the larvae and pupae were being collected. Two of these adults, both female, were collected, along with 35 larvae and pupae. The larvae and pupae were reared for identification as adults. The mosquitoes were maintained through one additional generation in a large mason jar, indicating stenogamy. The colony proved to be autogenous; viable eggs were produced without blood. *Culex restuans* Theobald larvae were also collected from the rock holes.

The rock holes were located on the surface of a granite outcrop about 8 m from the shoreline and elevated about 5 m above the water level. The largest of the three pools was 3 m long by 2 m across at the time of collection. The maximum depth of this pool was about 20 cm. The other pools were much smaller. The most prominent types of vegetation were grasses and moss growing along the edge of the largest pool and in cracks in the granite. All of the pools contained leaf litter and other organic material.

Recently, numerous reports of *Ae. atropalpus* utilizing discarded tires have appeared in the literature (Covell and Brownell 1979, Restifo and Lanzaro 1980, White and White 1980). The tire breeding habit brings this species into close proximity of human habitations and allows for large numbers to develop in a small area. Furthermore, tire breeding strains are avid blood feeders even though autogeny is maintained. *Aedes atropalpus* has been shown to utilize a wide range of host species, including humans, under natural conditions in a tireyard (Berry and Craig 1984). The demonstration of the ability of *Ae. atropalpus* to transmit La Crosse encephalitis virus orally and transovarially (Freier and Beier 1984) indicates that this species merits further study, especially with regard to the tire breeding strains.

Voucher specimens of adults discussed in this report have been deposited in the Iowa State Insect Collection at Iowa State University.

References Cited

Beadle, L. D. 1963. The mosquitoes of Isle Royale, Michigan. Proc. New Jersey Mosq. Exterm. Assoc. 50:133–139.

Berry, W. J. and G. B. Craig, Jr. 1984. Bionomics of *Aedes atropalpus* breeding in scrap tires in northern Indiana. Mosq. News 44:476–484.

Cassani, J. R. and H. D. Newson. 1980. An annotated list of mosquitoes reported from Michigan. Mosq. News 40:356–368.

Covell, C. J., Jr. and A. J. Brownell. 1979. *Aedes atropalpus* in abandoned tires in Jefferson County, Kentucky. Mosq. News 39:142.

Freier, J. E. and J. C. Beier. 1984. Oral and transovarial transmission of La Crosse virus by *Aedes atropalpus*. Am. J. Trop. Med. Hyg. 33:708–714.

Restifo, R. A. and G. C. Lanzaro. 1980. The occurrence of *Aedes atropalpus* (Coquillett) breeding in tires in Ohio and Indiana. Mosq. News 40:292–294.

White, D. J. and C. P. White. 1980. *Aedes atropalpus* breeding in artificial containers in Suffolk County, New York. Mosq. News 40:106–107.

Zavortink, T. J. 1972. Mosquito studies (Diptera: Culicidae). XXVIII. The New World species formerly placed in *Aedes (Finlaya)*. Contrib. Am. Entomol. Inst. (Ann Arbor) 8(3):1–206.

CULEX THEILERI AND SINDBIS VIRUS; SALIVARY GLANDS INFECTION IN RELATION TO TRANSMISSION

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The competence of a mosquito species as a vector of an arbovirus depends upon both extrinsic and intrinsic factors (Reeves 1967, World Health Organization 1972, Hardy et al. 1983). Extrinsic factors include, among others, the relative abundance and biology of the mosquito vector and its vertebrate host as well as environmental conditions. The latter affect not only the bionomics of both invertebrate and vertebrate hosts of the virus but also the duration of the extrinsic incubation period of the virus in the mosquito. The main criteria pertinent to a quantitative laboratory assessment of vector competence are the susceptibility of the insect to infection, the length of the extrinsic incubation period and the transmission rate. The transmission rate is defined in our laboratory as the proportion of infected mosquitoes which successfully transmit the virus. In transmission tests to determine this rate we expose individual susceptible animals to individual infected mosquitoes, the choice of animal depending on the virus in question. However, tests with large numbers of animals have the disadvantage of being expensive, cumbersome and time consuming. Furthermore, certain species of mosquitoes may be reluctant to take a second blood-meal, notably aedine species in the subgenera *Ochlerotatus, Neomelaniconion* and *Aedimorphus* (Jupp, unpublished work). Alternative ways for determining the transmission rate are the *in vitro* capillary method of Aitken (1977) or testing the salivary glands of each mosquito for infectivity (Chamberlain 1968).

According to Chamberlain (1968), the presence of virus in the salivary glands of mosquitoes indicates the probable ability of the species to transmit by bite. The experiment reported below was undertaken to investigate whether individual mosquitoes with infected salivary glands always transmitted virus by bite.

The mosquitoes used were aged 2–18 days, the 3rd generation of a laboratory colony of *Culex theileri* Theobald which originated from gravid females collected at Lake Chrissie in the eastern Transvaal highveld. The experiment was undertaken in an insectary where the mosquitoes were maintained at 75–85% RH and 24–26°C. They were infected by feeding on a blood-Sindbis virus mixture through a chicken skin membrane. The virus used was the AR86 strain of Sindbis virus (Weinbren et al. 1956) at its 4th intracerebral mouse passage level. Methods for preparation and titration of the infective blood-meal, feeding the mosquitoes, and determining the infection rate were the same as described previously (Jupp and McIntosh 1970) except that the infecting titer of 7.2 logs is expressed as the log mouse LD_{50} per ml.

Fourteen days after their infective feed the mosquitoes were allowed to feed singly on individual 2-week-old chicks. Three weeks after this transmission feed the chicks were bled and their sera tested for antibody against Sindbis virus by the haemagglutination inhibition test (Clarke and Casals 1958). The presence of antibody indicated successful transmission. On the day following the transmission feed those mosquitoes which had fed were frozen at −70°C and 12 days later were removed from storage and allowed to thaw at room temperature. The salivary glands were removed from the mosquito, rinsed in a drop of normal saline, and then triturated with small needles in a second drop of saline. The suspension was then transferred to a tube containing 0.3 ml of bovine phosphate albumin. This salivary gland suspension and another prepared from the decapitated body of each mosquito were tested separately for virus by inoculating them intracerebrally into 2–3-day-old mice.

The decapitated bodies of all 23 mosquitoes tested were found to be infected but only 9 had infected salivary glands. Only 2 of these 9 mosquitoes transmitted virus to the chicks. Thus it appears in the case of *Cx. theileri* with Sindbis virus that, although salivary glands are infected, transmission will not necessarily follow when the mosquito bites the susceptible host. Hence the salivary gland infection rate cannot be used as a measure of the transmitting ability. This result is shown in Table 1 together with the results obtained previously by other workers using other virus-mosquito vector combinations. It can be seen that a good agreement between salivary gland infectivity and transmission by bite (transmission rate) has usually been demonstrated in experiments, with the notable exception of California encephalitis virus and *Aedes aegypti* (Linn.).

It can be concluded from the present experiment and the results of others, that although salivary glands infectivity would often be a reliable expression of the transmission rate, this is not always the case. Why are some mosquitoes unable to transmit virus while feeding even though infectious virus or viral antigens can be detected in the salivary glands? Hardy et al. (1983) suggested 3 possible explanations: (1) Viral infection is at an early stage so that little or no infectious virus has been released into the salivary glands, (2) the mosquito could have re-

Table 1. Experiments comparing salivary gland infection rates and transmission rates.

Virus	Mosquito vector	Salivary gland infection rate	Transmission rate	Reference
Alphavirus				
Sindbis	*Culex theileri*	9/23[1]	2/23[2]	Present report
Whataroa	*Aedes australis*	6/10	6/10	Miles et al. 1973
Western equine encephalitis	*Culex tarsalis*	4/10	3/10[3]	Thomas 1963
Western equine encephalitis	*Culex tarsalis*	17/17	17/17	Kramer 1981
Flavivirus				
Dengue 2	*Aedes aegypti*	11/11	11/11	McLean et al. 1974 (Table 3)
Bunyavirus				
California encephalitis	*Aedes aegypti*	16/23	4/22	McLean et al. 1974 (Table 1)

[1] Numerator = number of mosquitoes with salivary glands infected; denominator = number tested.

[2] Numerator = number of mosquitoes transmitting by bite, denominator = number of infected mosquitoes feeding.

[3] This rate could have been 2/10 but actual result unclear as groups of more than one mosquito fed on chicks in the transmission attempts (Thomas 1963).

cently ejected the virus while feeding on a carbohydrate source and the virus has yet to be replenished into the salivary duct, and (3) there might be a "salivary gland escape barrier" which prevents virus leaving these glands. This would be the situation when the production of infectious virus has been modulated to low levels. Such an escape barrier could explain the lowered transmission rates which have been obtained after prolonged incubation periods (McIntosh and Jupp 1970, Mangiafico 1971).

I wish to thank the Director-General of National Health and Welfare, Pretoria, for permission to publish.

References Cited

Aitken, T. H. G. 1977. An *in vitro* feeding technique for artificially demonstrating virus transmission by mosquitoes. Mosq. News 37:130–133.

Chamberlain, R. W. 1968. Arboviruses, the arthropod-borne animal viruses. Current Topics Microbiol. Immunol. 42:38–58.

Clarke, D. H. and J. Casals. 1958. Techniques for haemagglutination and haemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 7:561–573.

Hardy, J. L., E. J. Houk, L. D. Kramer and W. C. Reeves, 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annu. Rev. Entomol. 28:229–62.

Jupp, P. G. and B. M. McIntosh. 1970. Quantitative experiments on the vector capability of *Culex (Culex) pipiens fatigans* Wiedemann with West Nile and Sindbis viruses. J. Med. Entomol. 7:353–356.

Kramer, L. D., J. L. Hardy, S. B. Presser and E. J. Houk. 1981. Dissemination barriers for Western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. Am. J. Trop. Med. Hyg. 30:190–197.

Mangiafico, J. A. 1971. Chikungunya virus infection and transmission in five species of mosquito. Am. J. Trop. Med. Hyg. 20:642–45.

McIntosh, B. M. and P. G. Jupp. 1970. Attempts to transmit chikungunya virus with 6 species of mosquito. J. Med. Entomol. 7:615–618.

McLean, D. M., A. M. Clarke, et al. 1974. Vector capability of *Aedes aegypti* for California encephalitis and dengue viruses at various temperatures. Can. J. Microbiol. 20:255–262.

Miles, J. A. R., J. S. Pillai and T. Maguire. 1973. Multiplication of Whataroa virus in mosquitoes. J. Med. Entomol. 10:176–185.

Reeves, W. C. 1967. Factors that influence the probability of epidemics of Western equine, St. Louis and California encephalitis in California. Vector Views 14:13–18.

Thomas, L. A. 1963. Distribution of the virus of Western equine encephalomyelitis in the mosquito vector *Culex tarsalis*. Am. J. Hyg. 78:150–165.

Weinbren, M. P., R. H. Kokenot and K. C. Smithburn. 1956. Strains of Sindbis-like virus isolated from culicine mosquitoes in the Union of South Africa. S. Afr. Med. J. 30:631–636.

W.H.O. 1972. Vector Ecology. Tech. Rep. Ser. 501:1–38.

TOXORHYNCHITES MOCTEZUMA, A POTENTIAL BIOLOGICAL CONTROL AGENT IN TRINIDAD AND TOBAGO, W. I.[1]

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Toxorhynchites moctezuma (Dyar and Knab) [as *theobaldi* (Dyar and Knab) in Knight and Stone (1977)] has a wide distribution from Mexico in Central America to Venezuela in South America (Rubio et al. 1980; S. J. Heinemann, personal communication). In the Caribbean Basin, it has a limited distribution and is found only in Trinidad and Tobago (Heinemann et al. 1980). It should be noted that *Tx. theobaldi* is now considered a synonym of *Tx. moctezuma* (S. J. Heinemann, personal communication).

In Trinidad and Tobago, three *Toxorhynchites* species have been recorded. *Toxorhynchites moctezuma, Tx. iris* (Dyar) [as *mariae* (Bourroul) in Knight and Stone (1977)] and *Tx. superbus* (Dyar and Knab), of which *Tx. iris* and *Tx. superbus* are found primarily in terrestrial and epiphytic bromeliads (Heinemann et al. 1980). *Toxorhynchites moctezuma,* on the other hand, has been collected from bromeliads, treeholes, in tires and cans (Heinemann et al. 1980, Chadee et al. 1984).

In 1976, the Insect Vector Control Division (IVCD) of the Ministry of Health and Environment embarked on an *Aedes aegypti* (Linnaeus) Eradication program in Trinidad, W.I. Tobago, however, is relatively free of *Ae. aegypti* but routine vigilance surveys are still conducted twice yearly (Chadee et al. 1984). During surveys, larvae and pupae of *Tx. moctezuma* were collected in artificial containers in both Trinidad and Tobago. All immature stages were collected and transported to the IVCD laboratory, St. Joseph, Trinidad, where they were subsequently identified by the author.

Table 1 summarizes the total collection of *Tx. moctezuma* from artificial containers in Trinidad

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