# AN *IN VITRO* TECHNIQUE FOR THE BIOASSAY OF REPELLENTS AGAINST BLACK FLIES

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The attempt to control attacks of biting flies by insecticides alone has come under increasing scrutiny in most Western countries. As well, there are circumstances such as in the high northern latitudes where pest control is impracticable except under highly unusual conditions. Indeed, the current technological forays into desolate areas for the exploitation of non-renewable resources has highlighted the severity of the biting fly problem in parts of the Canadian North. The search for more effective protection of personnel from these insects has re-emphasized the need for long-lasting and non-toxic repellents.

A screening program to bioassay the effectiveness of candidate repellents can be both lengthy and subject to statistical problems such as the individual attractiveness of experimental hosts. Screening procedures are doubly difficult with respect to black flies, due to their well-known reluctance to feed reliably under laboratory conditions, so that it is ordinarily necessary to await the next "biting season" to test the repellents in the field. However, the development of artificial membrane systems suitable for use with at least those simuliid species which *will* feed reliably has provided opportunities for more closely examining the possibility of using an *in vitro* device for testing repellents against these pests.

A blood/membrane system (Mokry 1976) was used as the surrogate host in these experiments. The exposed surface area of each of the 4 membranes was 4.5 $cm^2$. The tests were originally conducted using DEET (N, N-diethyl-m-toluamide) Shoo-bug Space-Shield II® (75% active ingredients) as a standard. Later, preliminary comparisons were done with experimental repellents supplied by Dr. Walborg Thorsell, National Defence Research Institute, Stockholm, Sweden. Dilutions of the repellents were applied by means of micropipettes. Five minutes drying time was allotted for the repellent-treated membranes and ethanol-washed control membrance before allowing adult female black flies access to them.

In the initial trials with DEET-treated membranes, *Simulium vittatum* females were lab-reared from eggs in a stirbar system (Colbo and Thompson 1978). Emergent adults were kept overnight in vials with free access to a sucrose solution. All females were tested for their willingness to feed on blood through the treated or untreated membrances within 24 hr of emergence. The test period was 20 min, after which the females were dissected and the number with ingested blood, even if in trace amounts only, was recorded.

Later trials were undertaken with *Prosimulium mixtum*, netted in the field and transported to the laboratory in styrofoam coolers. The females were given an opportunity to feed through the membrances within 2 hr of capture. DEET again served as the test repellent. Preliminary comparisons with compound 703 and 2 plant extracts, (all supplied by Dr. Walborg Thorsell), were undertaken.

*S. vittatum* females fed well through untreated control membranes, 58/73, 79%. There was a marked drop in feeding as the amount of DEET applied to the membrane was increased); 0.15 mg/$cm^2$, 47%; 0.30 mg/$cm^2$, 32%; 0.75 mg/$cm^2$, 4%.

Field-collected *P. mixtum* also fed readily through untreated membranes, 103/129, 80%. The results for DEET-treated membranes showed the same trend as those recorded for *S. vittatum;* 0.03 mg/$cm^2$, 71%; 0.15 mg/$cm^2$, 45%; 0.75 mg/$cm^2$, 4%. Compound 703 gave similar results; 0.03 mg/$cm^2$, 68%; 0.15 mg/$cm^2$, 47%. The 2 plant extracts tested also showed some repellent activity; 0.11 mg/$cm^2$, 44% and 57%.

These results indicate that an artificial membrane system may be used in the screening of repellent compounds for activity against black flies, as has already been done for mosquitoes (Bar-Zeev and Smith 1959, Rutledge et al. 1976). In this connection a major problem in the past has been inducing simuliids to feed reliably on blood in the laboratory. Despite this, common, and in most regards "typical," species often prove suitable. Thus field-collected *S. venustum* (Sutcliffe and McIver 1975), and both field-collected *P. mixtum* and lab-reared *S. vittatum* are all suitable North American examples. The fact that the latter feeds readily while unmated and only 24 hr old, and is easily reared in the laboratory, makes it especially attractive for the bioassay of candidate repellents.

As pointed out by Rutledge et al. (1976), much higher repellent concentrations are required in a "no choice" situation to achieve results similar to a "free choice" context. The concentrations used here gave very similar results to those of Bar-Zeev and Smith (1959) for

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.

ACKNOWLEDGMENTS. The author is grateful to Dr. Marshall Laird and Dr. Jean Finney who critically reviewed this manuscript and to Dr. Walborg Thorsell who originally suggested these experiments. This work was supported by grants to the Research Unit on Vector Pathology from the National Sciences and Engineering Research Council of Canada and the International Development Research Centre of Canada.

*Literature Cited*

Bar-Zeev, M. and C. N. Smith. 1959. Action of repellents on mosquitoes feeding through treated membrances or on treated blood. J. Econ. Entomol. 52:263–267.

Colbo, M. H. and B. H. Thompson. 1978. An efficient technique for laboratory rearing of *Simulium verecundum* S. & J. (Diptera: Simuliidae). Can. J. Zool. 56:507–510.

Grunewald, J. and H. P. Wirtz. 1978. Künstliche Blutfütterung einiger afrikanischer und paläarktischer Simuliiden (Diptera). Z. Ang. Entomol. 85:425–435.

McMahon, J. P. 1968. Artificial feeding of *Simulium* vectors of human and bovine onchocerciasis. Bull. Wld. Hlth. Org. 38:957–966.

Mokry, J. E. 1976. A simplified membrane technique for feeding blackflies (Diptera: Simuliidae) on blood in the laboratory. Bull. Wld. Hlth. Org. 53:127–129.

Rutledge, L. C., M. A. Moussa and C. J. Belletti. 1976. An *in vitro* blood-feeding system for quantitative testing of mosquito repellents. Mosq. News 36:283–293.

Sutcliffe, J. F. and S. B. McIver. 1975. Artificial feeding of simuliids (*Simulium venustum*): Factors associated with probing and gorging. Experientia 36:694–695.

Wenk, P. 1965. Über die Biologie blutsaugender Simuliiden (Diptera) I. Besamungsrate der ♀♀ beim Blutenbesuch und Anflug auf den Blut wirt. Z. Morphol. Okol. Tiere. 55:656–670.

## POSSIBLE SITE OF ENTRY OF THE REGULAR MOSQUITO IRIDESCENT VIRUS (RMIV) IN *AEDES TAENIORHYNCHUS* LARVAE[1]

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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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