

DEET against *Aedes aegypti* in a "no choice" context. Although a "free choice" probably furnishes a more realistic appraisal of repellent activity, such a situation has certain drawbacks when used with simuliids. The problem lies in the fact that black flies have only been successfully induced to feed in the laboratory when confined fairly closely to the membrane or host (Wenk 1965, McMahon 1968, Sutcliffe and McIver 1975, Mokry 1976, and Grunewald and Wirtz 1978). This drastically limits the choices that can be offered to the females. Although it is possible to treat different areas of the same membrane with two different concentrations, the tabulation of results must then rest on the "observed feeding rate" which is inherently less accurate than the "total feeding rate" as determined by post-experimental dissections. Adding to this the fact that a membrane system would ideally be used simply to assess whether or not a compound showed any promising repellent activity within reasonable concentration ranges, it is submitted that the system used here is probably adequate to that purpose.

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POSSIBLE SITE OF ENTRY OF THE REGULAR MOSQUITO IRIDESCENT VIRUS (RMIV) IN *Aedes taeniorhynchus* LARVAE¹

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Early interest in the mosquito iridescent virus (MIV) of *Aedes taeniorhynchus* as a biological control agent was fostered by observations that virtually all overtly infected larvae died before or during the pupal stage (Chapman et al. 1966, Hall and Anthony 1971). However, these hopes were subverted by failures to achieve more than 10-20% transmission of the virus by *per os* exposures, regardless of dosage. Attempts to explain the low infectivity of MIV by determining its mode of entry were unsuccessful (Hall and Anthony 1971, Stoltz and Summers 1971). Because cuticle lines both the foregut and hindgut of the mosquito, it is unlikely that any object or organism lacking active invasive powers would enter by these routes.

¹ Opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. Use of proprietary names does not constitute endorsement.

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However, Hall and Anthony (1971) found replicating virus in foregut tissue in well developed infections. The midgut is the most likely site of entry, but virus particles have never been found in midgut epithelium. Stoltz and Summers (1971) observed that most, if not all, MIV particles were degraded shortly after entering the midgut. The possibility that infections were established by DNA molecules crossing the gut barrier has not been excluded. In this paper we describe an attempt to locate the site of entry of MIV at the anterior end of the midgut of *Ae. taeniorhynchus*.

The RMIV strain of MIV was used (Matta and Lowe 1970). The *Ae. taeniorhynchus* were from a laboratory colony known to be free from MIV. Two-hundred 1st stage larvae were exposed in a 10 cm petri dish to an inoculum of 10 triturated, overtly infected 4th stage larvae. Samples of exposed larvae were removed from the exposure medium after 5, 10, 20 and 30 min and 1, 1.5, 2, 3, 4 and 5 hr of exposure. Head capsules and air tubes were excised and the larvae were fixed for 4 hr at room temperature in 4% glutaraldehyde. Larvae were post-fixed in 1% osmium tetroxide and embedded in epon-araldite according to Mollenhauer (1964). Sections were cut at the level of the anterior end of the midgut with a Sorvall Porter-Blum MT-2 Ultramicrotome and stained according to Venable and Coggeshall (1965). Sections were examined and photographed with a Hitachi 125 E electron microscope using accelerating voltages of 50 to 75 KV.

In a specimen fixed 5 hr after the initiation of exposure, virus like particles were found

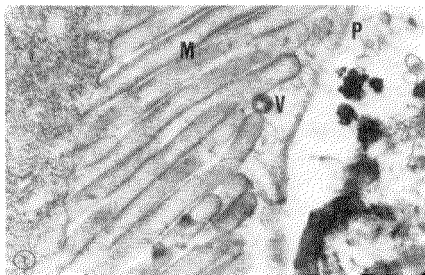


Fig. 1. Virus like particle between peritrophic membrane and midgut epithelium, 45,000X. (M-microvilli on midgut epithelium; P-peritrophic membrane; V-virus like particle).

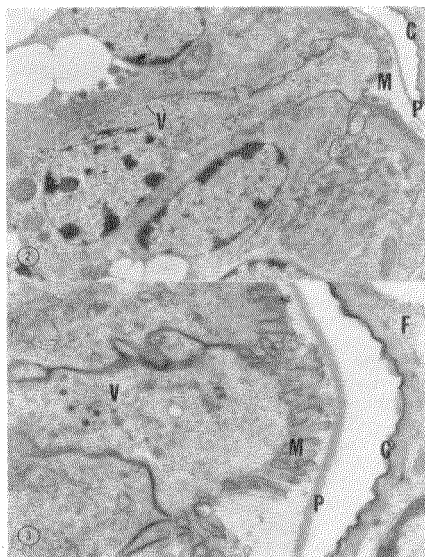


Fig. 2. Midgut epithelial cell containing RMIV particles, 10,000X. (M-microvilli on midgut epithelial cell; P-peritrophic membrane; V-virus particles).

Fig. 3. Higher magnification of same field as seen in Figure 2, 25,650X (C-cuticle on foregut invagination; F-foregut invagination; M-microvilli on midgut epithelial cell; P-peritrophic membrane; V-virus particles).

outside the peritrophic membrane against microvilli of the midgut epithelium (Figure 1). Numerous virus particles were also seen within 1 midgut epithelial cell (Figures 2 and 3). The infected cell was lateral to the foregut invagination into the midgut as can be determined by the presence of cuticle on the foregut invagination. These particles were of the same size and shape as RMIV particles and were not seen in adjacent cells. RMIV replicating in *Ae. aegypti* cell cultures does not produce intact particles until about 48 hr after uptake (Webb et al. 1976). Therefore, it appears unlikely that the virus particles observed were the product of a recently established infection.

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United States, Ontario and Quebec in Canada, in Mexico, and in the West Indies (Amrine and Butler 1978, Carpenter and LaCasse 1955, Carpenter 1968, 1970, 1974). The species has been recorded to the north of Colorado in Scotts Bluff County of western Nebraska (Edmunds 1958) and to the south in New Mexico (Sublette and Sublette 1970); it has not been reported from states farther west.

The record specimen was collected in a New Jersey light trap near Building 529 on the Army installation. The area surrounding the trap site is flat prairie with some small bushes. The Arkansas River is located about 2 mi. away, and there are several smaller semi-permanent and permanent bodies of water within the same distance of the trap site. The climate of the Pueblo area is semi-arid with warm summers and comparatively mild winters. Precipitation is 11 in. per year.

The identification was made by this Agency and confirmed by E. L. Peyton, Medical Entomology Project, US National Museum of Natural History (USNM), Smithsonian Institution, Washington, DC, for which we are grateful. The specimen is deposited in the USNM.

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NEW RECORD FOR *URANOTAENIA SAPPHIRINA* IN COLORADO¹

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On 25 September 1979, one female *Uranotaenia sapphirina* (Osten Sacken, 1868) was collected on Pueblo Army Depot Activity, Pueblo, Colorado, by Roy Shrove, Environmental Health Technician with the US Army Civilian Employee Health Clinic. This finding is the first record of the species in Colorado.

Uranotaenia sapphirina has previously been reported from 37 states and the District of Columbia in the eastern two-thirds of the

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