FIELD EVALUATION OF SEVERAL REPELLENTS AGAINST BLACK FLIES (DIPTERA, SIMULIIDAE) [1]

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ABSTRACT. Deet (N, N-Diethyl-meta-toluamide) and twelve promising experimental repellents impregnated in mesh polyester-cotton jackets were tested against black flies in the central valley of Costa Rica and northern New York during 1975. Experimental repellents were paired with a control and a deet standard in each series of tests. Ratios of effectiveness were calculated for relative comparisons. The best compounds tested were 2 hydroxyethyl cyclohexane carboxylate (AI3-70087) and tetrahydrofurfuryl octanoate (AI3-8118).

INTRODUCTION. With recognition of the spatial repellency of impregnated mesh netting, considerable attention has been directed to the use of mesh jackets as a means of providing individual protection from biting Diptera. In the search for effective repellents for impregnation of netting, the majority of research effort has been directed toward mosquitoes both in the laboratory (Gouck et al. 1967b, Schreck et al. 1970) and in the field (Gouck et al. 1967a, Grothaus et al. 1972, 1974). The mesh jacket concept would assume additional importance if adequate protection against black flies (Simuliidae) could be demonstrated. Black flies present a severe nuisance in temperate regions and are involved in the biological transmission of pathogenic organisms to man in tropical areas.

Jackets fabricated of polyester and cotton impregnated with deet were shown to be highly effective compared to topical applications of the same compound against several nearctic species of black flies during the spring of 1974 at Camp Drum, New York (Frommer et al. 1975). It is recognized, however, that deet does not provide all the desired characteristics of the optimum insect repellent. Deet is becoming increasingly difficult to obtain, it does not provide the longevity of many other compounds, and its effectiveness is greatly reduced after wetting (Gouck et al. 1971). It is therefore necessary to screen additional compounds, utilizing the jacket concept, which might prove to be as effective as deet while providing more desirable characteristics of an optimum repellent. The purpose of this paper is to report on two studies designed to evaluate deet and 12 promising experimental repellents against tropical and temperate species of black flies.

METHODS AND MATERIALS. Polyester-cotton, waist length, long sleeved overjackets of .635 cm mesh dyed Army olive drab, shade 107 were used. Each jacket weighed approximately 130 g and was impregnated with 0.25 g of repellent per gram of fabric weight by placing it into a measured solution of the technical grade repellent compound in acetone carrier until the solution was absorbed. After treatment, the jackets were air dried and sealed in plastic bags. Impregnation was accomplished 3 three weeks prior to field testing. The repellent compounds used are shown in Table 1.

Tests were conducted in two locations: in the central valley of Costa Rica during February 1975, and in northern New York during May 1975. In Costa Rica, approximately 30 sites were examined and the 4 sites having the greatest adult black

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Table 1. Repellent compounds and location at which tested.

USDA ENT Code No.	Compound definition	Costa Rica	New York
AI3-2830-Gc	Diisopentyl malate	x	
AI3-3775b	1,3-Propanediol, 2-butyl-2-ethyl 1-ethyl		x
AI3-5974q	1-Butyryl 1-1, 2,3,4-Tetrahydroquinoline		x
AI3-7627Gg	1-Butyl-4-methyl-carbostyril		x
AI3-8118-Gd	Tetrahydrofurfuryl octanoate	x	x
AI3-14825-Gc	1,3-bis (Butoxymethyl)-2-imidazolidinone	x	
AI3-18059-Gd	3-Chloro-1-propanol carbanilate	x	x
AI3-19083-Gb	o-Ethoxy-N, N-dipropylbenzamide	x	
AI3-19084-Gc	N, N-Dibutyl-o-ethoxybenzamide	x	x
AI3-20364-a	1-(o-Ethoxybenzoyl) piperidine	x	x
AI3-20573-Gb	2-[(p-Methoxybenzyl)oxy]-N, N-dipropylacetamide	x	
AI3-22542	N, N-Diethyl-meta-toluamide (deet)	x	x
AI3-70087bGh	2 Hydroxyethyl cyclohexane carboxylate		x

fly activity were selected for the tests. The selected sites were located near Aserri (San Jose Province), Carrizal (Alajuela Province), Navarro (Cartago Province) and Orosi (Cartago Province). The sites at Aserri and Carrizal have been described in greater detail during earlier black fly bionomics studies by Vargas and Travis (1973) as their study sites numbers 4 and 31. In New York, the single study site was located on the military reservation at Fort Drum in Jefferson County near Watertown, New York.

Eight experimental repellents were compared to deet and to an acetone treated jacket (control) in both Costa Rica and New York. The four best repellents from the Costa Rican study plus four additional promising repellents were evaluated in New York. The areas at which the various repellents were tested are shown in Table 1. In each study, local code numbers were assigned to each of the 9 repellent-treated jackets (8 experimental repellents plus deet) and to the control jackets. These numbers were drawn at random to determine the sequence of testing for both participants and repellent jackets.

The procedure for evaluation of the jackets involved four people (2 pairs) seated on stools approximately one meter apart with each participant observing his partner. Two unknown repellents, one standard (deet), and one control were evaluated at one time. Each test consisted of two timed observations of black fly landing counts for the 10 treatments at one location. After each timed observation period, subjects changed locations so the second count was made by a different observer. Ten-minute periods were used in Costa Rica and 5-minute periods were used in New York. After the two observation periods, jackets were changed according to preselected random assignment, and this process continued until all 10 treatments had been tested. Ten new cotton utility shirts, laundered several times, were matched to each repellent mesh jacket to avoid masking of repellents as jackets were changed by participants. The mesh jackets were worn over the shirts and both were stored separately in plastic bags when not in use. Landing counts were recorded in 3 areas of the upper ventral body: (1) jacket only; (2) unprotected face and hands only; and (3) total above waist. These categories allowed statistical comparisons for both spatial and contact repellency. The tests were replicated 10 times in Costa Rica and 12 times in New York.

An additional comparison of the experimental compounds as affected by wetting was conducted in New York. Four replications of observations on the unwashed mesh jackets were made; the jackets were then exposed to the equiva-

lent of 2.54 cm rainfall followed by 4 replications of observations. This treatment was continued for the equivalent of 5.08 cm simulated rainfall. Rainfall was simulated by exposing the jackets in a shower bath. Approximately 30 seconds was the time required to reach an equivalent of 2.54 cm rainfall, approximately 1 m from the shower head. The exact time was determined for each treatment from 3 timed trials by using a standard meteorological rain gauge.

For the purpose of calculating ratios of effectiveness and subsequent statistical analysis of the relative effectiveness of the repellent compounds, a value of one was added to each observation. This was necessary to eliminate occasional negative landing counts on the repellent jacket and to permit the necessary mathematical calculations. Ratios of effectiveness of each repellent to the untreated control and to the deet standard were calculated by dividing the landing count for the two timed observations on the control and deet standard by the paired repellent in each of the 3 areas of observation: landings on jacket only; landings on face and hands only; and total landings above the waist.

An additional transformation of the New York data was made by converting to square roots to compensate for an abnormal distribution. The ratios were reconverted after analysis. The ratios were analyzed using standard analysis of variance of a randomized block design and Duncan's Multiple Range Tests (Duncan 1955).

RESULTS AND DISCUSSION. The tropical species of black flies against which the jackets were tested in Costa Rica included *Simulium metallicum* Bellardi (56%), *S. quadrivittatum* Loew (37%) and *S. callidum* (Dyar and Shannon) (7%). The percentages were determined from collecting samples of landing black flies at each site and are based on a total of 177 identifications. Travis (1974) reported *S. quadrivittatum* to be the predominant species in the central valley of Costa Rica. Both *S. metallicum* and *S. callidum* have been implicated in the transmission of onchocerciasis in Guatemala (Dalmat 1954). The black fly population tested in Costa Rica was much smaller than that observed at Fort Drum, New York. The Costa Rican sites were rather typical of tropical ecosystems where relatively small populations of individual species can occur over long periods of time. The total number of black flies observed landing during the 10 tests in Costa Rica were 2,688. Of these, 2,104 fly landings were made on subjects wearing control jackets.

The predominant temperate zone species of Simuliidae against which the jackets were tested in New York were members of the *Prosimulium hirtipes* complex. Twelve hundred larvae collected 1 week prior to the study from the single study site showed 54% *P. hirtipes* complex and 46% *Cnephia mutata*. However, *C. mutata* rarely attacks man. The total number of black flies observed landing during the 12 tests in New York was 10,756 with 6,399 landing on subjects wearing control jackets.

Black flies are extremely sensitive to environmental changes, and the number of landings varied greatly within a given day and from one day to the next. To minimize the effect of environmental fluctuations, and to provide a more meaningful measure of effectiveness, a random experimental repellent was always paired with the control or the standard repellent. The environmental parameters measured were temperature, relative humidity, and wind. In Costa Rica, the average temperature during the test periods was 22.0°C with a relative humidity of 60.2%. Winds were light and variable from 0–8 km/hr. At the New York test site the average temperature was 26.1°C with a relative humidity of 50% and wind velocity from 0–16 km/hr.

The mean relative effectiveness of the repellent treated jackets to the untreated control and to the standard repellent, deet, is shown in Table 2 for the neotropical species and in Table 3 for the nearctic species.

Table 2. Ranked mean ratio of effectiveness for 20 observations of 10 repellent treatments to control and deet against Costa Rican black flies.

Repellent	Ratio to control			Ratio to deet		
	Total Landings*	Jacket	Face and Hands	Total Landings	Jacket	Face and Hands
8118	7.08 b	5.63 b	3.07 a	1.17 a	0.87 ab	1.40 a
14825	3.51 cd**	3.75 bc	1.90 a	0.65 b	0.69 bc	0.83 bc
18059	5.77 bc	5.67 b	2.68 a	0.37 bc	0.49 d	0.54 cd
19083	3.12 cd	4.19 bc	1.43 a	0.47 bc	0.64 cd	0.57 cd
19084	4.10 bcd	5.49 b	1.42 a	0.79 ab	0.71 bc	0.75 bcd
20364	5.13 bc	5.88 b	2.18 a	0.61 bc	0.78 bc	0.67 cd
20573	2.57 cd	3.39 bc	1.39 a	0.39 bc	0.53 cd	0.51 cd
20830	3.82 bcd	3.50 bc	2.16 a	0.44 bc	0.48 d	0.72 bcd
22542	10.71 a	9.13 a	3.33 a	1.10 a****	1.05 a	1.05 b
control	1.60 d***	1.27 c	1.83 a	0.10 c	0.12 e	0.42 d

* Landings on jacket, face, and hands.
** Means with same letter do not significantly differ (5% level, Duncan's Multiple Range Test).
*** Mean ratio between two control jackets.
**** Mean ratio between two deet jackets.

In the tropical site, deet (AI3–22542) was significantly better than the other 8 repellents tested in preventing black flies from landing on the upper ventral aspect of the body, as well as on the jacket when ranked with the control (Table 2). When ranked with deet, tetrahydrofurfuryl octanoate (AI3–8118) and N, N-Dibutyl-o-ethoxybenzamide (AI3–19084) appeared not significantly different from deet in preventing total black fly landings. Also, when ranked with deet, tetrahydrofurfuryl octanoate showed more spatial repellency than deet as indicated by fewer landings on the unprotected hands and face. This compound was also shown to be the most effective chemical against black flies in Maine during the screening of 24 compounds by USDA investigators in 1971 (Gouck, personal communications).

In New York, a previously untested repellent against black flies, 2 hydroxyethyl cyclohexane carboxylate (AI3–70087) was much more effective than other repellents

Table 3. Ranked mean ratio of effectiveness for 24 observations of 10 repellent treatments to control and deet against New York black flies.

Repellent	Ratio to control			Ratio to deet		
	Total Landings*	Jacket	Face and Hands	Total Landings	Jacket	Face and Hands
3775	2.51 cd**	2.74 c	2.36 bc	0.55 cd	0.64 cd	0.57 cd
5974	3.53 c	5.54 b	2.30 bc	1.26 bc	1.95 b	0.99 cd
7627	1.85 cd	1.93 cd	1.32 c	0.38 e	0.46 d	0.40 d
8118	2.62 cd	2.56 c	2.65 bc	0.98 cd	1.04 c	1.13 bc
18059	2.06 cd	2.68 c	1.62 c	0.50 cd	0.74 cd	0.46 cd
19084	1.65 cd	2.07 cd	1.53 c	0.50 cd	0.64 cd	0.52 cd
20364	2.51 cd	2.50 c	1.37 c	0.52 cd	1.02 c	0.48 cd
22542	6.33 b	4.85 b	5.32 b	2.18 b****	1.73 b	1.92 b
70087	18.16 a	10.88 a	13.81 a	5.62 a	4.20 a	3.76 a
control	0.90 d***	0.85 d	0.93 c	0.44 e	0.37 d	0.42 d

* Landings on jacket, face, and hands.
** Means with same letter do not significantly differ (5% level, Duncan's Multiple Range Test).
*** Mean ratio between two control jackets.
**** Mean ratio between two deet jackets.

when ranked with the control (Table 3). Deet was the second best repellent tested in preventing landings on the upper ventral torso. Also, when ranked with the control, 1-Butyryl 1-1, 2, 3, 4-Tetrahydroquinoline (AI3-5974) was as effective as deet in preventing landings on the jacket, but permitted significantly more landings on the face and hands than did deet. When compared to deet, only 2 hydroxyethyl cyclohexane carboxylate was consistently better than deet in preventing landings on the jacket and exposed face and hands.

Rainfall, as expected, had a definite adverse affect on the repellent jackets' effectiveness. Simulated rainfall of 2.54 cm caused an overall 26% reduction in the jackets' effectiveness compared to the control. The effectiveness of deet decreased with wetting more than the other repellents combined. Examination of the landing ratios for each comparison showed 3 compounds whose effectiveness did not appear to be reduced by the total 5.08 cm simulated rainfall. These were repellents 1-Butyl-4-methylcarbostyril (AI3-7627), 3-Chloro-1-propanol carbanilate (AI3-18059), and 2 Hydroxyethyl cyclohexane carboxylate (AI3-70087). Gouck et al. (1971) reported Compound AI3-7627 to remain more than 90% effective after exposure to 6.86 cm rainfall.

This investigation showed that jackets provided better protection in New York than in Costa Rica. Differences in the biting habits of the two groups of black flies attributed to this difference. The Costa Rican species prefer to bite the lower extremities and gradually move to the upper torso only if they are unable to bite in the lower regions. Therefore, additional protection would have to be provided to protect the legs. The small number of flies reaching the face and hands during the Costa Rican test prevented obtaining enough data to clearly show differences in the spatial repellency of those compounds tested. Repellents tested on the much larger black fly population in the New York study area showed significant reductions in black fly landings both on the jacket and on the face and hands.

The repellent 2 Hydroxyethyl cyclohexane carboxylate (AI3-70087) appears to be the best compound for use in impregnating mesh jackets for protection against black fly bites in the temperate zone. Although deet (AI3-22542) and two other compounds adequately protected the upper body against bites from tropical species of black flies, they did not provide adequate protection from black fly bites because of the different biting habits of those flies.

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