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COLONIZATION OF NORTH AMERICAN *AEDES TOGOI*

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ABSTRACT. Asian *Aedes togoi* (Theobald) has been colonized. Procedures for the successful colonization and laboratory maintenance of *Ae. togoi* from British Columbia, Canada, are described.

INTRODUCTION

Aedes (Finlaya) togoi (Theobald) occurs in Siberia, China, Formosa, Korea, Japan, Marcus Island, Ryukyu-Retto, Ogasawara Gunto, Malaysia, U.S.S.R., Bonin Island and Thailand in Asia (Knight and Stone 1977). Larvae develop in saline, rockpools along rocky sea shores. In Korea, larvae have also been recorded from freshwater, man-made containers in port cities and in settlements hundreds of kilometers inland (Petrishcheva 1948). *Ae. togoi* has recently been collected along the south-west coast of Canada. Meredith and Phillips (1973) collected larvae from rock-pools at Victoria, Vancouver Island, and we have collected larvae from rockpools in West Vancouver, at Horseshoe Bay, and on South Pender Island. *Ae. togoi* was probably collected earlier from rockpools in the Vancouver area by Hearle (1926) as *Aedes (Ochlerotatus) dorsalis* (Meigen).

Ae. togoi is a natural vector of bancroftian and brugian filariasis in China and Japan and of Japanese B encephalitis in the Far-eastern U.S.S.R. (Smith 1973). Gerberg (1970) described procedures for laboratory maintenance of *Ae. togoi* from Asia. This paper describes the colonization and laboratory maintenance of North American *Ae. togoi*.

COLONIZATION

Our colony was established using large numbers (>300) of 4th-instar larvae and pupae collected from rock-pools at Lighthouse Park, West Vancouver (49°20'N., 123°15'W.), during August and September 1977. Adults were held in a 23×23×23 cm cage and provided with water, dry sucrose and a guinea pig for blood. Twilight was not simulated. No mating was observed, although viable eggs were laid.

LABORATORY MAINTENANCE

Seventy-five adults of each sex are held in a 23×23×23 cm plexiglass cage. Dry sucrose is provided as carbohydrate. Water-soaked paper toweling held in a 100-ml beaker (water-wick) provides water and maintains the RH in the cage at 55–60%. Temperature is 25°C and the photoperiod (17.5L:06.5D) approximates

the longest day of the year, including civil twilight, at Lighthouse Park (List 1971). Twilight is not simulated.

When all females are at least 2 days old, a shaved guinea pig is restrained on the screened cage-top for 15 min. When the females have fed, a plexiglass cover is placed over the screen to retard evaporation of water from the wick. Three days after the 1st blood-meal the wick is removed from the cage and 4 100-ml beakers lined with a 5×18 cm strip of paper toweling and partially filled with distilled water are placed in the cage as oviposition sites. Eggs are laid on the paper toweling just above the water surface. We have found toweling more suitable than filter paper (Gerberg 1970) as an oviposition substrate. Eggs do not adhere well to filter paper and easily wash off, with the result that many fall on the water surface when the beakers are disturbed.

Beakers are removed daily from the cage, covered, and incubated for 24 hr at 25°C. Eggs are incubated an additional 24 hr after removing all water from the beakers and replacing the covers. Paper toweling strips with attached eggs are then removed from the beakers and air-dried until the paper is almost dry. Eggs are stored at 20°C, 85% RH, and 17.5L:06.5D. Eggs may be stored up to one month, but if the paper toweling is too moist, these stored eggs may hatch. Gerberg (1970) suggested incubating the eggs under saturated conditions for 4 days, but we found that this procedure resulted in many of them hatching before they could be dried for storage.

Eggs are hatched by inundating them with distilled water at 20°C. A small amount of fish food (Tetra® Conditioning Food) is provided for newly hatched larvae. All eggs do not hatch simultaneously and after 24 hr submersion, those unhatched are removed, air dried and stored for 2nd and 3rd 24-hr inundations.

Larvae are reared in 7.5 cm-high, 9.5 cm-diam, covered glass dishes containing ca. 500 ml of rearing medium prepared by adding equal parts of distilled water and filtered ocean water. The salinity of the rearing medium ranges from 10–12 gm salts/liter. Fifty larvae are placed in each dish and provided with fresh, wetted fish food each day. The amount of fish food added varies with the instar. There should be enough to provide some excess, but not so much as to facilitate bacterial growth and clouding of the water. The larval rearing medium is changed if it becomes fouled with bacteria. Larvae should be maintained at 20°C; we observed almost 100% mortality of 1st-instar larvae at 25°C. At 20°C, larval development is completed in 15–18 days.

Pupae are removed from the rearing medium daily, washed and placed in distilled water at 25°C. At this temperature the pupal stage lasts 4–5 days.

At 25°C, oviposition begins 72–96 hr after the initial blood-meal. We blood-feed adults daily for 7 days and collect eggs over an 8–9 day period. During this period 75 females lay 6653±592 ($\bar{x}$±S.E.; n=9) eggs.

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AERIAL APPLICATION OF *ROMANOMERMIS CULICIVORAX* (MERMITHIDAE:NEMATODA) TO CONTROL *ANOPHELES* AND *CULEX* MOSQUITOES IN SOUTHWEST FLORIDA

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ABSTRACT. An experimental release of preparasites of the mosquito nematode *Romanomermis culicivorox* Ross and Smith (=*Reesimermis nielseni* Tsai and Grundmann, auct., partim.) was made in 3 ponds via helicopter at the rate of ca. 4,629/m^2 of water surface to control natural populations of *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann, and *Culex erraticus* (Dyar and Knab) larvae. Mean levels of parasitism of 52.4, 66.7 and 43.8% of the 1st-4th instar *Anopheles* spp. and 39.2, 40.0, and 53.3% of 1st-4th instar larvae of *Cx. erraticus* from 3 ponds, respectively were obtained.

The aerial spray tests indicated that a helicopter equipped with a Simplex low profile spray system with TeeJet^R spray nozzles can be an effective means of disseminating the preparasitic stage of *R. culicivorax* in mosquito control operations.

Control of natural populations of mosquito larvae with a pathogenic agent applied by air has not been demonstrated. However, Levy et al. (1977) reported encouraging results in tests using a helicopter spray system to deliver the mosquito nematode *Romanomermis culicivorax* Ross and Smith. Tests were conducted at ground level with a Bell 47G helicopter equipped with a Simplex low profile aerial spray system having a boom fitted with TeeJet^R nozzles at 25 psi and no adverse effects on preparasite infectivity or nematode development were detected. These tests suggested the potential use of this aerial spray system for the dissemination of *R. culicivorax* in mosquito control operations.

The susceptibility of larvae of *Anopheles quadrimaculatus* Say, *An. crucians* Wiedemann and *Culex erraticus* (Dyar and Knab) to field parasitism by preparasites of *R. culicivorax* applied by conventional compressed air hand sprayer has been demonstrated by Petersen and Willis (1972; 1974). We are now reporting on the aerial application of preparasites of *R. culicivorax* via helicopter to control field populations of larvae.

METHODS AND MATERIALS

Three semipermanent ponds (30.5 × 15.2 × 0.5-1.0 m deep) constructed in 1976 at the Lee County Mosquito Control District were used as the sites for the tests (Fig. 1). The ponds contained rain and standing ground water (0.3-0.6 m deep) and vegetation scattered throughout but concentrated primarily around the edges. Pre-treatment sampling showed large populations of larvae and pupae of *An.*

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