SPECIAL REPORT*

BIONOMICS OF IMPORTANT MOSQUITO VECTORS IN MALAYSIA

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INTRODUCTION

Diseases such as malaria, dengue, filariasis and Japanese encephalitis are still major problems in the tropics. These are the four important vector borne diseases in Malaysia. The vectors responsible for the four diseases belong to the genera *Anopheles, Aedes, Mansonia* and *Culex*. The present report is a summary of recent findings from studies conducted on the vectors from Malaysia.

MALARIA VECTORS

Anopheles maculatus Theobald is the important vector of human malaria in Peninsular Malaysia and Southern Thailand but it is of no importance elsewhere throughout its range of distribution in the Oriental zoogeographic region. Morphologically *An. maculatus* is a variable species. Studies carried out in Thailand indicate that there is a complex of 6 species, however to date, studies have indicated that only the form E is present in Malaysia (Loong *et al,* 1988).

The other vectors in Peninsular Malaysia are *An. sundacius, An. campestris, An. letifer* and *An. dirus* (Sandosham and Thomas, 1983); however due to limited distribution they are less important. *An. balabacensis* and *An. leucosphyrus* are the important vectors in Sabah and Sarawak, respectively.

Larval biology

Anopheline eggs are boat-shaped with one end blunter than the other. The eggs are provided with lateral floats and are suspended on the surface of water. *An. maculatus* larvae are found in slow flowing water which is exposed to direct sunlight. The larva manages to anchor itself to any rough surface by means of hooked caudal hairs. *An. leucosphyrus* larvae was found in clear seepage pools beside a jungle stream and in swampy patches along the foothills; most pools were well shaded but some received at least one hour's sunlight daily (Colless, 1956a). *An. balabacensis* larvae were found breeding in clear spring water in tiny seepages at the source of streams or along foothills and always in complete shade (McArthur, 1947). Reid (1949) and Colless (1956), on the other hand, recorded that this species was capable of breeding in more open situations although a certain amount of shade seemed to be necessary. In Sabah the *An. balabacensis* larvae have been found breeding in slow running streams (Cheng, 1968), buffalo hoof prints, temporary pools on the margins of stream banks after a dry spell, blocked earth drains in rubber plantations and wheel ruts (Rajapaksa, 1971).

Laboratory culture

Colonization of strains of *An. balabacensis* from Sabah proved extremely difficult to achieve because of the reluctance to feed on man or laboratory animals (Esah and Scanlon, 1966). *An. maculatus* has been successfully bred in the laboratory by artificial mating (Ow Yang *et al,* 1963). The food for the larvae consists of about 300mg of pulverized food comprising a mixture of 300g casein, 30g liver powder, 50g wheat germ, 300g oat, 15 vitamin B complex and 30g dried yeast. Adult females are exposed to white mice for their blood meal (Loong and Cheong, 1986).

Adult biology

Host preference

An. maculatus has long been recognized as

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having a tendency to bite cattle more than man (Wharton, 1951, 1953; Reid 1968; Loong *et al.* 1988). The preference for cattle to man is 2:1 (Loong *et al,* 1990). Studies carried out by Wharton (1951) showed that *An. maculatus* are attracted to both goats and dogs and to dogs more so than man. *An. balabacensis* prefers to bite man more than water buffalo; the ratio being 1.8 : 1 (Hii, 1987). *An. leucosphyrus* is anthropophilic: 78.8% fed on humans while the rest fed on dogs, pigs and fowls (Colbourne *et al,* 1959).

Resting places

In Malaysia *Anopheles* mosquitos in general do not remain indoors long after feeding with the exception of *An. campestris* (Sandosham and Thomas, 1982). *An. maculatus* rests mostly on upper part of walls of houses and on grass stems and bushes around cattle sheds at nights (Sandosham and Thomas, 1982; Wharton, 1951). During the day it is found resting in low vegetation and under trees. It also rests on the outside of the house before entering (Moorhouse, 1965) and it attacks on entering and does not rest on the walls until after feeding (Wallace, 1950; Reid and Wharton, 1956; Moorhouse, 1965). Similarly *An. balabacensis* rests indoors after feeding and exits the same night or early morning (Hii, 1985). *An. leucosphyrus* also rest on the walls before and after feeding and are usually found low down on the walls (Colless 1956a). These mosquitos emerged from their resting places early in the night but spent long hours on foliage around the house before entering to feed (Colless, 1956a).

Seasonal abundance

An. maculatus has shown to exhibit definite seasonal prevalence in studies carried out by Sandosham and Thomas (1982). The numbers increase at the end of March and the begining of April, which is the primary peak, and the secondary rise is in September. The minimum incidence occurs in June-July and January-February. According to Loong *et al* (1988) the density peaks coincided with rainfall pattern but the high infective rate appear to coincide with period of low rainfall. In Sabah work carried out by Hii *et al* (1988) showed that the number of *An. balabacensis* was higher in March compared to November. The seasonal preference of *An. balabacensis* is closely related to the rainfall, because the breeding places are highly dependent on the rainfall pattern.

On Labuan Island, Sabah, Colless (1952) found that monthly catches of *An. balabacensis* were at the peak in July and reached their lowest point during the January-February dry season, the correlation coefficient between catch and rainfall being 0.8. In the inland areas of Sabah, *An. balabacensis* could be found throughout the year and its peak density coincided more or less with the two wet seasons of the year ie the northeast (October-January and southwest (May-August) monsoons. Between the monsoons, small pools dry out and seepages are reduced to small flowing channels. The vector density thus dropped to a minimum during the dry period (Colless, 1952).

In Sarawak it was observed that the numbers were much higher during the wet periods than in the dry periods. This was not due to increased breeding, but due to the favorable effect of higher humidities on adult survival, particularly in day time resting places (Colless, 1956b).

Biting activity

The biting activity of *An. maculatus* occurs throughout the night with a major peak around midnight (between 2200-0100 hours) (Loong *et al,* 1988). Wallace (1948) noted the peak biting time of *An. maculatus* was between 2100 and 2400 hours. The same was also observed by Wharton (1951). Available evidence suggests that for *An. maculatus* the curve of biting activity on man outdoors is said to be similar to that indoors (Moorhouse and Wharton, 1965; Davidson and Ganapathipillai, 1956). *An. balabacensis* bites more outdoors than indoors (Hill *et al,* 1988). The peak biting activity of *An. balabacensis* is much later than *An. maculatrus,* being between 2400 and 0400 hours (Colless, 1956b). For *An. leucosphyrus* the peak biting time was between 2200 and 0400 hours (Colless, 1956b).

Gonotrophic cycle

The duration of the gonotrophic cycle of *An. balabacensis* in the field as determined by mark release recapture experiments was found to be three days (Hii, 1985), while that of *An. maculatus* was found to be 2.3 days (Loong *et al,* 1990; Chiang *et al,* 1991). This may have been due to the availability of breeding sites close to the study area (Loong *et al,* 1990).

A majority of the mosquitos collected on the second and third days after release had 'a-sacs', while only a small proportion of the females returning to feed after the third day had 'a-sacs'. It thus appeared that the majority of the females oviposited two days after a blood meal but a varying proportion returned that night to feed (Loong *et al,* 1990).

Survivorship

The survivorship of *An. balabacensis* was high in Sabah ranging between 0.719 to 0.7874 (Hii and Vun, 1985). It was also similar to that of *An. maculatus* which had a survivorship of 0.71 to 0.761 (Loong *et al,* 1990) and 0.699-0.705 (Chiang *et al,* 1991).

Flight range

The flight range of *An. maculatus* has been estimated to be about 1.25 km (Sandosham and Thomas, 1982). However, later studies showed that the mean dispersal distance estimated was 0.373 + 0.467 km and the maximum flight range recorded was 1.6 km (Chiang *et al,* 1991), *An. balabacensis* has a narrow-ranged dispersion and tends to move freely within the confines of its own forest fringe (Hii and Vun, 1985). McArthur (1947, 1949) first pointed out the strong negative correlation between distance from forest margin and malaria incidence on the Tambunan plains of Sabah. Using McArthur's epidemiological data, Colless (1952) described an indirect method for estimating the effective flight range of *An. balabacensis* which was about 1.5 mile.

Vector parasite relationship and transmission

One of the methods employed to determine the species transmitting malaria is to establish the relative susceptibility of different species under experimental conditions. However there are limitations to laboratory infectivity experiments in determining the relative importance of anopheline species under natural conditions. Results of experimental feedings in the laboratory indicate that *An. maculatus* and *An. sundiacus* are efficient vectors (Hodgkin, 1956).

Vector control

In Malaysia DDT residual house spraying using emulsifiable concentrate at $2g/m^2$ has been carried out as part of the malaria control program since 1967. The present policy is to continue applying DDT (2 cycles per year) as the principal antimalaria measure in malarious areas and in focal spraying as a remedial measure in new foci of malaria (VBDCP, 1988).

There is also strong evidence to suggest that the effect of DDT house spraying reduces man-vector contact through its deterrent and irritant effects rather than through its effect on the vector's longevity (Colless, 1952; Cheng, 1968). Thus small scale field trials have been carried out with other insecticides such as malathion and sumithion in Sabah and results showed that the mortality achieved was nearly 100% in the first month after sumithion spraying and 100% for 3 months after malathion spraying (Hii and Chin, 1979). In Sarawak *An. leucosphyrus* was susceptible to both DDT and dieldrin (Colbourne, 1959).

Laboratory evaluations of cyfluthrin, fenitrothrin, DDT and malathion on *An. balabacensis* showed that cyfluthrin possess excellent toxicity against *An. balabacensis* (Tan and Yap, 1986). Cyfluthrin has also been tested in the laboratory against *An. maculatus* and found to be effective (Vythilingam et al, 1992). Vinicide paint also had good adulticidal effect against *An. maculatus* (Chiang *et al,* 1990a).

Of the many alternates that could be tried, the reduction of man/vector contact by the use of bed-nets has promise but this gives no protection until a person goes to sleep inside the net. A more recent concept of treating bed-nets with an insecticide which would kill mosquitos alighting on the net or entering it and being trapped, thereby not only reducing man/vector contact but also reducing overall vector density has been considered (Hii *et al,* 1987; Loong *et al,* 1985). Field trials carried out in Sabah showed that unimpregnated bednets has much greater numbers of live and bloodfed *An. balabacensis* in them than bednets impregnated with permethrin (Hii *et al,* 1987).

Field tests were also conducted to compare the degree of protection from bites of *An. maculatus* by applying two repellent/insecticidal bars, Mosbar and Moskill to exposed arms and legs. Mosbar was more effective and gave 5 hours of protection against *An. maculatus* (Chiang *et al,* 1990b).

Environmental management, which in the past constituted one of the main methods for the prevention and control of malaria, became of reduced importance with wide-scale application of chemical control measures. Williamson and others attempted various naturalistic control methods including sluicing, flushing, automatic siphon, fascine drainage, herbage packing and larvae eating fish for the control of *An. maculatus* in inland rural areas (Williamson and Scharff, 1936). Some of these naturalistic measures are still in existence and functional in Penang (Jit Singh and Tham, 1988).

A petroleum based oil has been used for over 50 years in the urban areas of Peninsular Malaysia as a larvicide for the control of *An. maculatus* (Thevasagayam *et al,* 1979). Temephos 500E has replaced oil as the larvicide against *An. maculatus* in Peninsular Malaysia since 1975 (Thevasagayam *et al,* 1979).

Studies carried out on the use of *Bacillus thuringiensis* and *Bacillus sphaericus* on *Anopheles* larvae showed that it was not very effective (Foo and Yap, 1982; Cheong and Yap, 1985).

The efficacy of sumithion used in ultra low volume applied to *An. balabacensis* in Sabah gave sustained control for two to three weeks (Hii, 1980). Thus space spraying could be carried out during epidemics to break the chain of transmission of malaria.

DENGUE VECTORS

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are serious *Aedes*-borne viral diseases commonly found in Southeast Asian countries including Malaysia ever since their first description in Penang in 1902 by Skae. Dengue is endemic in Malaysia and is being maintained by the indigenous *Aedes albopictus* before the introduction of the alien *Ae. aegypti* during the turn of the century (Smith, 1956a). In the early days *Ae. aegypti* was found only in the coastal towns (Daniels, 1908; Leicester, 1908). However, by 1920 *Ae. aegypti* had already moved inland and was found in abundance in Kuala Lumpur. By 1990, Lee and Hishamudin found that *Ae. aegypti* has completed its spread in Peninsular Malaysia.

Life history

Studies conducted in IMR insectarium indicated that the females of both mosquitos were ready to oviposit 4-5 days after copulation. Females of *Ae. aegypti* and *Ae. albopictus* laid an average of 102 and 79 eggs/female respectively. In both species, the eggs hatched within 1-48 hours at ambient temperature. The duration of the larval period was 6-8 days, while that of pupae was 1-2 days. The complete life cycle from egg to adult at ambient temperature was 9-10 days.

The combined mortality of eggs which failed to hatch and larval mortality was about 9% for both species. However, pupal mortality was about 8% for *Ae. aegypti* and 4% for *Ae. albopictus.* The longevity of *Ae. aegypti* males was 10-29 days and 12-56 days for females (mean: 19 and 34 days). For *Ae. albopictus,* the female life span was 12-40 days and 10-22 days for males (mean: 26 and 16 days). The sex ratio of males : females was about 1.3 : 1.0 and 1.4 : 1.0 for *Ae. aegypti* and *Ae. albopictus* respectively.

Larval biology

In Malaysia, *Ae. aegypti* is a domestic species and the larvae are found breeding in man-made containers. Cheong (1967) reported that *Ae. aegypti* larvae were found to prefer ant-traps (26.5%) and earthen jars (25.2%) in urban towns. In addition, other artificial containers especially tires and flower pots were preferential breeding habitats. However, 2 decades later it was found that *Ae. aegypti* larvae seemed to prefer miscellaneous containers such as buckets, basins, bowls etc (27.8%) and concrete tanks (21.2%) and no breedings were detected in ant-traps, tires and flower pots (Lee and Cheong, 1987a). These changes in preference may not be attributed to genetic or biologic reasons, but rather to the persistent and constant emphasis of vector control agencies on the risk and potential of these containers as breeding habitats. Recent nationwide larval survey had similar findings (Lee, 1991a).

Ae. aegypti generally prefers indoor artificial containers while *Ae. albopictus* breeds in mostly natural water receptacles found outdoors. In a survey conducted in a rural area it was shown that *Ae. aegypti* was found breeding outdoors in earthen jars (Ho and Vythilingam, 1980). In a nationwide survey in urban towns of Peninsular Malaysia in 1988-1989, Lee (1991a) reported that both *Ae. aegypti* and *Ae. albopictus* were found breeding both indoors and outdoors in a variety of containers. The dominant indoor breeder appeared to be *Ae. aegypti* while both species showed equal preference for outdoor containers.

In ovitrap studies carried out in dengue endemic areas of Kuala Lumpur, it was found that *Ae. albopictus* was more dominant than *Ae. aegypti* as a domestic mosquito species inside premises of houses, shophouses and factories (Sulaiman *et al,* 1991). This finding was contradictory to that found by Yap (1975) where *Ae. albopictus* was found to prefer outdoor ovitraps over indoor ovitraps.

Larvae were found breeding even in partially covered containers. These observations therefore confirmed that oviposition of *Aedes* is not deterred by the presence of partial shade. Water quality was also another important limiting factor affecting breeding. *Aedes* larvae required clear, but not necessarily clean water (Lee, 1991a).

Adult biology

Host preference

Ae. aegypti feeds principally on man (Macdonald, 1956). Host preference, however, did not influence the amount of blood meal taken. More eggs were produced from blood of chicken and guinea pig but not with man or monkey. In the field *Ae. aegypti* is probably dependent on man for its blood meal since its fore-fathers are thought to originate from forms frequenting coastal houses in east Africa.

Resting places

Ae. aegypti rest only indoors. Most of them were found resting on temporary objects, such as clothing, bed covers and mosquito nets while a smaller percentage was found resting on furniture and other semi-permanent articles (Macdonald, *et al,* 1965).

Biting activity

Ae. aegypti is active and bites principally by day. During two 24-hour catches in a Kuala Lumpur slum house, the *Ae. aegypti* were captured between sunrise and sunset and period of greatest activity were mid-morning and late afternoon (Macdonald, 1956). However, *Ae. albopictus* have also been observed to possess some night-time activity (Lim, 1979). During the day, both mosquitos have peaks of landing and biting activity. Lim (1979) found that the highest landing and biting frequency of *Ae. albopictus* was at 1800 hours. The morning peak varied and occurred at 0600, 0800 and 1000 hours.

While *Ae. aegypti* remains biting mainly indoors, *Ae. albopictus* bites both indoors and outdoors.

Gonotropic cycle

Macdonald (1956) reported that in about 1% of a test population of *Ae. aegypti,* the gonotropic cycle lasted 1-2 days, in 51% 2-3 days, in 32% 3-4 days and in 4% 4-5 days. The remaining mosquitos oviposited more than 5 days after the blood meal. After all females had oviposited, they were re-fed. It was noted that there was a reduction in the duration of the cycles after the first.

Flight range

Knowledge about the flight range of *Aedes* vectors is important for control. Presently, the flight range of these mosquitos is assumed to be 200 m which implied that premises within a diameter of 200 m are at risk of dengue transmission. The flight range of 200 m presumably was a result of observation of Smith (1956b) in Kuala Lumpur. He noticed that no cases were reported from houses more than 200 m from the outbreak area (a school).

Vector-virus relationship and transmission

Sabin (1952) first isolated dengue virus types 1 and 2 from human serum, while Hammon *et al* (1960) first succeeded in isolating dengue virus type 3 from *Ae. aegypti* collected in the Philippines and Thailand. In 1965, Rudnick and Chan isolated dengue 2 virus from a pool of *Ae. albopictus* from Singapore. Subsequently, Rudnick (1982) succeeded in isolating the virus from *Ae. aegypti* and *Ae. albopictus* in Malaysia.

In studying the ecology of dengue in Malaysia, Rudnick (1978) showed clearly that dengue was a zoonotic disease of monkeys maintained by *Ae. pseudoniveus/subniveus* at canopy level. Transmission of dengue was thought to include a silent rainforest cycle, a rural endemic mild form maintained by *Ae. albopictus,* an urban cycle involving *Ae. aegypti* and *Ae. albopictus* and a severe form involving *Ae. aegypti.*

The possibility for transovarian transmission of dengue virus in *Aedes* was investigated in Malaysia by Ramalingam *et al* (1986). Tests on F-1 progeny collected from ovitraps and parents fed with blood from dengue patients did not indicate presence of dengue antigen. These results however, were inconclusive because of the small sample size of mosquitos tested.

Vector control

Reslin 10/10 has been used by the Ministry of Health since 1974 as the insecticide for ground thermal fogging against *Ae. aegypti* in accordance with the National Plan of Action for the Control of Dengue Hemorrhagic Fever in Malaysia. Subsequently field trials have been carried out to evaluate the effectiveness of Reslin 10/10 and other new insecticides. Lo *et al* (1980) carried out field trials using Reslin 10/10 on *Ae. aegypti* and obtained an outdoor mortality of 89.5% and an indoor mortality of 61.1%. Further trials carried out later gave an overall mortality ranging from 40% to 76% (Vythilingam and Cheong, 1982).

Fogging trials with Actellic 25% EC gave an overall mortality of 100% against *Ae. aegypti* (Lo *et al,* 1981). Field trials with Resigen and fenitrothion gave good adulticidal as well as larvicidal effects on *Ae. aegypti* (Yap *et al,* 1988; Vythilingam 1988).

During dengue epidemics ultra low volume (ULV) application of malathion 96% TG is the recommended control method against adult *Ae. aegypti* in Malaysia as in the guidelines in the Plan of Action for DF/DHF Control of the Ministry of Health (1986). Over the last few years studies have been carried out to evaluate the efficacy of ULV operations (Lam and Tham, 1988; Vythilingam and Panart, 1991). Malathion 96% TG gave highest mortality in cages placed outdoors and decreased in living room and kitchen (Vythilingam and Panart, 1991).

Two new ULV aerosol generators were evaluated for the control of *Aedes* (Lee *et al,* 1991a; Lee, 1992b). In these trials, ULV grade (96%) malathion was used at a discharge rate of 50 ml/minute to fog the entire area and resulted in a mortality of 100%.

Larviciding with temephos (Abate 1% sg) has been reommended at a dosage of 1 mg/l. Although it has been used for more than 2 decades, studies on wild populations of *Ae. aegypti* revealed only very low temephos resistance (Lee *et al,* 1984; Lee and Lime, 1989).

The possibility of using *Bacillus thuringiensis* serotype H-14 has been investigated in Malaysia. Lee and Cheong (1987b) reported that a re-formulated sand granule formulation of *B. thuringiensis* H-14 was effective for 13 weeks at a dosage of 5 mg/l in suppressing *Ae. albopictus* population breeding in outdoor artificial water receptacle.

Source reduction ie the destruction of potential breeding places of the vectors is one of the strategies of the Ministry of Health.

Long term control measures include health education. This is implemented progressively by means of talks given by health staff and through the mass media like radio, newspapers and TV.

Environmental management includes physical measures to manipulate physical environment of *Aedes,* eg covering of containers to prevent oviposition. However, Lee (1991a) noted that complete covering of containers was required to deter oviposition.

The Destruction of Disease Bearing Insects Act was implemented in 1977. This is effective as a strong deterrant to mosquito breeding in houses. However, legislation should only be enforced as a last resort when other methods have failed.

Despite very low larval indices, dengue transmission continues to occur. The vector density threshold required for dengue transmission is unknown in Malaysia. In an attempt to estimate the threshold, Lee (1992b) analysed ovitrap data using sequential sampling techniques and determined that an ovitrap index of 10% was critical. Lee and Inder Singh (1991, 1992) similarly estimated the adult threshold density. The validity of these techniques can be tested by suppressing vector populations to levels below the threshold. A corresponding reduction in dengue incidence following such control would then favorably justify integration of these decision-making techniques into current *Aedes* surveillance system.

Conventional house-to-house larval survey is still used extensively in routine dengue vector surveillance in Malaysia. However, it was observed that at low *Aedes* population levels, it became increasingly more difficult to detect and locate positive containers using conventional larval survey techniques. Obviously, the possible use of other more sensitive indicators such as ovitrap index should be re-examined.

Lee (1991d) conducted a series of surveys in several suburban communities near Kuala Lumpur

City for the purpose of dengue seroepidemiology and dengue vector control studies. Since ovitraps as well as larval surveys were conducted in these localities, such studies offered an opportunity to compare these 2 *Aedes* surveillance techniques. Initial and subsequent monthly larval surveys indicated generally low *Aedes* House Index (HI) and Breteau Index (BI). However, weekly ovitrap survey data indicated high *Ae. albopictus* and *Ae. aegypti* populations in these communities. Ovitraps also generate continuous weekly data useful for the monitoring of population fluctuation. On the other hand, weekly larval surveys are considered impractical and uneconomical. The possibility and effectiveness of widespread use of ovitraps remain to be seen.

To date, only organophosphates (malathion and temephos) are widely used for *Aedes* control in Malaysia. Resistance to these compounds is most likely due to detoxifying enzymes, especially esterase and acetylcholinesterase. The screening of these enzymes would indicate possible emergence of resistance. Lee (1991b, 1991c) successfully developed rapid colorimetric tests for the large scale screening of *Aedes* populations.

MANSONIA VECTORS

The genus *Mansonia* is divided into two subgenera, *Mansonia* and *Mansonioides*. The subgenus *Mansonioides* includes the important vectors of lymphatic filariasis caused by *Brugia malayi* in South and Southeast Asia. Its status as a vector of *Wuchereria bancrofti* in New Guinea has also been documented (Van Dijk, 1958).

Mansonioides is predominantly oriental in distribution although its range extends from New Guinea and Japan in the east, to Africa in the west. Six species of this subgenus occur in the oriental region and they are vectors of the two types of Brugian filariasis, periodic and subperiodic. All the six species, *viz Mansonia bonneae, Ma. dives, Ma. uniformis, Ma. annulifera, Ma. annulata* and *Ma. indiana* are present in Malaysia.

Larval biology and life cycle

The eggs of all species are laid in clusters on the under surface of floating leaves and the larvae hatch directly into the water by breaking off the apical portion of the egg. The larvae and pupae attach themselves by means of their siphon to the roots of aquatic plants.

A large number of aquatic plants in different breeding habitat are associated with *Mansonia* in Malaysia (Wharton, 1962). In open swamps, ponds, rivers and canals, various floating plants such as *Eichornia, Salvinia* and *Pistia* spp, or rooted plants such as *Cyrtosperma, Isachne, Panicum* are the host plants of *Mansonia* spp. In swamp forests, the plants include the rattan *Plectocomia,* the trees *Dillenia, Eugenia* and grasses, herbs and arums (Wharton, 1962). Recently several additional species of host plants not reported earlier were found positive for *Mansonia* larvae. Three species belonging to the Family Araceae, *Homalomena cordata, Homalomena rostrata* and *Hydrostemma motleyi* were reported positive for *Ma. bonneae/dives* in Sarawak (Chang *et al,* 1988). In a larval survey in Malaysia, *Setaria geniculata,* a tall grass plant resembling lalang, was positive for *Ma. dives, Ma. bonneae,* and *Ma. uniformis,* and a tall succulent type of plant called *Hanguana malayanum* was positive for *Ma. uniformis* and *Ma. indiana* (IMR Annual Report, 1986).

Detailed monitoring of the productivity of *Ma. bonneae* and *Ma. dives* in a swamp forest with *H. cordata* as the host plant in Sarawak revealed no significant monthly fluctuations in larval density per plant nor the proportion of positive plants (Chang *et al,* 1988). The output of adults per plant was approximately halved during the dry season (June-August) compared to the wet season (December-February).

Laboratory cultures

Colonization attempts in Malaysia have been made by Wharton (1958), Chiang *et al* (1985) and more recently by Chang *et al* (1991b). Wharton (1958) had varying success with *Ma. uniformis, Ma. annulifera* and *Ma. indiana* but little success with others. Cheong *et al* (1981) and Chiang *et al* (1985) had good success with *Ma. uniformis, Ma. indianna, Ma. bonneae* and *Coquillettidia crassipes* but as with Wharton, had difficulties with *Ma. dives.* Chang *et al* (1991b) were able to colonize *Ma. dives* for only a few generations. Generally the life-cycles are very long. At 28-29°C the larval development period is around 18-45 days, pupal period 3-4 days, gonotrophic cycle of 3-5 days and the incubation of eggs 5-7 days, depending on the species. *Ma. uniformis* has shorter life-cycle and it is easier to colonize compared to the other species (Chiang *et al*, 1985). Larval culture medium used successfully for *Mansonia* are ground guinea pig dung-yeast infusion (Wharton, 1958) and Bacto liver yeast infusion (Chiang *et al*, 1985). Chang *et al* (1991b) used an albino rat dung infusion. The larval attachment substrates that were utilized for the inmature stages of *Mansonia* spp. were either aquatic plants such as *Eichornia crassipes, Pistia stratiotes, Jussiaea repens* and a special paper called 'keaykolour' ruffia snow white paper. In all the species mating occurred readily in 30.5 × 30.5 × 30.5 cm cages. The adult emergence rates reported for the different species were very low, normally not more than 30%.

Adult biology

Host preferences

Observations in West Malaysia by Wharton (1962) suggested that *Mansonia* are attracted to a wide range of hosts and normally man is not the preferred host. In outdoor resting catches, Wharton (1962) showed that *Ma. bonneae/dives* and *Ma. uniformis* fed predominantly on bovids and only 10% *Ma. dives/bonneae* and 2% *Ma. uniformis* were recorded as having fed on man. Recently, Chang *et al* (1991b) recorded an average human blood index (HBI) of 0.25%, 0.26 and 0.69 for *Ma. bonneae, Ma. dives* and *Ma. uniformis*, respectively, in a subperiodic *B. malayi* area in Sarawak. A study in Peninsular Malaysia showed that the HBI in an nonendemic area for *Ma. bonneae* and *Ma. uniformis* was 0.04 and 0.09, respectively, but in another area where brugian filariasis was endemic, the HBI was 0.50 and 0.40, respectively (Chiang, unpublished data). The low HBI in the nonendemic area was due to the presence of a large number of cows which served as a readily available source of blood to the *Mansonia*, hence diverting the mosquitos away from humans. Samarawickrema (1968) in a comparative trapping experiment found the man:calf ratio for *Ma. uniformis* to be 1 : 49.6 in Sri Lanka. However, in Peninsular Malaysia and Sabah (Wharton, 1962; Chiang *et al*, 1984a). *Mansonia*, in particular *Ma. uniformis*, appeared to be less attracted to cattle than in Sri Lanka.

Wharton (1962) found that adults received less than three times the number of bites by *Ma. bonneae/dives* and *Ma. uniformis* than a 2 year old child.

Resting places

All species of *Mansonia* rest outdoors during the day (Wharton, 1962; Chiang, 1984b). Wharton and Santa Maria (1958) observed that 90% of *Mansonia* were caught in window traps as they flew out of huts at night. Outdoor resting places include rock crevices, grasses and ferns under the shade of trees and bushes and damp moist shady places (Wharton, 1962).

Seasonal abundance

In a detailed study by Wharton (1962) in Pahang, largest number of *Ma. dives/bonneae* were caught towards the end of the year at the time of heavy rains of the north-east monsoon. Recently, the seasonal abundance of *Mansonia* was studied in two ecotypes, a swamp forest in Tanjong Karang and an open swamp rice field in Lubuk Pusing, Malaysia (Chiang *et al*, 1984c). In the swamp forest, the number of *Mansonia* taken was very high over a long period from July to December while there was a steady rainfall in the area during the same period. The peak of *Ma. annulata* was during the single month November coinciding with the highest rainfall. In Lubuk Pusing high numbers of *Ma. uniformis* prevailed at two peaks, July-September and again in December-January coinciding with the rainfall pattern. *Ma. indiana, Ma. bonneae* and *Ma. annulifera* followed a similar trend as *Ma. uniformis*. It is interesting to note in this study the abundance of the vector species and its seasonal variation has a direct bearing on disease transmission. In Tanjong Karang, the transmission of the infection in *Ma. annulata* was observed in October/November coinciding with its peak density and highest rainfall. Chang *et al* (1991a) reported that there was no clear correlation between the biting density of *Ma. bonneae, Ma. dives* and *Ma. uniformis* and the total rainfall in a coastal swamp area in Sarawak.

Biting activity

Mansonia mosquitos are nocturnal, they bite shortly after dusk and biting continues through the night. Their peak biting times, occurring in the late evening and early morning hours, appeared to coincide closely with the nocturnal subperiodicity of *B. malayi* (Wharton, 1962; Chiang *et al*, 1984a, 1984d; Chang *et al*, 1991a). *Mansonia* spp. are also known to bite during the day in shaded situations and bite outdoors more than indoors. Studies in

Sarawak by Chang *et al* (1991a) showed that the *Mansonia* mosquitos were less exophagic in behavior than those in Peninsular Malaysia and Sabah. They suggested that the different behavior observed could be due to differences in the architecture of Sarawak's long houses whose bamboo flooring has gaps through which mosquitos can gain access into the houses. The characteristic form of the biting cycle of *Mansonia* is not related to the age composition of the biting population.

Gonotrophic cycle

The duration of the gonotrophic cycle of *Ma. uniformis* in the field determined by mark-release-recapture technique was 3-4 days (Chiang *et al*, 1988b). A cycle of 4 days was also estimated for *Ma. bonneae, Ma. dives* and *Ma. uniformis* by Chang *et al* (1991a) using the same technique.

The estimates in the laboratory were comparable to the field estimates. The mean duration from feeding to oviposition at 28-30°C and RH of 80% for *Ma. uniformis, Ma. indiana* and *Ma. bonneae* was 3.14, 4.25 and 3.71 respectively (Chiang, 1987). The estimate for *Ma. dives* was 4.04 days (Chang *et al*, 1991b).

The presence of 'asacs' and mature eggs in the ovaries of recaptured females suggested that *Ma. uniformis* seeking oviposition sites on the same evening return to feed after oviposition (Chiang *et al*, 1988b).

Survivorship

Generally *Ma. uniformis* has a lower survivorship compared to the other five vector species of *Mansonia*. In a study on the natural survival of *Mansonia* vectors in two sites in Peninsular Malaysia, survivorship ranged from 0.650 in *Ma. uniformis* to 0.721 in *Ma. annulifera* (Chiang *et al*, 1984d). In an area endemic for subperiodic *B. malayi* in Sarawak, Chang *et al*, (1991a) reported that the daily survival of *Ma. bonneae, Ma. dives* and *Ma. uniformis* was 0.900, 0.892 and 0.863, respectively. Estimates of daily survivorship of *Ma. bonneae* and *Ma. dives* in an endemic area in Sabah was also observed to be high, 0.926 and 0.903, respectively (Chiang *et al*, 1984a). A remarkably constant daily survivorship was demonstrated by the *Mansonia* spp. throughout the year in both study areas (Chiang *et al*, 1984a; Chang *et al*, 1991a). The reduced longevity of the mosquitos could be responsible for the low Annual Infective Biting Rate (AIBR) and Annual Transmission Potential (ATP) estimated for the *Mansonia* mosquitos in Peninsular Malaysia (Chiang *et al*, 1984d) compared to that observed in Sarawak where the longevity of the mosquitos were higher and hence making them more efficient vectors with high AIBR and ATP (Chang *et al*, 1991a).

Flight range

Earlier through indirect observation, it was estimated that *Ma. bonneae/dives* travelled a distance of 1.6 - 3.2 km in the forest (Wharton, 1962). More recently, in an open swamp in Malaysia, many marked *Ma. uniformis* were recaptured at 1.5 km from the point of release and the longest flight distance was 3.5 km. The mean dispersal distance for the blood-fed and unfed females was 1.45 and 1.71 km, respectively (Chiang *et al*, 1988b). In another area in Malaysia endemic with *B. malayi*, individual marked *Ma. annulifera, Ma. bonneae, Ma. dives, Ma. indiana* and *Ma. uniformis* were found to have travelled up to a distance of 2.4 km (Macdonald *et al*, 1990). All the above observations indicated that *Mansonia* have strong flying abilities and the importance of reinvasion must be recognized when control operations are restricted to small areas.

Vector-parasite relationship and transmission

Experiments with laboratory bred *Mansonia* mosquitos fed on infected cats showed that *Ma. uniformis, Ma. bonneae* and *Ma. dives* were equally efficient vectors but *Ma. indiana* was a poor vector for subperiodic *B. malayi* (Chiang *et al*, 1988a). Five strains of *Ma. uniformis* from endemic and non-endemic areas in Malaysia were found to be equally susceptible to subperiodic *B. malayi*. The susceptibility rates were directly related to the microfilarial densities in the carrier at the time of feeding (Chiang *et al*, 1989). This confirmed the earlier work of Wharton (1958) using subperiodic *B. malayi* in *Ma. dives/bonneae*. In Sabah and Sarawak, Malaysia, *Ma. bonneae, Ma. dives* and *Ma. uniformis* were incriminated as vectors of subperiodic *B. malayi* (Chiang *et al*, 1984b; Rubis *et al*, 1981; Chang *et al*, 1991a).

Vector control

To date, the control of Brugian filariasis in Malaysia has been through mass drug treatment using diethylcarbamazine citrate (DEC). Even through chemotherapy has done much to reduce filariasis in this region, it has been shown in Malaysia where there was intense zoonotic transmission of subperiodic *B. malayi* and *Mansonia* spp. were the vectors, infections still persisted with the finding of new human cases and mosquito infections with infective larvae (Mak *et al*, 1982; Cheong *et al*, 1981) despite several mass treatments with DEC. In such places mass chemotherapy supplemented by vector control measures may be the best approach in controlling the disease. However, at present there have been no operational control measures undertaken against the vector *Mansonia*.

Control of the mosquito vectors is possible in the larval stages by removal of aquatic vegetation that they are associated with at regular intervals in irrigation canals and ponds. This method is not possible in the extensive areas of the swamp forest, as in Malaysia, where the *Mansonia* breeds profusely.

The susceptibility of *Mansonia* larvae to *Bacillus thuringiensis (Bti)* and *Bacillus sphaericus* has been well studied in the laboratory (Foo and Yap, 1982; Cheong and Yap, 1985; Lee, 1988). Recently, Foo and Yap (1983) and Yap *et al* (1991) reported on the field applications of *Bti* H-14 Teknar and *B. sphaericus* against *Ma. uniformis* and *Ma. indiana* breeding in swampy ditches on Penang island, Malaysia. They found that high dosages of *Bti* were required to achieve control of *Mansonia* larvae. In Sarawak, Malaysia, field simulated studies by Chang *et al* (1990a,b) using *Bti* and *B. sphaericus* against *Ma. bonneae* showed that the emergence of *Mansonia* adult mosquitos increased at week 6 and week 7 post-treatment following a marked decrease during the first 4 weeks after exposure to *Bti* Skeetal and Bactimos briquette treatment. Bactimos briquettes and *B. sphaericus* RB80 had higher residual capability than *Bti* Skeetal against *Ma. bonneae*. The results are encouraging even though both bacterial larvicides sustained suppression of *Mansonia* larvae/adult emergence for over 4 weeks in the field.

Space spray trials for control of adult *Mansonia* mosquitos using malathion 2% thermal fog showed that short-term control in a limited area can markedly reduce the number of *Ma. bonneae* and *Ma. dives* for about 2 weeks (Chang, 1980). However, a recent study conducted in an open swamp in Selangor, Malaysia, using lambdacyhalothrin 5 and 10 g thermal fog did not appreciably reduce the *Mansonia* adult population (Lee and Salleh, 1990).

In a field trial using residual formulation of deldrin against *Mansonia* over a two-year period, Wharton *et al* (1958) reported that they were not successful in bringing down the microfilarial rates in the experimental areas. Laboratory susceptibility tests of *Ma. uniformis* to insecticides have shown that the adults are susceptible to DDT, decamethrin and chlorpyrifos (Lee *et al*, 1986). Vinycide, an insectividal paint containing deltamethrin has good adulticidal effect against *Ma. indiana*, with high mortality in the test mosquitos up to ten months after treatment on both wooden and cement surfaces (Chiang *et al*, 1990). Cyfluthrin has also been evaluated in the laboratory and was found to be effective against *Ma. uniformis* (Vythilingam *et al*, 1992).

Deet impregnated anklets and headbands and a cheap repellent insecticidal bar MOSBAR gave good protection against the bites of *Mansonia* species in the field (Chiang *et al*, 1990a, 1991).

Response of *Mansonia* males to different frequencies of the female wingbeat was analysed by Kanda *et al* (1987, 1988) with the intension of developing a trap which could be used in the field for controlling the species. It was found that sound at a wingbeat frequency of 350 Hz was markedly more attractive than the other frequencies. At this sound frequency together with dry ice and a hamster as attractants, large number of *Mansonia* were collected in two areas in Malaysia (Kanda *et al*, 1988). However the feasibility and cost of using the sound trap for control purposes requires further studies.

JAPANESE ENCEPHALITIES VECTORS

In Malaysia the principal vectors of Japanese encephalitis appear to be *Culex gelidus* and *Culex vishnui* group mosquitos (Marchette, 1967). Simpson *et al* (1970) working in Sarawak in the late sixties have isolated the virus from *Mansonia annulifera*, *Cx. tritaeniorhynchus*, *Cx. gelidus* and *Cx. pseudovishnui*. These are rural mosquitos feeding primarily on domestic animals and breeding in paddy fields and in pools of standing water (Gould, 1962).

Larval biology

Eggs are always found as adherent masses or rafts floating on the surface of water. All sorts of water surfaces may bear eggs, but it was found that the optimum breeding site for *Cx. tritaeniorhynchus* in Sarawak was water which was becoming stagnant with scum, particularly when fresh organic matter was constantly being added (Heathcote, 1970). The outburst of breeding occurs just before paddy planting (Heathcote, 1970). The larva emerges from the submerged anterior end of the egg directly into the water. There is just one generation of larvae, reaching the pupal stage almost simultaneously and only 4-5 days after oviposition, with very low predation (Heathcote, 1970).

Laboratory culture

Cx. gelidus was reared in a large cage 6ft × 4 ft × 7 ft tall, at a temperature of 27-28°C and a humidity of 80%. Pans of tap water placed in the cages for egg deposition. Larvae were reared in enamelled pans, containing tap water or a mixture of tap water and water taken from a breeding place. The larval food was a mixture of baker's yeast and a pellet of a high-protein sheep feed. Human beings and pigs were offered as blood meals on alternate days. Occasional offerings of rabbits and chickens were also made (Barnett, 1962). The larval food for *Cx. tritaeniorhynchus* was powdered yeast and the water was changed daily. Pupation occurred after 5-6 days. Adults were allowed to emerge in lamp chimneys in batches of about 50. Chickens were provided as blood meals (Hale, 1957).

Adult biology

Host preferences

Cx. tritaeniorhynchus tends to bite pigs more readily than it bites man (Macdonald *et al,* 1965, Hill *et al,* 1969). The four species of *Culex* - *Cx. annulus, Cx. gelidus* (1 : 1.7), *Cx. nigropunctatus* and *Cx. tritaeniorhynchus* (1 : 2.6) - all showed preference for the pig (Macdonald *et al,* 1967). It has been found that both *Cx. gelidus* and *Cx. tritaeniorhynchus* also feed on fowl and buffalo (Hill *et al,* 1969). Humans, dogs and birds were also found to be attractive to *Cx. tritaeniorhynchus.* The partiality of *Cx. tritaeniorhynchus* for the blood of dogs, together with high incidence of antibody to JE found in these animals (Simpson *et al,* 1970) and their abundance and association with man throughout Sarawak, makes the dog a potentially important host of Japanese encephalitis virus in that country (Bendell, 1970).

Resting places

The mosquitos were seen resting beside poultry, pigs and cattle in the early evening. At night they were seen resting on grass, wire netting and fences (Macdonald *et al,* 1967).

Seasonal abundance

Cx. tritaeniorhynchus shows two peak periods of increase in population throughout the year, One in September-November and the other in April-May (Heathcote, 1970; Macdonald *et al,* 1967; Hill *et al,* 1969), while *Cx. gelidus* is present throughout the year.

Biting activity

Most *Culex* mosquitos are nocturnal. *Cx. tritaeniorhynchus* usually bites just after sunset for a three hour period the peak being between 1830 to 2130 hours, after which the numbers decreased sharply and continued at a lower level until biting activity ceased abruptly at 0630 hours (Hill *et al,* 1969). Studies carried out by Bendell (1970) showed that *Cx. tritaeniorhynchus* does enter attap huts to bite man.

Gonotrophic cycle

The duration of the gonotrophic cycle of *Cx. tritaeniorhynchus* in the field determined by the mark-release-recapture technique was 5-8 days (Reisen *et al,* 1978). The estimate of the length of the gonotrophic cycle in nature agreed well with laboratory life table experiments, where the maximum age-specific fertility was attained between six and seven days after emergence (Reisen *et al,* 1977). (This work has not been carried out in Malaysia, it was done in Pakistan).

Survivorship

The survivorship estimates for *Cx. tritaeniorhynchus* males and females were low, 0.595 and 0.615 respectively. There was no significant difference between the survivorship of the males and females. (Reisen *et al,* 1977).

Flight range

Bailey and Gould (1975) showed that *Cx. tritaeniorhynchus* dispersed up to 1.8 km from the site of release within 48 hours and the dispersion was random. In other studies dispersal within 48 hours was 1.22 km (Reisen *et al,* 1978) and 5.1 km (Wada *et al,* 1969). (Work done in Pakistan and Japan).

Vector-virus relationship and transmission

Thirty-six isolations of JE virus were obtained from *Cx. tritaeniorhynchus* while only 7 were obtained from *Cx. gelidus* in Sarawak (Simpson *et al,* 1974). A high proportion of pigs in Malaya possess neutralizing antibody to JE virus and are undoubtedly acting as amplifier hosts for the virus (Marchette, 1967). Laboratory experiments have shown that in *Cx. tritaeniorhynchus,* after an infected blood-feed, there is a multiplication of virus, and that after a period of 10 days the mosquito is capable of transmitting the infection to fresh hosts (Hale *et al,* 1957). Study by Gould (1962) has demonstrated that JE virus multiplies readily within *Cx. gelidus* and that this mosquito is capable of transmitting the virus to susceptible hosts. More than one host could be infected by a single infective mosquito in a short period of time if the mosquito was interrupted during the act of feeding (Gould, 1962).

The demonstration of the efficiency with which these mosquitos transmit the virus under controlled conditions, combined with evidence from field studies, provides the data necessary to incriminate *Cx. gelidus* and *Cx. tritaeniorhynchus* as important vectors of Japanese encephalitis.

Vector control

So far no attempts have been made to control JE vectors in Malaysia. The need for experimental assessment of vector control measures in this country is great. The large scale production of vector densities and immigration of mosquitos suggest that the limited coverage that can be achieved by ground application space- spraying cannot be expected to achieve a significant impact on the overall mosquito population per se in these areas.

An entomological approach to Japanese encephalitis control is the use of the light trap (Mogi, 1978, 1984; Wada, 1974). The light traps at the pigsties attract many bloodfed females and thus the chances of those just fed on viremic pig getting killed is great. Mosquito proof pigsties would also decrease the transmission of JE virus. Insecticide impregnated netting can also be hung around the pigsties to control the vectors.

CONCLUSION

Despite a large amount of information obtained on these vectors, the question of how to control some of them remain unsolved. This could be due to lack of field research carried out for the evaluation of different control tools already available. Thus there is a pressing need to incorporate new and more effective techniques in our National vector programs to effect a more favorable outcome in the control of these vector-borne diseases.

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