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DENGUE

Vector biology, transmission and control options
in Mexico

by

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SUMMARY

The importance of dengue as a national public health problem is described. The present state of knowledge on the biology and behavior of the vector, the Aedes aegypti (L) mosquito is also presented. Emphasis is placed on the relationship between the biology of the vector and the virus transmission mechanisms, and the different types of control currently employed around the world, chiefly chemical and biological, are reviewed. Based upon the technological panorama of vector control, research options are suggested, to generate information leading to establishment of integrated A. aegypti control in Mexico, with its own characteristics, maximum efficiency and minimum dependence on insecticides.

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INTRODUCTION

The mosquito Aedes aegypti (L) (Diptera: Culicidae) is important in medical entomology because it is a pantropical vector of viral diseases such as yellow fever, dengue and dengue type hemorrhagic fever (Mattingly, 1969). In the urban areas of Southeast Asia, the Western Pacific and practically the entire American Continent, the children are exposed to one or more of the four serotypes of the dengue virus and can develop hemorrhagic fever from dengue; this latter will be fatal to some of them unless they receive adequate medical care. The syndrome appeared in Cuba in 1982, when 344,203 cases and 158 fatalities were officially recorded (Gratz, 1985). It appeared in Brazil in 1986, with 300,000 cases of the disease reported (López-Antuñano, personal letter, 1986).

As of 1963, A. aegypti had been eradicated in Mexico, but it reappeared in 1978, the first case of dengue being reported in Chiapas. By 1983, the disease extended to 23 states, including the country's largest metropolitan areas. The annual peak infection period is during October and November, following the rains, with local epidemics cropping up from March to June. In 1985, a total of 17,706 cases of classic dengue were reported throughout the country (Koopman, 1986).

In this paper, we present a panorama of the vector's biology, the means by which the dengue arbovirus is transmitted, and current research on its control. In conclusion, we include some ideas for future research thrusts for establishment of integrated

vector control of the A. aegypti mosquito in Mexico.

I. ORIGIN AND ECOLOGY OF DENGUE AND BIOLOGY OF ITS VECTOR

Many of the viruses which cause diseases in humans were in existence before the appearance of Homo sapiens (Matsumoto, 1985). These viruses survived by maintaining transmission chains in various species of wild animals, infecting man or his predecessors as soon as they appeared on the evolutionary scene some 10 million years ago, starting with Australopithecus.

There are about 500 formally recognized arborviruses (Karabatsos, 1985), and we know or suspect that more than 200 of these are transmitted by mosquitoes; these include the etiological agents of frequently studied diseases such as yellow fever, Japanese and San Luis encephalitis and dengue. Most of these viruses are originators of zoonosis, and man is infected only tangentially, having no primary participation in the basic transmission cycle which perpetuates the virus. The fact that today's arborvirologists use a variety of animal species as well as culture of mosquito cells (A. albopictus and Toxorhynchites spp) for laboratory production of the virus (DeFoliart et al 1987), is an indication that it can exist in nature without being associated with man. In fact, it has been found that most of the monkeys which inhabit the Malaysian jungles have high concentrations of dengue-neutralizing antibodies (Rudnick, 1965). Based upon this, and though there is no conclusive evidence, many specialists accept the hypothesis that

dengue infection is widely distributed, even in the absence of its vector, the a. aegypti mosquito.

A. aegypti originated in Africa, and having spread around the world, is now universal. The spread of its original area of dispersion, like that of other insects harmful to man, grew in tandem with technological development in transportation means and increased international commerce during the second half of the nineteenth century. It is considered to have been originally of sylvan (forest) habitat, becoming adapted later to the domestic and urban environment of modern times.

The newly emerged adult spends its first 24 hours at rest, sitting on shady walls or vertical surfaces which are closest to the breeding ground. It then initiates a period of short flights in search of the opposite sex to mate, and of a host to provide it with nourishment. There is no particular order of mating and feeding. During the rest period, a partial follicular development takes place, the completion of which, in most mosquitoes, is subject to the ingestion of blood (anautogenesis). The female, whether inseminated or not, ingests the same quantity of blood; between 2 and 3 μ l on the second day of adult life (Klowden, 1979). This quantity is regulated by the insect's abdominal proprioceptors (Gwadz, 1969). The female orients herself by detecting the heat, carbon dioxide, and in particular, the lactic acid given off by the potential host, her search behavior commencing at between 24 and 72 hours of age. During this period the female's response increases gradually, stabilizing itself at 97 hours, and remaining so

stabilized until the death of the insect (Davis, 1984).

Insemination of the females under laboratory conditions produces autogenesis (not requiring food in order to lay eggs) and monogamy, and acts as a stimulus for oviposition. Acoustic communication is the determinant of mosquitoes' reproductive behavior (Roth, 1948). The male A. aegypti locates the female at between 15 and 24 hours of age, but displays the greatest attraction to sound when the greatest fertility has been attained, between the second and fifth days of age (Ikeshoji, 1985). A recently emerged (24 hours) young female produces a low frequency sound and is not pursued by males, but a mature (over 48 hours) female, once having fed, produces higher frequency sounds (700 to 800 hertz) and is strongly attractive to the male.

The females possess an ovary with meristic ovarioles (Postlethwait and Giorgi, 1985). After a blood meal there is a drop in the levels of the juvenile hormone (HJ) (Shapiro et al, 1986) and the neurosecretory hormone (HNDH) for development of eggs is released. This hormone is produced in the brain and released into the heart in response to ingestion of blood (Greenplate, 1985), and brings about development of the oocytes in the ovary. On the other hand, the host searching behavior is inhibited by a factor which circulates in the hemolymph and which is liberated from the vitellogenic ovary, which in turn receives a signal from the fatty body (Klowden et al, 1987). This takes place simultaneously with a depression in the sensitivity of the neurons to lactic acid. The maximum inhibitory effect on the search for a host occurs approxi-

mately 24 hours after the blood meal, at which time the sensitivity to lactic acid is almost ten times lower than in fasting females (Davis, 1988). On the other hand, there is an antigonadotropine or hormone which inhibits development of the egg (oostatic hormone) in A. aegypti. When injected into females, this hormone inhibits the formation of the vitellus and biosynthesis of vitellogenine. Activity of the hormone in the ovaries increases rapidly after the blood meal, reaching a maximum in 48 hours, and while it does not block liberation of the HNDH hormone, it does inhibit biosynthesis of alkaline proteases and trypsin in the middle intestine, which substances drop to a minimum after 55 hrs (Borovsky, 1988).

As to the feeding process, when landing on a host and piercing the skin, a female is stimulated to feed by the cellular portion of the blood; but she is stimulated by adenine nucleotides (ATP), which, in order to exert its effect, must in turn be dissolved in an isotonic solution with a sodium carbonate acid buffer (Galun, 1975), though albumin frequently strengthens the phagostimulant effect of the ATP. Adenylmethylenediphosphate (AMP-PCP) and adenyl-limidophosphate (AMP-PNP), for example, are three to five times more effective than ATP as phagostimulants for the female A. aegypti (Galun, 1987).

II. TRANSMISSION OF THE DISEASE

Vector competence, or capability, is defined as the product of all factors which play a part in producing an infection in the vector and enabling it to transmit the infection of the host (WHO,

1972). In other words, it is related to the capability to permit reproduction of the virus and to the total quantity of viral particles. It represents an operational method to evaluate the relative importance of a specific vector with respect to a given disease. Vector capability includes the following interactions: 1) physiological and biochemical factors which determine the susceptibility of an arthropod vector to be made infectious with a pathogen and 2) ecological factors such as population density, longevity, dispersion, flight range, host preferences and feeding patterns, which determine the probability of a successful contact with the host (Metcalf, 1975).

It has been found that the virus' susceptibility to oral infection varies among the different geographical populations of A. aegypti, but the susceptibility patterns are the same in the four serotypes of the virus. During the period 1960-1970, a worldwide effort to eradicate the insect was made. Many countries, including Mexico, were declared free of A. aegypti, but with cessation of the eradication program, the mosquito reinfested Mexico. A study was conducted to analyze the genetic differences among populations coming from the six geographic regions and between two subspecies, including the northern zone of Mexico. The population from this latter region is genetically very similar to the populations of the Caribbean, and suggests that it may have been introduced from the islands, rather than by colonization from the USA or South America. This is an interesting point, as the recent epidemics in the Caribbean suggest a potential hazard to Mexico (Powell et al,

1980).

The blood ingested in a mosquito bite is deposited in the posterior mesenteron, which consists of a layer of epithelial cells surrounded by a porous membrane of several layers (basal membrane). The viral particles invade the epithelial cells and reproduce there, passing then to the hemocoele (Figure 1). The time which elapses between the blood meal (infection) and viral transmission is called the extrinsic incubation, and may last until the mosquito is 4 or 5 days old or up to two weeks old (Hardy et al, 1983). Thus, high concentrations of dengue viral antigens have been detected in the nervous tissue of A. albopictus, as well as in its ventriculus, fatty body, salivary glands and ovarian covering six days after feeding. Under laboratory conditions it has been found that 43% of A. aegypti mosquitoes infected orally, transmit detectable quantities of the dengue virus after 15 or 16 days of incubation. This is related to the quantity of tissue infected in the salivary glands; this susceptibility is apparently hereditary (Gubler et al, 1979). It was demonstrated in a recent study that dengue virus, serotype 2 (DEN 2) was transmitted to monkeys only by A. aegypti females maintained at about 30°C. The extrinsic incubation period was 12 days for mosquitoes maintained at 30°C, and dropped to seven days when they were kept at 32 to 35°C. These results imply that variations in the A. aegypti efficiency induced by temperature might be determinants in the annual cyclic pattern of dengue hemorrhagic fever epidemics in Bangkok (Watts, 1987).

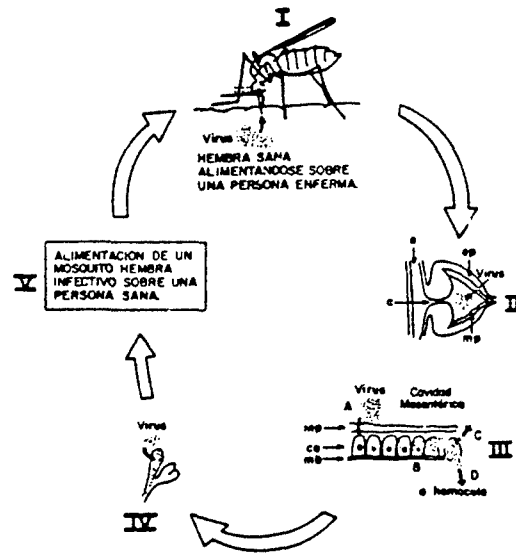


Figure 1. Transmission cycle of the dengue virus (DEN). The extrinsic period of the virus comprises Phases I, II, III and IV. The virus is absorbed together with the blood of a diseased person and by the stomodeal route (e), penetrates the middle intestine or mesenteron, through the cardia (c). In the mesenteron, the virus penetrates the peritrophic membrane (mp) and invades the epithelial cells (ep, ce), where it reproduces until it breaks them and spreads into the mesenteron (C) and hemocele (D). A septicemia is thus produced, invading the salivary glands (IV), and is finally passed to a healthy person through horizontal transmission. mb = basal membrane.

I- HEALTHY FEMALE FEEDING ON A DISEASED PERSON; II- ; III- Mesenteric Cavity; IV-; V- INFECTIOUS FEMALE MOSQUITO FEEDING ON HEALTHY PERSON.

Now, under natural conditions, endemic dengue in different regions of the world is related to the hibernation mechanisms of the arborvirus. The hypotheses advanced to explain how the arborvirus survives during the winter, that is, when the host mosquito is inactive, can be divided into two categories; those which suggest that the virus hibernates locally, and those which hold that the virus is reintroduced into the locale every year (Rosen, 1987a). Ascertaining how and where the virus hibernates is

a factor of great importance in controlling the disease.

The oldest hibernation theory is that of Carlos Finlay (1899), who hypothesized that the yellow fever virus might hibernate through vertical transmission, remaining during any metamorphic stage of the insect: egg, larva, pupa or adult. In A. albopictus, males experimentally infected with the four serotypes of the dengue virus transmit the virus sexually to the females. This transmission is enhanced when the females feed between two and seven days after mating. The males also infect the F1 with the virus. Infected offspring are found among those individuals hatched from eggs laid 72 hours after mating or later, but not in those hatched from eggs laid prior to that time. This suggests that the virus is not transmitted directly to the egg, but after it is reproduced in the genital tract. A. albopictus females experimentally infected with serotype 1 do not transmit the virus sexually to the males. These results support the hypothesis that the males naturally infected with the dengue virus acquire their infection vertically (Rosen, 1978b). By vertical transmission, we mean that the virus is transmitted by the female to her offspring through the egg, in addition to transmitting it through feeding when she bites a healthy host (human, monkey, etc.); horizontal transmission, on the other hand, refers exclusively to the second process, that is, the case in which the virus is transmitted when the female feeds. A recent explanation of the relative rarity of vertical transmission of the flavivirus in mosquitoes (DEN) is based upon entrance of the virus into the already formed egg through the micropyle at the time

of fertilization. It is inferred from this that the transmission is vertical, but not transovarial as occurs in nature (Rosen et al, 1987c). Also, the dengue virus (DEN) has been isolated from mosquitoes captured in the field (Cornet et al, 1984), as well as from larvae collected in the field or from adults bred from these larvae (Khin and Than, 1983; hull et al, 1984).

Since the epidemiology of mosquito-transmitted diseases is intimately related to the natural feeding habits of the vector, the development of techniques capable of evaluating the feeding backgrounds of these insects has attracted considerable interest. In the absence of transovarial (vertical) transmission, a female mosquito must feed at least twice to acquire and transmit a pathogen. Consequently, the methods used to determine the number of gonotrophic cycles which have occurred in the insect, or its physiological age, constitute the key factor in ascertaining the present age of natural populations, and thus, the possibilities of infection. These estimates assume that a normal gonotrophic cycle consists of blood feeding to repletion (satiation), being followed by the processes of digestion, oocyte maturation and oviposition. However, serological studies of the blood being digested in the mosquito's stomach show a tendency toward multiple feeding. In other words, they show stomachs filled with blood coming from two or more bites, wherein the second has been added before the blood ingested during the first feeding has been digested sufficiently to prevent its serological identification. A possible explanation of these interrupted meals might be the defensive behavior of the

vertebrate hosts, which exerts a significant influence, and even induces the mosquito to feed on a less defensive host. Thus, these observations on multiple feeding and defensive behavior of the host suggest that the estimates of vector capability based on the concept of a single host per gonotrophic cycle might be underestimating the insect's true potential as a vector. Seasonal periods which are accompanied by large mosquito populations are characterized by explosive outbreaks of dengue, in clear disproportion to the insect density. The above inference is based upon studies carried out under laboratory conditions. A mosquito bred with a poor larval diet (simulating a water catchment with a low content in organic material) is smaller in weight and size than any mosquito bred with a rich larval diet, and the tendency of the A. aegypti, one hour after the first partial feeding, to attempt once again, to feed on the host, is a function of the size of this meal, which in turn, depends on the bodily dimensions. It has been calculated that the volumes of blood ingested in which 50% of the mosquitoes do not return to the host, are from 1.6 to 2.5 μ l for small mosquitoes and from 2.6 to 4.5 μ l for large ones (Klowden and Lea, 1978). In addition to this, it has been found that the small females are not well represented in the population of hungry females, which suggests that the larger individuals are more successful in obtaining blood meals and in reproducing (Nasci, 1986). Likewise, these small females, even though well nourished with carbohydrates, exhibit a minimal host-seeking behavior, as compared to those who were bred with a rich larval diet (Klowden et

al, 1988). On the other hand, a large proportion of pregnant females continue to look for a host (Klowden, 1986) after the blood meal, and maintained in the absence of saccharose.

On the other hand, the lack of a virus to initiate infection of the mesenteron (middle intestine) of the mosquito, once the blood has been ingested, is related to various biophysical and biochemical factors. These factors may operate in the mesenteric lumen or on the surfaces of the microvilli membranes of the epithelial cells. All insects have a thin coating of chitin (nitrogenated mucopolysaccharide) with a high chemico-mechanical resistance which protects the mesenteric epithelium. This is known as the peritrophic membrane. The pore diameter of this membrane might explain differences in the susceptibility of mosquitoes to arborviral infections (Chamberlain and Sudia, 1965). Another barrier to viral invasion is the enzymatic complex which exists in the stomach. Some arborviruses are rapidly deactivated by proteases, chemotrypsin and trypsin. Differences in the concentrations of these can affect susceptibility to infection. Some researchers operate on the hypothesis that viral absorption in the epithelium is a function of the distribution of positive and negative electrical charges on the surface of the cells. This distribution is affected by changes in the stomach pH, which are common at the individual level in any species of mosquito (Marker and Jahrling, 1979). Finally, there are reports in the literature of what is known as permeable mesenteron ("leaky midgut"); On very rare occasions, fortunately, the presence of viral particles has

been detected in hemolymph samples a few hours after ingestion of infected blood. If these observations are valid, though, it can be inferred that the virus infiltrates the epithelium intercellularly to the lumen, without going through the reproductive process in the epithelial cells (Hardy et al, 1983).

III. CONTROL

Dengue is the most frustrating of all the arboviral diseases because, while it is apparently vulnerable to simple control methods, the disease continues to break out and recur throughout the world (DeFoliart et al, 1987).

In a recently published study on epidemiological surveillance of dengue in two Puerto Rican populations, Waterman et al (1985) reported that a greater incidence of the disease and the continued presence of viral antibodies are significantly associated with the lower social strata. The chief incidence occurs in those persons whose dwelling areas had trees over 7 m high, outside privies, domestic animals, no mosquito netting on windows and doors, and houses of wood construction. On the other hand, given the diagnostic difficulties, the number of cases officially reported in any area is a multiple of the real number. During the 1977 epidemic in Puerto Rico, it was reported that, even with good organization of dengue prevention programs, it took 20 days from outbreak of the epidemic to implement an efficient counter-virus response capability (Morens et al, 1986).

A. Chemical Control

Rapid intervention to eliminate some viral epidemics, specifically with dengue, requires a good knowledge of the sensitivity of A. aegypti to the various products which must be used. In 1976, the World Health Organization (WHO) suggested that there were two regions where A. aegypti resistance and the need for alternate insecticides are of special interest. In America, the attempt to eradicate this species has brought about an almost universal resistance to the organochlorated and organophosphate pesticides. In Southeast Asia, where hemorrhagic dengue is an ongoing threat, resistance to the organochlorates was so generalized that they were not usable for combatting A. aegypti. Control of this vector was accomplished through use of a variety of organophosphorated compounds. But, in 1980, the WHO reported a resistance of this species to the following organophosphorates: Malathion, Phenitotrion, Phenthion and Temephos (Abate). These were distributed widely in the Caribbean and neighboring countries and Malathion and Temephos in a number of Asian countries.

The use of diagnostic doses to simplify test execution should permit a more rigorous surveillance of the resistance of this mosquito to insecticides, particularly the organophosphorated compounds, which are the most widely used. In this connection, the WHO (1976) indicated the probability that changes in the regression line, due to incipient resistance, are manifested with high dosages. Resistance indices should therefore be sought in this sector rather than in the change in LC_{50} (mean lethal concentra-

tion). It was thus recommended that systematic surveillance tests for early determination of resistance should be carried out using concentrations capable of killing all susceptible insects. Thus, in Cuba, Tang Chiong et al (1985) reported that the A. aegypti stock is susceptible to Temephos and Phenthion, with a diagnostic dosage of 0.03 mg l⁻¹ of both insecticides. With respect to Malathion as an adulticide, a study was conducted in New Orleans, Louisiana, USA, to detect susceptibility of A. aegypti to Malathion, DDT, Permethrin and Resmethrin. Larval tests were conducted in accordance with the standard WHO method, and the four field population centers tested yielded results showing high susceptibility to Malathion and Resmethrin and high resistance to DDT. Resistance to DDT was lower in the forest tree hole population than in the domestic population. The LC₅₀ of Malathion to susceptible A. aegypti larvae is 0.1 ppm (part per million) (Brown and Pal, 1971) and the diagnostic concentration to determine resistance on larvae of the vector to Malathion is 1.0 ppm (WHO, 1981). In these tests, there were no survivors when concentrations above 0.625 ppm were used (Beard et al, 1985). In spite of all this, the effectiveness of Malathion has not been proven. The efficiency of sequential applications of aerosol at ULV (ultra-low volume) at a dose rate of 48 ml active ingredient (AI) per hectare, from a LECO-HD fogging machine mounted on a trailer drawn at 16 km/hr was tested in New Orleans as an emergency measure for control of A. aegypti adults. The machine was calibrated manually to produce a droplet having a mean diameter of 10 to 15 µl. Each application

required 1.5 hours, and two were administered daily, at 6:00 A. M. and 5:30 P.M.. The operation continued for 5.5 days and the mean adult capture and oviposition rates during the period of administration were 73 and 75%, respectively. But, one week later, the density of adults once again reached the initial level (Focks et al, 1987). These authors assume that the oviposition rate was not reduced completely because the females with developed eggs remained hidden during the treatment or tolerated the insecticide. Also, a simulation model using the results obtained suggests that, under conditions of crowded housing and abundant vegetation, a single application of aerosol will kill an average of 88% of the males and only 30% of the females present.

In another earlier study, the same insecticide applied in the same manner and only twice a week, only reduced the A. aegypti densities by 29% (Focks et al, 1986). Also, contradictory results have been reported in other cases. A five-year study in Puerto Rico, which monitored adult population by means of human bait one day prior to, and four days after the application, evaluated the effectiveness of Malathion to be 96% when applied at ULV with a fogging unit, at a dose of 7.57 l/hectare. There were no consistent changes in the number of mosquitoes attracted to the bait during the days of the application itself, and so the statistical analysis did not indicate any significant affect of the spraying on the number of mosquitoes observed (Fox and Specht, 1988). Finally, in a study carried out in Surinam, applying the insecticide at 95% at ULV, it was demonstrated that the spraying campaign had no

apparent effect on the A. aegypti infestation index or on the incidence of dengue (Hudson, 1986). And in Trinidad, Malathion applied with a ULV fogger mounted on a truck, and with a dose of 460 ml/km. proved to be even less efficacious against the A. aegypti population than in Surinam (Chadee, 1985).

Based on the foregoing, the literature indicates that Malathion at ULV is effective against A. aegypti in certain situations, depending upon the dose, application technique and frequency of treatment (Pant et al, 1971). A treatment at ULV, using doses approved in the USA, should kill at least 70% of the mosquitoes in a 91 m radius; if this mortality does not occur, another insecticide should be used (CDC, 1977). There is also disagreement on the degree of correlation between the recorded population of adults and the cases of dengue (Moore et al, 1978). In concluding this section, we might mention some research which is currently being conducted in the search for insecticides of natural origin which have a potential for chemical control in the immediate future. The Antelea azadirachta (L), a tree native to India is a source of a large number of polycyclic triterpinoids, which have an antinutritional, or growth regulatory effect on insects. The compound being studied the most is azadirachtine, which strongly limits the growth of the adult A. aegypti when applied to fourth stage larvae, as well as demonstrating insecticidal effects on adult mosquitoes (Zeibitz, 1986). More recently, it has been reported that extracts of the mature fruit of the African tree Melia volkensii (Gurke) inhibit the growth of A. aegypti larvae as

well as producing high mortality rates. There is speculation that the active compound of this plant is more potent than azadirachtine (Mwaugi and Rembold, 1988).

B. Biological Control

Although the most promising biocontrol agents for vector mosquitoes, whether culicides or simuliocides according to WHO experts (1987), are the entomopathogenic bacteria Bacillus thuringiensis, serotype H-14 and B. sphaericus. These microorganisms can operate only against vectors which breed in large bodies of water, such as marshes, ponds and rivers, which makes them undesirable as a practical matter, for control of vectors which breed in temporary water collectors, such as tires, pails, cans, etc. in the city and rock hollows, tree trunks and plant crotches in the country, as is the case with A. aegypti. These bacteria offer a potential for the control of those vectors which have already developed a resistance to organophosphorates, such as the oncocercosis vectors in Africa. B. sphaericus has shown itself to be very effective against Culex quiquefasciatus, which is a vector of filiariasis. Experimental formulations have proven effective for up to 10 weeks in habitats infested with this vector (WHO, 1985).

On the basis of the foregoing, it can be said that the Bacillus species act as biocides, and in this context they are comparable to insecticides. Chemical control of the immature stages of A. aegypti requires that field personnel locate a high

proportion of the breeding receptacles within a given area, and antimosquito campaigns are thus expensive, and often only partially effective.

On the other hand, another natural enemy of A. aegypti is represented by all species of Toxorhyncites, which are depredatory in the larval stage, while the adults feed on vegetable nectar and secretions (Steffan and Evenhuis, 1981).

The attractiveness of using this depredatory mosquito as a biocontrol agent of A. aegypti is not only its potential for use without insecticide, but also the labor savings associated with the ability of the females to find breeding receptacles faster and more frequently than antimosquito personnel can neutralize them (Focks, 1985). In New Orleans, Louisiana, USA, A. aegypti control is currently being carried out through combined use of chemical and biological control. The former consists of ULV application of malathion, and the latter, of releasing 100 pregnant females of T. amboinensis (Doleschall) per block, every week, both in urban zones. Of the two types of control the second is the more effective. It has been demonstrated experimentally that the depredatory activity alone reduces vector populations by up to 70%, while chemical control only reduces it by 26%; working together, they achieve a 96% reduction in the A. aegypti population (Focks et al, 1986).

Other natural enemies which have been discovered to attack A. aegypti larvae are the copepods of the Cyclopidae family. Hurlbut (1935) was the first to find a copepod as a mosquito larva

depredator. Referring specifically to A. aegypti, Suárez et al (1984) were the first to report the copepod Mesocyclops aspericornis (Daday), depredating immature stages in artificial breeding receptacles in Colombia. The copepod grasps the larva by the back; gnawing vigorously at the larva's body with its mouthparts, it tears off pieces of the muscular tissue and pushes them into its throat. This species has also been reported in Venezuela and the islands of Guadeloupe and Martinique (Dussart, 1982 and 1984).

More recently, in French Polynesia, a study was conducted with application of M. aspericornis into recesses in the shell of the Cardisoma carnifex crab and in trees, as well as in tires, pails and wells. The larval populations of A. aegypti and A. polynensis were successfully reduced by from 90 to 95%. The copepod remained in the crab recesses for 29 months and for up to 60 months (five years) in some artificial receptacles. On the other hand, since the copepod did not reduce Culex populations efficiently, suspensions of it were mixed with B. thuringiensis, israelensis strain, without harming the copepod and successfully killing the larvae of both mosquitoes. This makes the M. aspericornis a good candidate as an A. aegypti biocontrol agent, by reason of its oligophagous nutrition pattern, its ease of massive breeding and collection, its high fertility at temperatures of 23 to 30°C and a salinity of from 0 to 4%.

The copepod attacks the A. aegypti because it does not float and the larvae of this mosquito are bottom feeders, while the larva of the Culex are surface feeders (Rivière et al, 1987).

C. A. aegypti control through use of the sound of the wings

Ikeshoji (1981) conducted a study with male insects captured with recordings of the wing sound emitted by females of four different species. The sound trap consisted of a 100 ml glass, in which a speaker of 5 cm diameter was placed. The wall of the glass was covered with "stickum" and the sound had an intensity of 10 dB, and was emitted for 15-minute periods at different times of day. The trap used for A. aegypti emitted a sound of 446 Hz, which is the average frequency of the cage, and as a result only 15.3% of the females in the cages were inseminated. On the other hand, when the number of periods with sound was reduced from 48 to 24, 12, 6 and 1 per day, the insemination rate of the females also gradually increased from 15.3 to 20, 40, 55 and 77%, respectively.

The same author carried out extractions with hexane from the body of the females, and added them to the sound traps without observing any additional response. He concluded that no pheromone is included in the sexual attraction mechanism of this species, and he demonstrated the potential of the use of wing sound from the control viewpoint. In a more recent study, using a frequency of 460 Hz and 100 dB at 0 cm from the speaker, the same researcher found that 50% of the A. aegypti males which responded to the sound, had the capability to inseminate the females when confined with them after being caught by the sound, which is also an indicator that the acoustic trap may be an effective means of control (Ikeshoji, 1985).

IV. PROSPECTS FOR INTEGRATED CONTROL OF A. AEGYPTI in MEXICO

The concept of integrated pest management is based on a philosophy of coexistence. Many pest management techniques use non-lethal mechanisms and successfully control the pest population. It is necessary to stress here that the goal is precisely not to kill or eradicate the vector, because it is well established throughout the continent and would immediately be reintroduced. Eradication is not recommended even in the United States, where there are sufficient resources to accomplish this (Gratz, 1985).

In view of this and in the light of the current literature on A. aegypti, we propose certain research thrusts which might be developed in Mexico to generate a suitable management technology. Its purpose would not be eradication, but epidemiological control of the A. aegypti dengue vector, with minimum dependence on the use of insecticides and maximum efficiency from the control viewpoint.

A. Chemical Control

It is evident from the literature that the use of ULV Malathion as an adulticide form of control is a mistake. Furthermore, as has been demonstrated in a number of countries, its use could easily generate resistance in A. aegypti. Faced with the risk of resistance and an inconsistent degree of control, it makes sense to eliminate it as a control tool in integrated management of this vector, except where it has been field evaluated at precisely those points in the metropolitan areas where dengue outbreaks occur. This is proposed because until now, the national anti-

mosquito campaign has been limited to application of this poison without quantitatively evaluating its effects on the vector adult population. On the other hand, in comparison with Malathion, fewer outbreaks of resistance have apparently been caused by using the larvicide, temephos (Abate). If the specialists consider temephos to be the best larvicide in the world, it should be accorded good toxicological management to ensure continuity in the degree of control, without producing resistance.

An optimum toxicological use of temephos would necessarily have to consider the extrinsic period of the dengue virus in the mosquito, that is, its duration. Sublethal doses of temephos should be studied with respect to their effects on longevity and fertility of the adult. If a dose is applied which doesn't kill the mosquito, but cuts its lifespan to four days and significantly reduces its fertility, this would create a desirable situation from the integrated point of view. In addition to reducing the costs of insecticides, it would reduce the probability of generating resistance, since the susceptible genotypes would not disappear from the population.

Sublethal doses affect mating, host-search, feeding and other behavioral patterns, to the extent that this chemical control approach might offer new control options. For example, reduced oviposition might indicate one or more different effects, including repercussions in locating the opposite sex, mating, oviposition itself and associated physiological events, such as spermatogenesis, sperm mobility, oogenesis, ovulation and fertilization of the

egg (Haynes, 1988).

For the case of the hematophages, a close relationship has been reported between feeding and subsequent reproduction. Duncan (1963) reported that when A. aegypti was treated with a sublethal dose of Dieldrin after feeding, the first oviposition cycle was normal. Subsequent feedings were reduced, and as a consequence, oviposition was diminished in the later cycles. On the other hand, when the females were treated prior to their first feeding, very few eggs were laid. Feeding is the result of many ethological components, such as finding the host, landing on the host, and acceptance by the host. There is no information available in the literature on this subject, which we are pursuing at present in our laboratory, with respect to temephos. When insecticide applied at sublethal doses exerts strong effects on the insect's behavioral repertoire in a manner such as to permit derivation of control effects, it is called an insectistatic; a term proposed by Levinson (1975).

B. Biological Control

With regard to biocontrol, many organisms have been discovered and used to act as agents to regulate the A. aegypti population. Apart from the biocides B. thuringiensis H-14 and B. sphaericus, it is most likely and practical to use some native species of the depredatory mosquito Toxorhynchites. The species T. theobaldi has been found to be very common in northeastern Mexico, and has good possibilities as a biocontrol agent, as it lays its eggs in a good

percentage of artificial catchments infested with A. aegypti larvae (Reyes et al, 1987). The advantage in using T. theobaldi is that the female seeks out A. aegypti breeding grounds and lays her eggs there. We have observed in the laboratory that a single larva can devour 25 to 30 A. aegypti larva of its own size in one day. Evaluating the oviposition capability of pregnant females released in urban zones would also be a promising research thrust; their effectiveness would complement the control exerted by temephos. These two types of control are complementary because T. theobaldi would function in shady areas, while the insecticide, in sublethal doses, would be applied in sunny area breeding grounds. The foregoing, reinforced by a campaign of catchment destruction (elimination of stagnant water breeding grounds) in urban and semiurban areas, would constitute an integrated A. aegypti control program in Mexico.

Finally, the entire package would have to be accompanied by a good vector monitoring system, which would permit in-progress evaluation of the control measures employed. Only A. aegypti hungry females are directly included in the transmission of dengue, and so the most realistic method of monitoring hematophagous mosquitoes is to routinely collect mosquitoes attracted to human bait (Service, 1977). Egg trap data aid in making inferences as to the abundance of females that were present, though it cannot be inferred from absence of eggs in the trap that there were no hungry females in the area. Accordingly, the experts recommend the use of human bait as the best method for inferring population data as an

evaluation of some control method, such as for example, the application of ULV Malathion to a dengue concentration area. The use of traps is also recommended, and in this connection, experts of the Atlanta, Georgia Center for Disease Control in the U.S. have design several types, including the CDC trap for pregnant females. This trap operates with a battery-powered motor, which drives a small propeller creating suction, and is also equipped in its lower part with a black colored plastic reservoir. This tank is filled with water, to which an oviposition attractant is added; a 0.2% solution of methyl propionate has proven effective for A. aegypti (Klowden, 1987).

We must work seriously in the national antimosquito campaign (against A. aegypti) because apart from its strong capability as a vector and the lack of medical entomologists in Mexico, we already have at our northern borders the "tiger" mosquito, A. albopictus Skuse, which was introduced from Asia into the southeastern USA. And this mosquito transmits the dengue virus not only horizontally, but, unfortunately for us, vertically, thus constituting another potential threat to public health in Mexico.

[Except for the below items, the bibliography is in English, and does not require translation.]

"Organización Mundial de Salud (OMS)" = "World Health Organization (WHO)".

The two Spanish titles given are:

"Resistencia de vectores y reservorios de enfermedades a los plaguicidas", "Resistance of disease vectors and carriers to pesticides"

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