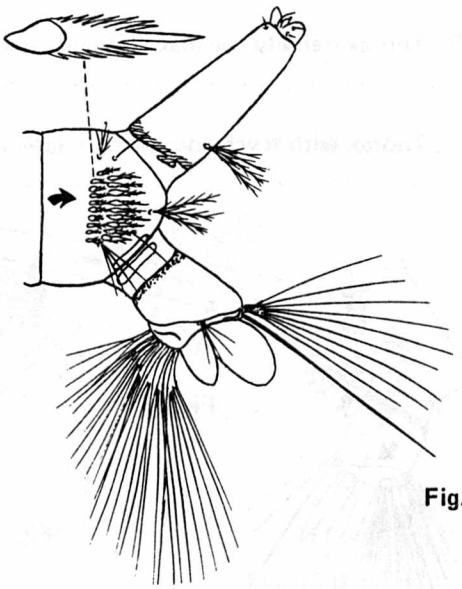


Fig. 20 B

*Usually not found in receptacles

22. Anterior row of comb scales about twice as wide as posterior row (17-23 scales to 6-10); in the 4th instar the eighth abdominal segment with large dorsal plate; lateral hair on anal segment single (Fig. 21 A). Larva usually pink . . . . . *Orthopodomyia signifera*

    Anterior row of comb scales only slightly wider than posterior row (12-17 scales to 8-12); dorsal plate absent on eighth abdominal segment in all stages; lateral hair on anal segment multiple (Fig. 21 B). Larva usually straw colored . . . . . *Orthopodomyia alba**

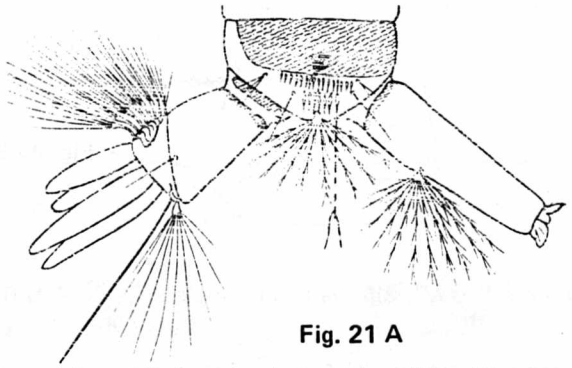


Fig. 21 A

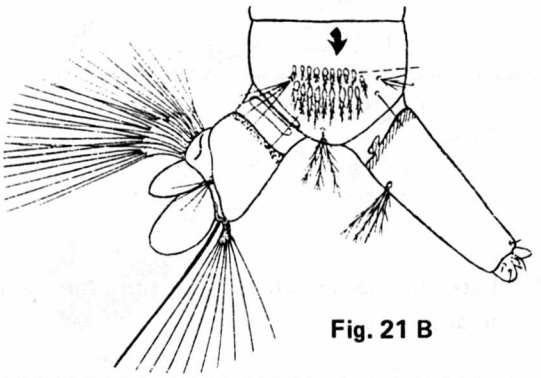


Fig. 21 B

23. Outer clypeal hairs densely branched (Fig. 22 A) 24

    Outer clypeal hairs simple (Fig. 22 B) 26

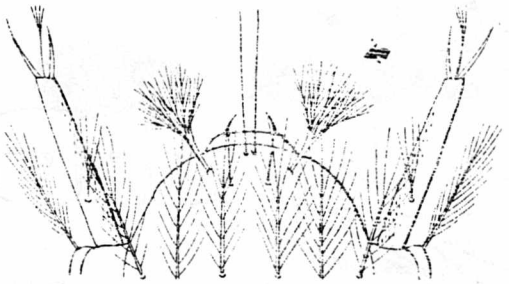


Fig. 22 A

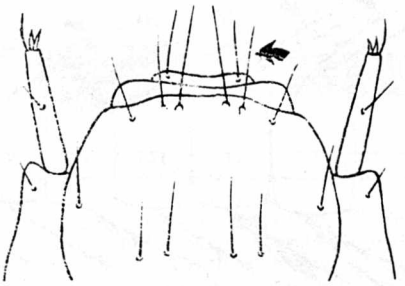


Fig. 22 B

24. Basal tubercles of inner clypeal hairs usually separated by at least the diameter on one tubercle; antepalmate hair usually single on segments IV and V (Fig. 23 A & B) . . . . . *Anopheles quadrimaculatus**

    Basal tubercles of inner clypeal hairs usually separated by less than the diameter of one tubercle; antepalmate hair usually double or triple on segments IV and V (Fig. 23 C & D) 25

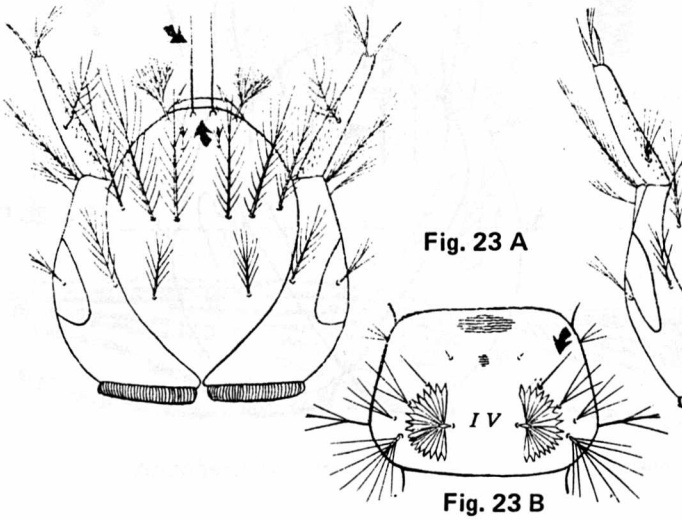


Fig. 23 A

Fig. 23 B

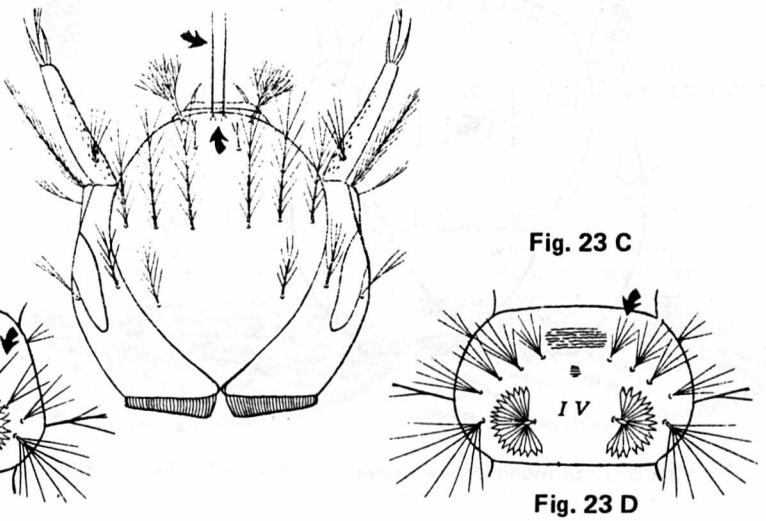


Fig. 23 C

Fig. 23 D

*Usually not found in receptacles

25. Abdominal segments 4 and 5 with several hairs with 3-5 branches or more in front of palmate hairs (Fig. 24 A) . . . . . *Anopheles crucians*

Abdominal segments 4 and 5 with a large single or double hair in front of palmate hairs (Fig. 24 B) . . . . . *Anopheles punctipennis**

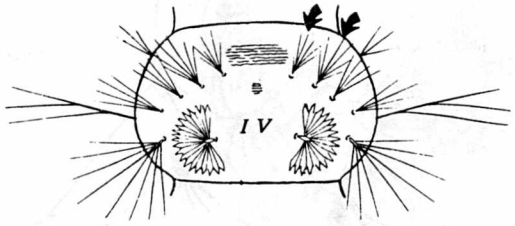


Fig. 24 A

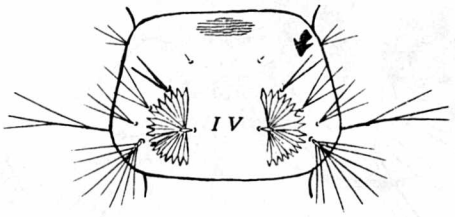


Fig. 24 B

26. Lateral abdominal hairs long and plumose on segments I to VI; all head hairs single (Fig. 25 A & B). Larvae small. . . . . *Anopheles barberi**

Lateral abdominal hairs long and plumose only on segments I to III; many of the head hairs branched (Fig. 25 C & D). Texas. . . . . *Anopheles pseudopunctipennis**

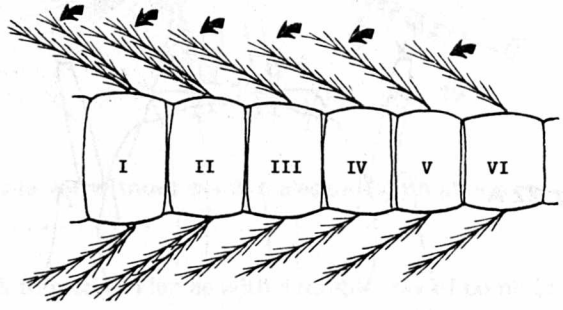


Fig. 25 A

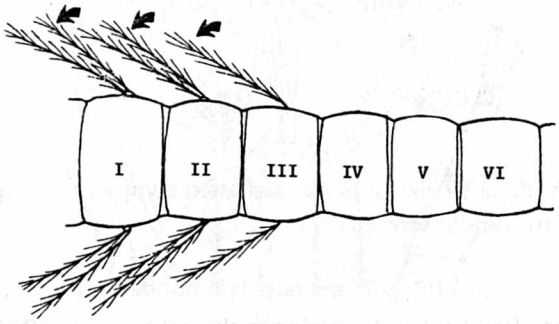


Fig. 25 C

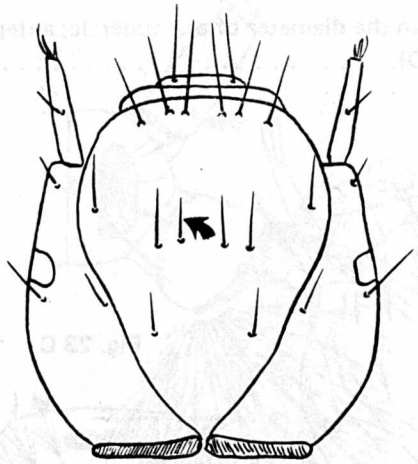


Fig. 25 B

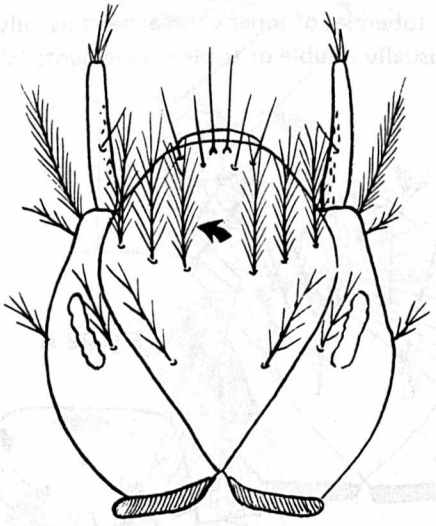


Fig. 25 D

*Usually not found in receptacles

3. Pictorial Key to Some Common Adult Mosquitoes Associated with *Aedes aegypti*

proboscis curved, large mosquitoes with brilliant greenish to purplish color

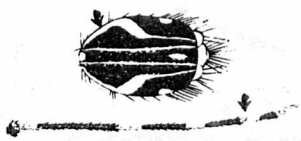
Toxorhynchites rutilus

proboscis straight, smaller mosquito usually with blackish or brownish color

hind tarsi with pale bands

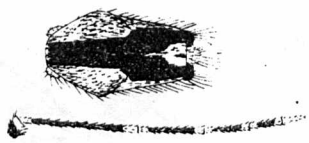
hind tarsi without pale bands

scutum with lyre-shaped marking, hind tarsi with pale basal bands



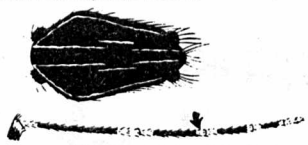
Aedes aegypti

scutum with dark, broad, median stripe, hind tarsi with pale basal and apical bands



Aedes atropalpus

scutum with parallel lines, hind tarsi with pale basal and apical bands

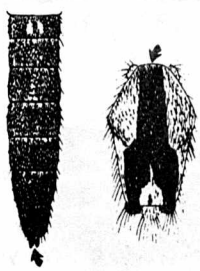


Orthopodomyia species

scutellum saddle-shaped, abdomen without scales or pale bands

scutellum tri-lobed, abdomen with scales and usually with pale bands

scutum with broad dark median stripe abdomen pointed

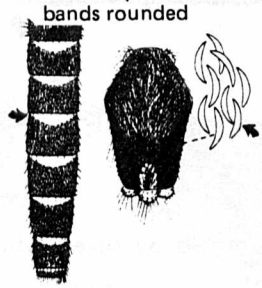


Aedes triseriatus

scutum with two pale spots and fine coppery scales, abdomen blunt with basal pale bands almost straight

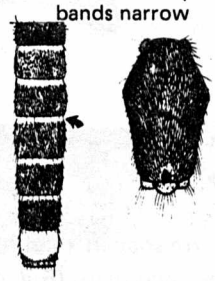
Culex restuans

scutum almost uniform, scales coarse and brassy; abdomen blunt with pale basal bands rounded



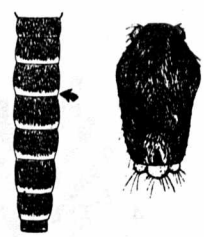
Culex quinquefasciatus

scutum almost uniform, scales fine and coppery; abdomen blunt with basal pale bands narrow



Culex salinarius

scutum almost uniform, scales fine and coppery; abdomen blunt with apical pale bands



Culex territans

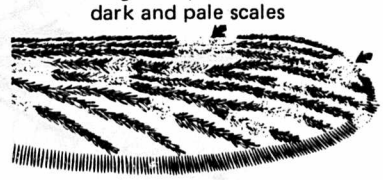
wing uniformly dark

Anopheles barberi

wing dark with four well-defined dark spots

Anopheles quadrimaculatus

wing with patches of dark and pale scales



Anopheles punctipennis

4. Key to Adults of Receptacle-Breeding Mosquitoes

1. Legs with white bands (Fig. 1 A) 2

   Legs unbanded, entirely dark or terminal segments white (Fig. 1 B) 13

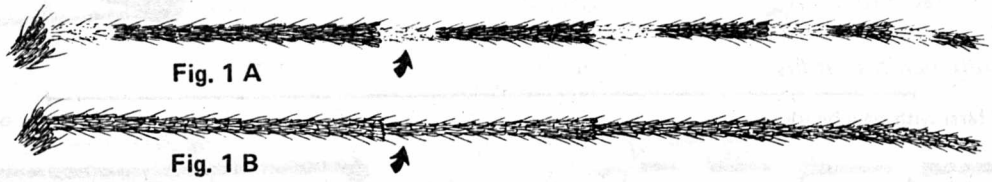


Fig. 1 A

Fig. 1 B

2. Proboscis unbanded (Fig. 2 A) 3

   Proboscis with white band (Fig. 2 B) 8

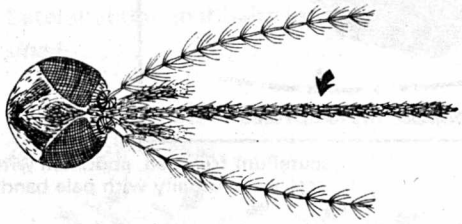


Fig. 2 A

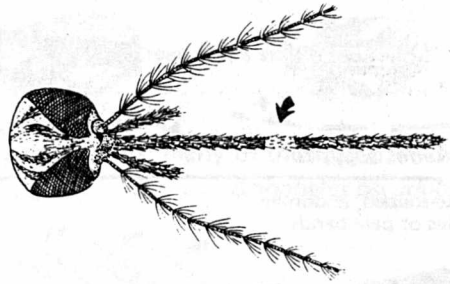


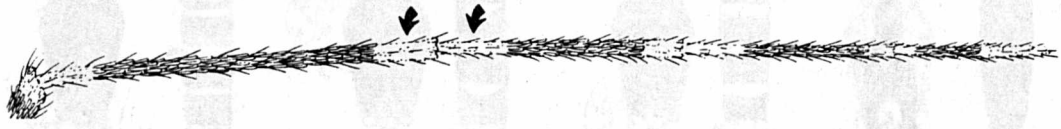
Fig. 2 B

3. Tarsal segments with white bands at basal ends (Fig. 3 A) 4

   Tarsal segments with white bands on both ends (Fig. 3 B) 6

Fig. 3 A

Fig. 3 B



4. Thorax with lyre-shaped marking formed by silvery-white scales on a blackish background; silvery-white scales on clypeus and palpi (Fig. 4 A) . . . . . YELLOW FEVER MOSQUITO *Aedes aegypti*

   Thorax without lyre-shaped marking; clypeus and palpi dark (Fig. 4 B) 5

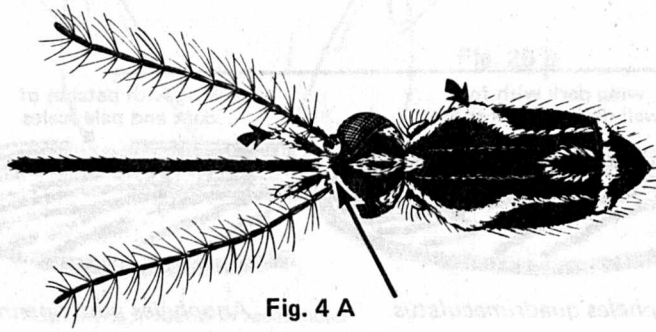


Fig. 4 A

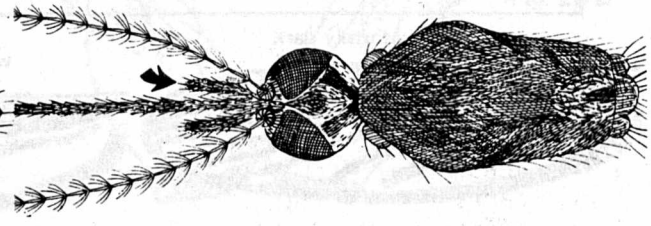


Fig. 4 B

5. Scutum with a narrow median longitudinal silvery line from anterior to prescutellar space. . . . . *Aedes mediovittatus*

   Scutum without such a line of silvery scales . . . . . *Aedes zoosophus*

Fig. 5 Deleted

6. Abdomen pointed, segment 7 of abdomen narrowed, segment 8 much narrowed and retractile (Fig. 6 A); scutum with a broad patch of pale scales on each side (Fig. 6 B). . . . . *Aedes atropalpus*

   Abdomen blunt, segment 7 of abdomen not narrowed, segment 8 short but not retractile (Fig. 6 C); scutum uniform or with fine lines of pale scales (Fig. 6 D) 7

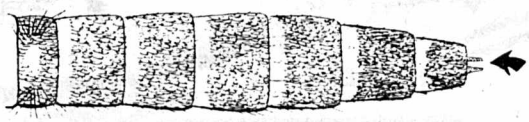


Fig. 6 A

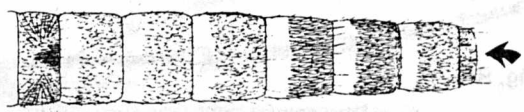


Fig. 6 C

Fig. 6 B

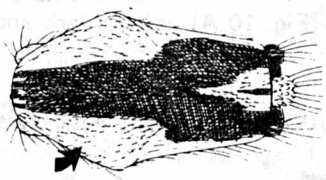
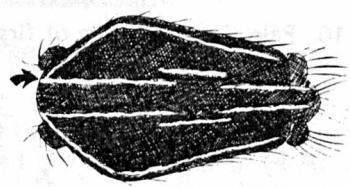


Fig. 6 D



7. Four fine longitudinal white lines on thorax (Fig. 7 A); scattered white scales on femur and tibia (Fig. 7 B); mixed dark and white scales on wings (Fig. 7 C) . . . . . *Orthopodomyia signifera* or *Orthopodomyia alba*

   No fine lines on thorax (Fig. 7 D); no scattered white scales on femur and tibia (Fig. 7 E); dark scales on wings (Fig. 7 F). Texas . . . . . *Culex coronator**

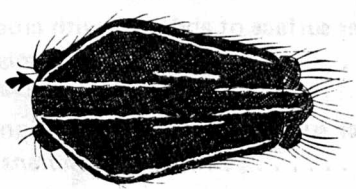


Fig. 7 A

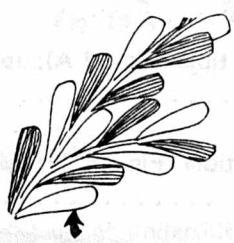


Fig. 7 C

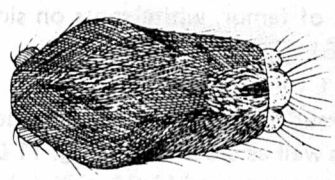


Fig. 7 D

Fig. 7 F

Fig. 7 B

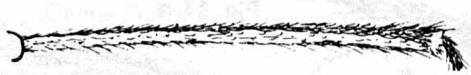


Fig. 7 E

*Usually not found in receptacles

8. Abdomen blunt at tip (Fig. 8 A). 9

Abdomen pointed at tip (Fig. 8 B) 10

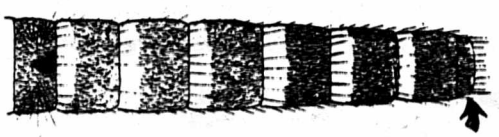


Fig. 8 A

Fig. 8 B

9. White stripe on side of femur, no pale band near apex of tibia, no pale ring at middle of first segment of hind tarsus (Fig. 9 A & B) . . . . . .*Culex tarsalis*

No stripe on side of femur, pale band present near apex of tibia, pale ring present at middle of first segment of hind tarsus (Fig. 9 C & D) . . . . . *Coquillettidia perturbans**

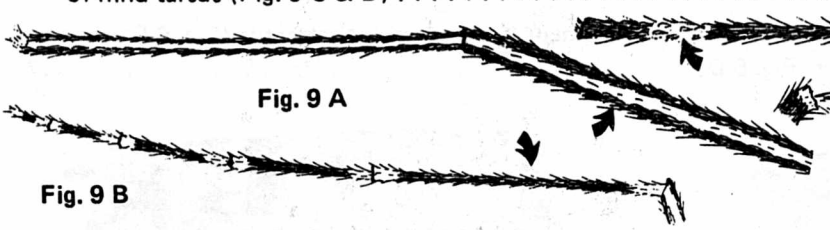


Fig. 9 A

Fig. 9 B

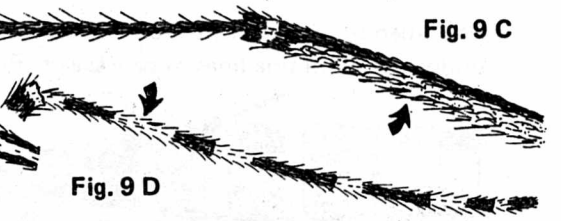


Fig. 9 C

Fig. 9 D

10. Pale ring at middle of first segment of hind tarsus (Fig. 10 A); mixed dark and white scales on wings (Fig. 10 B) 11

No pale ring at middle of first segment of hind tarsus (Fig. 10 C); only dark scales on wings (Fig. 10 D). . . 12

Fig. 10 A

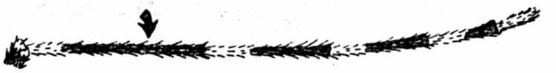


Fig. 10 C

Fig. 10 B

Fig. 10 D

11. Pale band near apex of femur, white spots on side of tibia (Fig. 11 A); upper surface of abdomen with cross bands only (Fig. 11 B) . . . . . .*Psorophora confinnis** *Psorophora columbia**

No pale band on femur or tibia, no spots on side of tibia (Fig. 11 C); upper surface of abdomen with long longitudinal stripe as well as cross bands (Fig. 11 D). . . . . .*Aedes sollicitans**

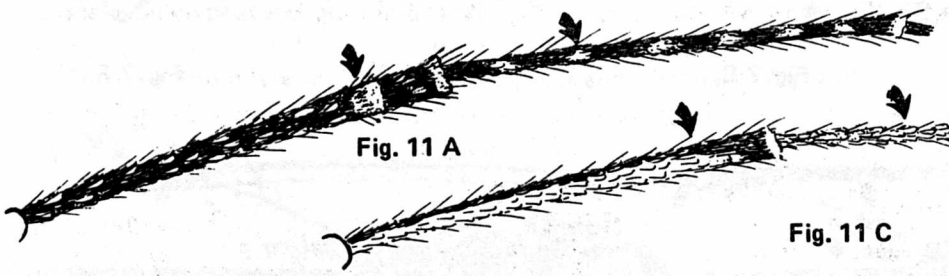


Fig. 11 A

Fig. 11 C

Fig. 11 B

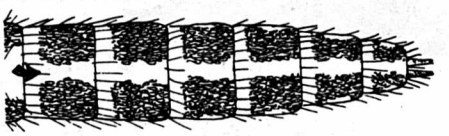


Fig. 11 D

*Usually not found in receptacles

12. Scattered white scales on legs (Fig. 12 A); upper surface of abdomen with median longitudinal pale stripe as well as cross-bands (Fig. 12 B) . . . . . *Aedes mitchellae**

    No scattered white scales on legs (Fig. 12 C); upper surface of abdomen with only pale basal cross-bands (Fig. 12 D) . . . . . *Aedes taeniorhynchus**

Fig. 12 A

Fig. 12 C

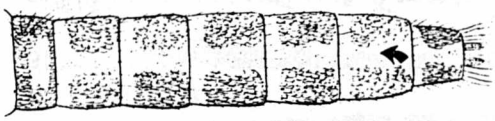


Fig. 12 B

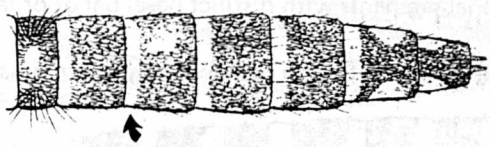


Fig. 12 D

13. Palpi much shorter than proboscis (Fig. 13 A) 14

    Palpi at least 1/2 as long as proboscis (Fig. 13 B) 21

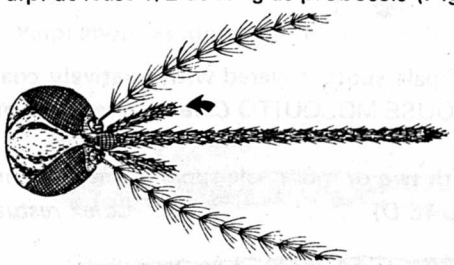


Fig. 13 A

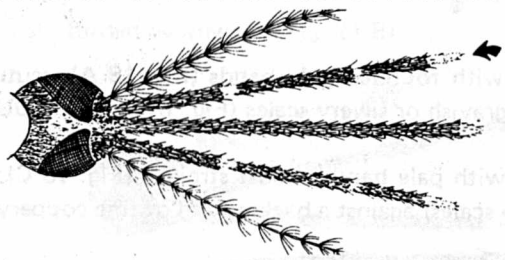


Fig. 13 B

14. Abdomen pointed, segment 7 of abdomen narrowed, segment 8 much narrowed and retractile (Fig. 14 A); scutum with a broad patch of pale scales on each side (Fig. 14 B) . . . . . *Aedes triseriatus*

    Abdomen blunt, segment 7 of abdomen not narrowed, segment 8 short but not retractile (Fig. 14 C); scutum without a patch of pale scales on each side (Fig. 14 D) 15

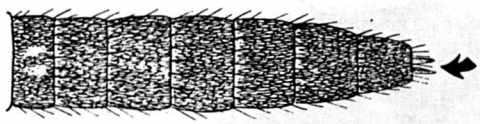


Fig. 14 A

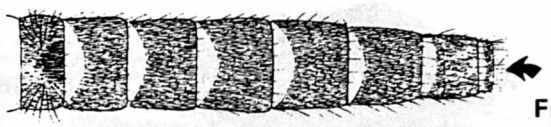


Fig. 14 C

Fig. 14 B

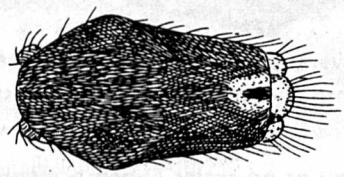


Fig. 14 D

15. Base of subcosta without a tuft of hairs on underside of wing (Fig. 15 A); spiracular bristles absent; proboscis short and straight . . . . . Genus *Culex* 16

    Base of subcosta with tuft of hairs on underside of wing (Fig. 15 B); spiracular bristles present (Fig. 15 C); proboscis long and recurved. . . . . Genus *Culiseta* 20

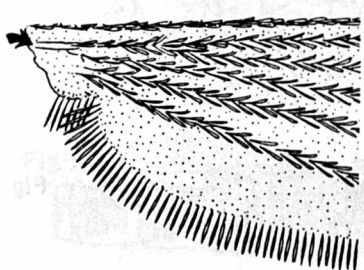


Fig. 15 A

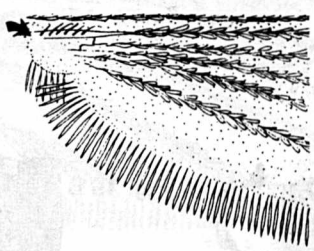


Fig. 15 B

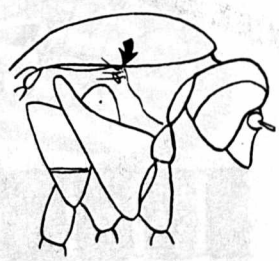


Fig. 15 C

*Usually not found in receptacles

16. Abdomen with white scales at apex of segments (Fig. 16 A) . . . . . *Culex territans*

Abdomen with white scales at bases of segments (Fig. 16 B) 17

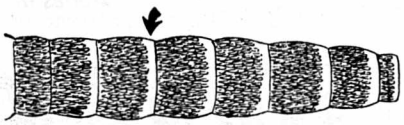


Fig. 16 A

Fig. 16 B

17. Abdominal segments with distinct basal bands or lateral spots of white scales (Fig. 17 A) 18

Abdominal segments with narrow dingy-white basal bands (Fig. 17 B) 19

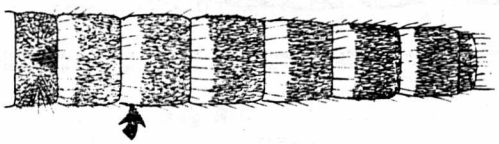


Fig. 17 A

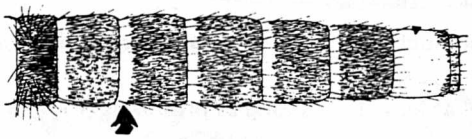


Fig. 17 B

18. Abdomen with rounded pale bands (Fig. 18 A); scutum without pale spots, covered with relatively coarse brownish, grayish or silvery scales (Fig. 18 B) . . . .SOUTHERN HOUSE MOSQUITO *Culex quinquefasciatus*

Abdomen with pale bands almost straight (Fig. 18 C); scutum with two or more pale spots (sometimes only one or two scales) against a background of fine coppery scales (Fig. 18 D) . . . . . *Culex restuans*

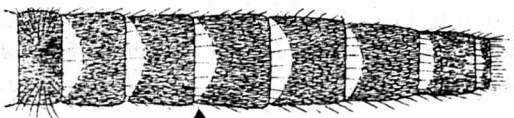


Fig. 18 A

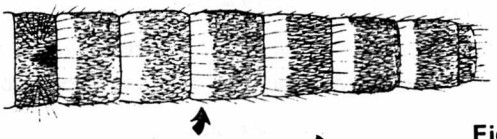


Fig. 18 C

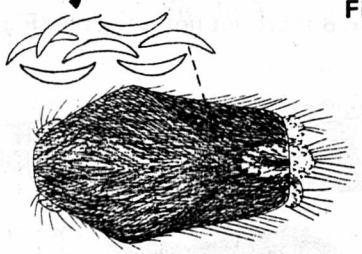


Fig. 18 B

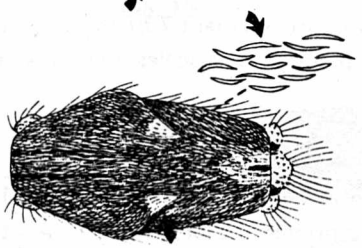


Fig. 18 D

19. Pleuron with several groups of broad scales, each group usually comprised of more than 6 scales (Fig. 19 A); seventh and eighth abdominal segments almost entirely covered with dingy-yellow scales (Fig. 19 B). . . . . *Culex salinarius*

Pleuron with few or no scales (when present, rarely more than 5 or 6 in a single group (Fig. 19 C); seventh and eighth abdominal segments banded (Fig. 19 D) . . . . . *Culex nigripalpus**

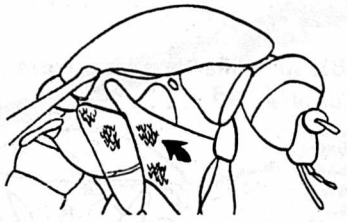


Fig. 19 A

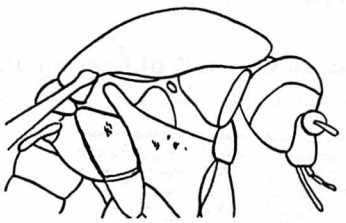


Fig. 19 C

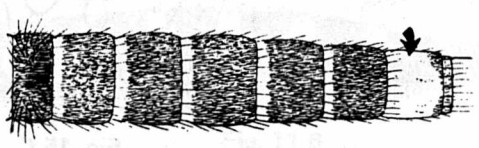


Fig. 19 B

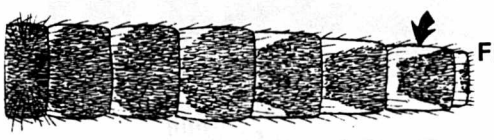


Fig. 19 D

*Usually not found in receptacles

20. With broad lightly scaled wings (Fig. 20 A); legs and wings sprinkled with white scales; a large species . . . . . *Culiseta inornata*

Wings and legs entirely dark scaled (Fig. 20 B); a small dark species. . . . . *Culiseta melanura*

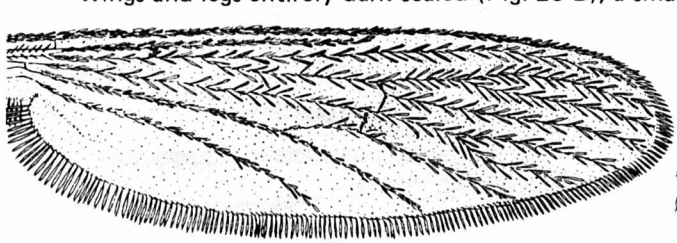


Fig. 20 A

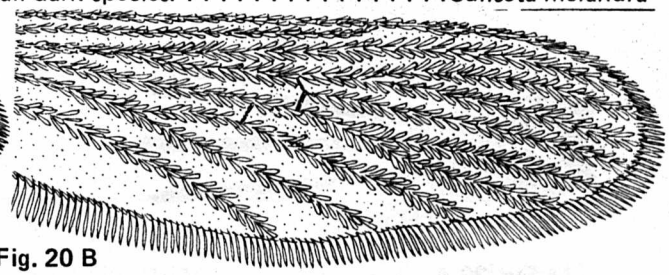


Fig. 20 B

21. Palpi about 2/3 length of proboscis; proboscis strongly turned downward (Fig. 21 A); large mosquito with brilliant metallic blue-green or purple coloring . . . . . *Toxorhyncites rutilus*

Palpi about as long as proboscis, proboscis not strongly turned downward (Fig. 21 B) 22

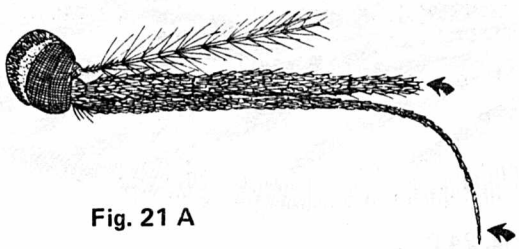


Fig. 21 A

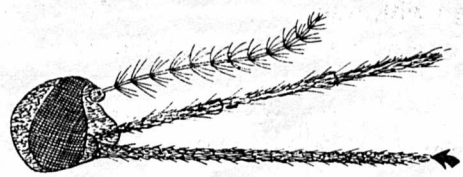


Fig. 21 B

22. Wings with definite areas of white or yellowish scales (Fig. 22 A) 23

Wings without definite areas of white or yellowish scales (Fig. 22 B) 25

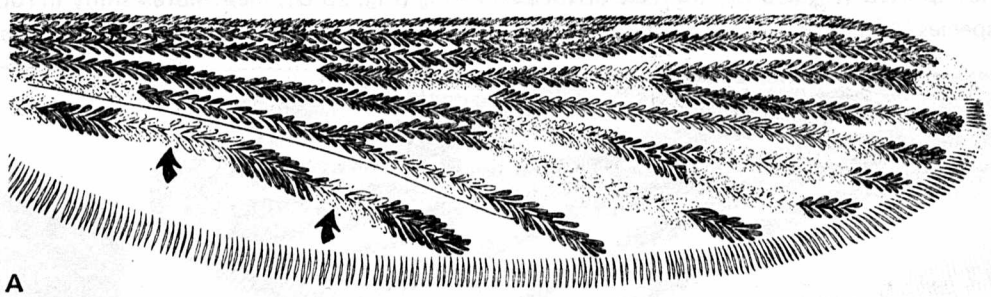


Fig. 22 A

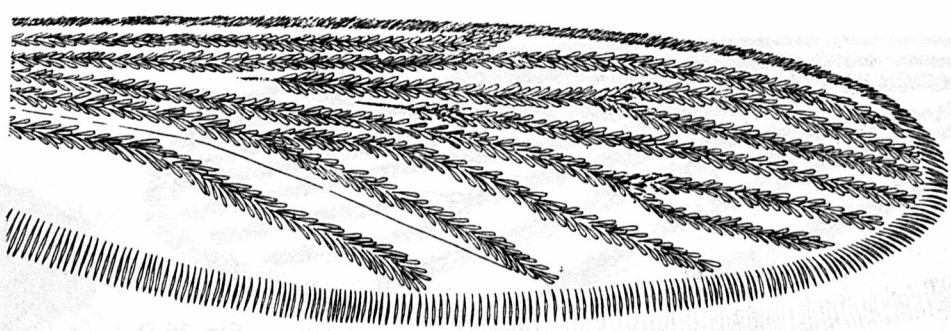


Fig. 22 B

23. Palpi entirely dark (Fig. 23A); costa with 2 white spots (Fig. 24 B). . . . . *Anopheles punctipennis*

Palpi marked with white (Fig. 23 B); costa with 1 or 2 spots (Fig. 24 A & B) 24

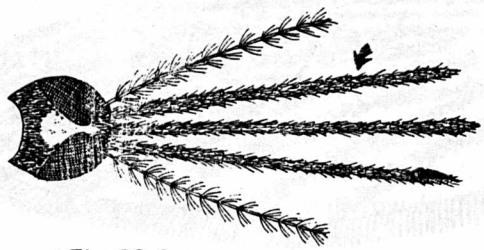


Fig. 23 A

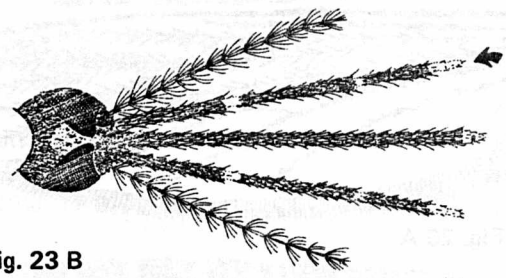


Fig. 23 B

24. Costa with white spot at tip of wing only (Fig. 24 A) . . . . . *Anopheles crucians**

Costa with 2 white spots (Fig. 24 B) . . . . . *Anopheles pseudopunctipennis*

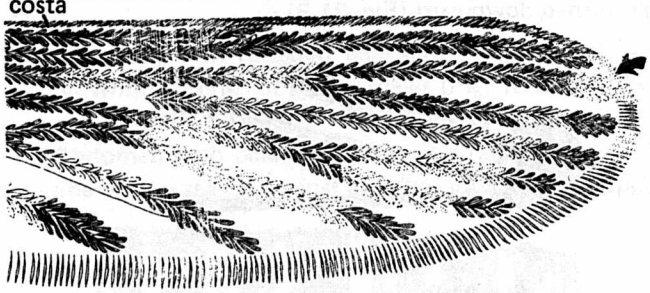


Fig. 24 A

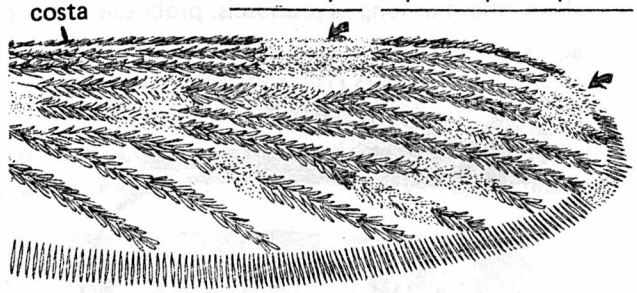


Fig. 24 B

25. Wing spotted (Fig. 25 A); thoracic bristles not very long (Fig. 25 B); mesothorax dull in rubbed specimens; medium sized species . . . . . *Anopheles quadrimaculatus**

Wing not spotted (Fig. 25 C); thoracic bristles very long (Fig. 25 D); mesothorax shiny in rubbed specimens; small species. . . . . *Anopheles barberi**

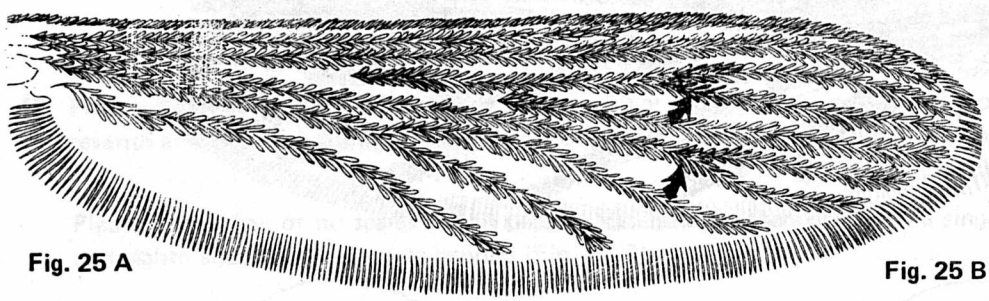


Fig. 25 A

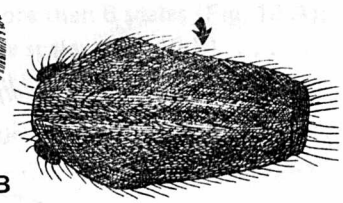


Fig. 25 B

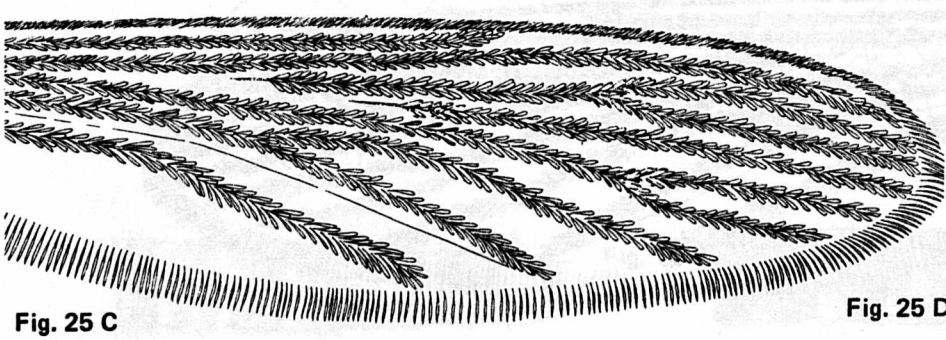


Fig. 25 C

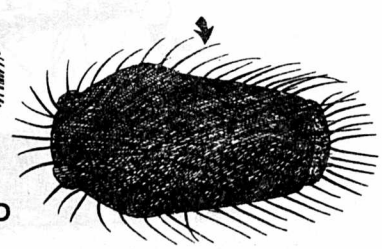


Fig. 25 D

*Usually not found in receptacles

APPENDIX II: Some Common Insects Found in and Around Natural as Well as Artificial Containers

Order		Family	
Scientific	Common	Scientific	Common
1. Collembola	Springtails	Poduridae	Springtails
		Sminthuridae	Globular springtails
2. Odonata	Damselflies	Agrionidae	Broad-winged damselflies
3. Coleoptera	Beetles	Dytiscidae	Predaceous diving beetles
		Hydrophilidae	Water scavenger beetles
		Staphylinidae	Rove beetles
		Helodidae	Marsh beetles
4. Diptera	True flies	Tipulidae	Crane flies
		Psychodidae	Moth flies
		Culicidae	Mosquitoes
		Ceratopogonidae	No-see-ums
		Chironomidae	Midges
		Stratiomyidae	Soldier flies
		Syrphidae	Syrphid flies
		Ephydridae	Shore flies
		Chloropidae	Stem flies
5. Hymenoptera	Ants, wasps, bees	Ichneumonidae	Ichneumons
		Formicidae	Ants
		Diapriidae	Diapriids

APPENDIX III: Insecticides for Use in Mosquito Control

These recommendations are guidelines only. User must ensure that insecticides are applied in strict compliance with the label, as well as local, State, and Federal regulations.

Only those pesticides approved by the U.S. Environmental Protection Agency can be considered for use in the United States. When applied according to label directions, these compounds are not considered hazardous to human health.

Table 1. Insecticides for Use as Mosquito Larvicides

Insecticide	Rate of application (AI/A*)	Formulation
Organophosphates		
chlorpyrifos (Dursban®)	0.0125-0.05 lb	2E (2 lb/gal emulsifiable conc.)
fenthion (Baytex®)	0.05 lb	4EC (4 lb/gal emulsifiable conc.)
malathion	0.4-0.5 lb	57E (57%; 5 lb/gal emulsifiable conc.)
temephos (Abate®)	0.05-0.1 lb	sand, celatom, 4E (4 lb/gal emulsifiable conc.)
Organochlorines		
methoxychlor	1 lb	50% WP (wettable powder)
Insect Growth Regulators		
methoprene (Altosid®)	0.025-0.05 lb	CR and others (controlled release, microencapsulated flowable, sand granular)
Oils		
diesel fuel oil No. 2	1-5 gal	with spreading agent only
proprietary mosquito control oils (Flit MLO, ARCO larvicide, GB-1313)	1-5 gal	with spreading agent only

*Active ingredient/acre

Table 2. Adulticides Applied Aerially or by Ground Equipment at Ultra-Low Volume (ULV)

Insecticide	Rate of application (AI/A*)	Formulation
	Aerial ULV	
malathion (Malathion ULV Concentrate®, Cythion®)	3 fl oz	ULV concentrate
naled (Dibrom®)	0.5-1.0 fl oz	ULV concentrate
	Ground-applied ULV	
Organophosphates		
chlorpyrifos (Dursban®)	0.005-0.01 lb	ULV concentrate
fenthion (Baytex®)	0.005 lb	ULV concentrate
malathion	0.08-0.1 lb/acre (3-4.6 oz/min at 5 miles per hour)	ULV concentrate
naled (Dibrom®)	0.01-0.02 lb	10% in heavy aromatic naptha
Pyrethroids		
pyrethrum (pyrocide®)	0.002-0.0025 lb	5% with 25% piperonyl butoxide
resmethrin (SBP-1382®)	0.007 lb	10% solution 40% oil-based ULV concentrate

*Active ingredient/acre

Table 3. Adulticides Used in Foggers, Misters and Dusters (Ground and Aerial)

Insecticide	AI/A**	Examples of concentrations of finished spray or dust*		
		Thermal fogs	Dusts	Emulsions***
carbaryl (Sevin®)	0.2-1.0 lb	-	2.5-5 lb/acre	-
chlorpyrifos (Dursban®)	0.025-0.05 lb	2 gal fogging conc/ 98 gal oil	-	3.2 fl oz/acre of 2E
fenthion (Baytex®)	0.01-0.1 lb	2 gal spray conc/ 100 gal oil	-	-
malathion	0.075-0.2 lb	-	-	2% spray (4.5-11.2 oz of 5E/gal water)
naled (Dibrom 14®)	0.02-0.1 lb	100 fl oz 14 conc/ 99 gal oil	-	100-230 fl oz 14 conc. in 100 gal oil
propoxur (Baygon®)	0.05-0.07 lb	-	-	4.25-6 fl oz 5E in 2-4 qt water for aerial use
pyrethrins (synergized)	0.002-0.0025 lb	-	-	5% oil-based ULV conc. with 25% piperonyl butoxide
resmethrin (SPB-1382®)	0.007 lb	-	-	10% solution

*Examples do not represent all acceptable pesticide formulations
**Active ingredient/acre
***2E = 2 lb/gal emulsifiable concentrate; 5E = 5 lb/gal emulsifiable concentrate

APPENDIX IV: Performance Requirements for Ultra-Low Volume Applications

I. Ground applications

Ultra-low volume (ULV) is defined as the application of less than 2 quarts of insecticide per acre. With ground equipment, dosage rates fall in the range of 0.3 to 3.0 fluid ounces per acre depending on the material. Performance requirements are:

a) The spray nozzle must have the capability of producing droplets with the correct diameter. For example, malathion requires droplets of 5-27 microns, with the average diameter not exceeding 17 microns. Droplets larger than 27 microns may cause permanent spotting of paint on automobiles, trucks, trailers, and boats.

b) When applicable, air pressure on the insecticide tank should be in accord with the manufacturer's instructions. The usual range is 2-6 pounds per square inch.

c) Flow rate must be regulated by an accurate flow meter. This measurement is the amount of insecticide dispensed, indicated as fluid ounces per minute. A record of the amount of insecticide dispensed and spraying time should be made after each application.

d) The nozzle of the ULV machine should be at the rear of the truck bed and pointed upward at a 45^{o} angle.

e) Vehicle speed is indicated on the insecticide label, and it is usually 10 miles per hour.

f) The ULV machine spray application must be stopped when the vehicle stops.

g) Atmospheric conditions greatly affect ULV operation. Spray applications should not be made when the wind speed exceeds 10 miles per hour or when the ambient air temperature exceeds $82^{o}F$ ($28^{o}C$). With some equipment, the temperature of the insecticide is also critical as the viscosity and thus the flow rates are altered as the temperature of the insecticide changes.

II. Fixed-wing aircraft

a) Multi-engine aircraft should be used in urban control situations for safety purposes in the event of engine failure and also for increased payloads.

b) The aircraft dispensing malathion should be operated at an altitude between 100-200 feet, at speeds of 150-200 miles per hour, and with an insecticide swath width of 300-1000 feet. For naled, 80 mph or more is required.

c) The pump pressure must be calibrated carefully in relation to air speed in order to get the correct dosage per acre. For malathion, the dosage is 3.0 fluid ounces per acre, and for naled the dosage is 0.5 to 1.0 ounces.

d) The number of nozzles, the size of their orifices, and their position are critical for correct droplet size. Labels state the nozzles should be at a 45^{o} angle, pointed down and into the wind.

e) Nozzles must be equipped with a diaphragm check valve which will terminate the insecticide flow immediately when the pump is disengaged.

f) Droplet size for malathion should be less than 50 microns (mass median diameter) and less than 10% of the droplets should exceed 100 microns. Naled requires nozzles capable of delivering droplets in the 30-80 micron range. Damage to automotive paint finishes have occurred when larger droplets were dispensed or when more than 10% of the droplets exceeded 100 microns.

g) To be effective against adult mosquitoes, at least 10 droplets per square inch are required. This evaluation can be made by using oil-sensitive dye cards or by glass slides.

h) Aerial spraying is usually performed in the early morning because of visability and safety factors. The temperature should be less than 80°F (27°C). the wind velocity below 10 miles per hour, and rainfall should not be imminent.

III. Helicopters

Helicopter ULV application is permitted by the malathion label; however, helicopter ULV applications have not been routinely used by mosquito control agencies because of the highly specialized nature of the pump-nozzle apparatus. Technical manuals for the insecticide, the nozzle, and aircraft must be consulted for the correct calibration and operational data.

APPENDIX V: Cholinesterase Determinations

The cholinesterases are enzymes which catalyze the hydrolosis of acetylcholine, the neurohormone of the nervous system. There are two types of cholinesterases. The first type is acetyl or true cholinesterase. This enzyme is found in the nervous tissues of all animals and in the red blood cells. The second type is found in smaller amounts in the nervous system of all animals and in the blood serum.

Many methods for the determination of these enzymes are available and are based on the rate of disappearance of acetylcholine or the rate of formation of acetic acid. The most widely used and reliable laboratory cholinesterase procedure is the Michel electrometric method (Michel, 1949). The procedure is based on the determination of the rate of production of acetic acid from acetylcholine by measuring the change in pH of a buffer with a pH meter. The washed red blood cells are incubated in a barbiturate buffer at pH 8.0. The pH is noted at zero time and at the end of a definite period of time. The cholinesterase activity is calculated from the difference in the 2 pH values and is expressed as Δ pH/hr at 25°C. The plasma cholinesterase is determined in the same way except that a higher acetylcholine concentration is used and the buffer is more dilute to compensate for the inherently weaker activity of the plasma enzyme.

Another more elaborate procedure is the titrimetric method of Nabb (1967). The principle is to titrate the acetic acid liberated during the hydrolysis of the choline ester with standard alkali. This method requires an automatic recording titrograph. The feasibility of the method requires considering the expense of the equipment and the time needed to learn to use the instrument.

In the field a procedure is needed that will permit rapid screening of a large number of samples so that persons with significantly low cholinesterase levels may be found quickly. Such methods should be easy to perform and should require only simple equipment with comparatively few manipulations and reagents. The tintometric method described by Limperos and Ranta (1953) and modified by Edson and Fenwick (1955) fulfills such requirements. This procedure is based on the determination of the production of acid from acetylcholine by using an indicator to measure the change in pH. The method has been improved by modifying the kit that is commercially available for the tests. This change resulted in greater accuracy and simplicity. The kits include a comparator which consists of 8 colored-glass standards. Acetylcholine perchlorate (substrate) is added to blood from a person with "normal" cholinesterase

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Michel, H.O. 1949. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. J. Lab. Clin. Med., 34:1964.

Nabb, D.P., and F. Whitfield. 1967. Determination of cholinesterase by an automated pH-Stat method. Arch. Environ. Health 15:147.

Limperos, G., and K.E. Ranta. 1953. A rapid screening test for the determination of the approximate cholinesterase activity of human blood. Science 117:453.

Edson, E.F., and M.L. Fenwick. 1955. Measurement of cholinesterase activity of whole blood. Brit. Med. J., 1:218.

activity. Bromo-thymol blue is used as indicator. Substrate is then added to the other blood samples at 1-minute intervals. The time required for the control reaction mixture to match the standard glass marked "100 percent activity" is noted. Usually this time is 20 to 30 minutes; the time of incubation is decreased at higher temperatures and increased at lower temperatures. At 1-minute intervals, after reading of the control, the remaining samples are read by matching against the disc in the comparator. This method is of great value for field determinations of whole blood cholinesterase activities provided that CO_2 is not introduced during collection and analysis of blood samples. The cholinesterase activities determined by the tintometric method closely agree with the plasma values obtained by the Michel method.

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APPENDIX VI: Technical Information and Assistance Sources

I. Status of disease problems

Center for Disease Control

Bureau of Laboratories

San Juan Laboratories
GPO 4532
San Juan, Puerto Rico 00936

Vector-Borne Diseases Division
PO Box 2087
Ft. Collins, CO 80522

Bureau of Epidemiology

Viral Diseases Division
Atlanta, GA 30333

Bureau of Tropical Diseases

Vector Biology & Control Division
Atlanta, GA 30333

DHEW, PHS Regional Offices, Division of Preventive Health Services

State Health Departments, State Epidemiologist

II. Mosquito control

Center for Disease Control, Bureau of Tropical Diseases, Atlanta, GA

State Health Departments, Vector Control Specialist

Agriculture Extension Agents

III. Aerial ULV spray applicators

Listing of applicators: U.S. Department of Agriculture

Center for Disease Control, Bureau of Tropical Diseases

IV. Supplies

Disposable bioassay cages (6" disposable mosquito cage kit, 3 rings each)

Tubes and Cores, Inc.
400 Paul Avenue
San Francisco, CA 94124

Silicone solution for coating microscope slides used in ULV aerosol sampling "DRI-FILM* SC-87"

Pierce Chemical Company
Box 117
Rockford, IL 67105

*Trademark General Electric Company

Teflon*-coated microscope slides for ULV aerosol sampling

Holiman Equipment Company
PO Drawer 3768
Jackson, MS 39207

*Trademark E.I. duPont deNemours & Co., Inc.

Oil-sensitive cards for aircraft spray evaluation

Home & Farm Chemical Company
PO Box 6055
Charlotte, NC 28207

Windgage

Windmeter (Catalog No. 63-193109-00)
Dwyer Instruments, Inc.
Michigan City, IN 46360

Mention of trade names and commercial sources does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

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Arnell, J. H., and L. T. Nielsen. 1972. The varipalpus group of *Aedes (Ochlerotatus)*. *Contributions Am. Entomol. Inst. 8*(2):1-48.

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