AREA PROTECTION BY USE OF REPELLENT-TREATED NETTING AGAINST *CULICOIDES* BITING MIDGES[1]

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ABSTRACT. Enclosures 2 m high and up to 12 m square covered with light-weight cotton-polyester netting were treated with either deet, diisopentyl malate (DPM) or indalone to determine if the biting midges, *Culicoides mississippiensis, C. furens, C. barbosai* and *C. floridensis,* attracted by CO_2 or human bait within the enclosures could be prevented from entering the area. The studies indicated that deet and DPM treatments provided good area protection to people for 4 and 5 days, respectively, while indalone in one test was somewhat erratic in its effectiveness. The residual effectiveness of the DPM treatment was greater than the deet treatment after exposure to rain.

Research on spatial or area repellency of various materials to hematophagous insects has been rather limited. Recently however, Grothaus et al. (1976) studied clothing and nets impregnated with spatially active repellents for mosquitoes and biting flies, and Sholdt et al. (1976) found that treating African dwellings with deet (*N,N*-diethyl-meta-toluamide) significantly reduced the numbers of anopheline mosquitoes entering the homes. Means (1978) and Wirtz et al. (1980) reported field and laboratory treatments using naphthalene, methylated naphthalenes, essential oils and repellents as area repellents of mosquitoes and black flies (*Simulium* spp.).

Studies with deet-treated net jackets made of light-weight polyester-cotton against several species of biting midges *Culicoides* spp. (Schreck et al. 1979, Harlan et al. 1983), indicated the spatial action of the repellent deet was effective in preventing the midges from passing through the treated jacket.

On the basis of these observations, we initiated a preliminary field trial in which a 3 × 3 × 2 m high (18 m^3 space) enclosure of deet-treated netting was successfully used to determine that a limited area could be protected from biting midge intrusion. This paper reports the methodology used, the efficacy of three repellents, and the duration of repellency observed when the treated perimeter of the space to be protected was increased several fold.

MATERIALS AND METHODS

LOCATION. The study site was an open area on an island in the salt marsh near Yankeetown, Levy County, Florida. The island is connected to the mainland by a narrow causeway over the marsh. The marsh is characterized by large expanses of black needlerush (*Juncus roemerianus* Steele) interrupted by tidal creeks bordered with smooth cordgrass (*Spartina alternifolia* Loisl.) and vegetated islands of limerock outcroppings. The major island vegetation is composed of live oak (*Quercus virginiana* Mill), southern red cedar (*Juniperus silicicola* (Small) Bailey), yaupon (*Ilex vomitoria* Ait.) and cabbage palmetto (*Sabal palmetto* (Walt.) Lodd.).

The major pest species are *Culicoides mississippiensis* Hoffman, in the spring and fall and *C. furens* (Poey) and *C. barbosai* Wirth and Blanton during the late spring and throughout the summer (Kline and Roberts 1981).

TEST PROCEDURES. For the first series of tests (April–May 1981), 2 square frames each 6 m on a side and 2 m high (72 m^3) were placed 20 m apart at the test site.

The frames were constructed of 2.54 cm (1 in) diam white polyvinyl-chloride pipe (PVC) using the appropriate numbers of elbow and "T" joints for pipe connections. Bleached, lightweight polyester netting with cotton strands running through it (four 1 mm cotton strands and nine 0.25 mm polyester strands/cm^2 with ca 40% open space) as described by Grothaus et al. (1976), was attached to the sides by stitching with a staple machine (Figure 1).

The netting was first treated at the laboratory by pouring a solution of deet in ethanol (sufficient to saturate the net) over it in a open plastic bag. The bag was then sealed and held for 2 days to allow the treatment to thoroughly penetrate the net. The treatment rate was 0.25 gm AI (Active Ingredient)/gm netting. A gm of netting occupies an area of ca 200 cm^2. The untreated control was saturated with an equivalent amount of ethanol only.

Treatments were made in the laboratory for the first 2 field evaluations. Thereafter all

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treatments were sprayed on the net while on the frame in the field using a hand pumped compressed air stainless steel pesticide sprayer. All evaluations were made 24 hr after treatment and repeated every 24 hr until repellency failed. The net was retreated when <80% effectiveness was reached for 2 consecutive tests.

A carbon dioxide (CO_2) baited sticky trap was centrally located in each framed area (Fig. 1). The traps were 23 × 25 cm Alsynite® panels marked with a 1.5 cm grid, coated with Tac Trap® and positioned 1 m above the ground. Carbon dioxide metered at a flow rate of 0.5 liter/min was released through perforated tubing above the sticky panel. Midges attracted to the CO_2, as it flowed down over the panel, were trapped in the sticky surface. The marked grid made it easier to count those trapped. Biting midge populations were monitored daily with a third CO_2 baited sticky trap set up in the open on the edge of the island 75 m east of the net covered units. This "outside" trap was operated at the same location throughout these tests. The 3 trap units were serviced every 24 hr by replacing the sticky panels and returning them to the laboratory where trapped midges were identified, counted and recorded.

For the second series of tests (May–July 1981), the 2 PVC) pipe frames were dismantled and then reassembled to create one square frame 12 m on a side and 2 m high (288 m^3). A new net was stitched to this frame and then was sprayed with the deet solution at 0.25 gm

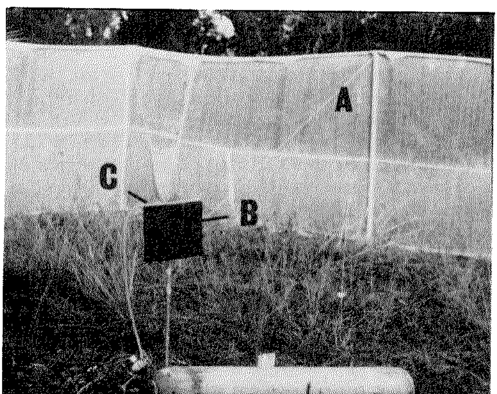

Fig. 1. Carbon dioxide baited sticky trap centrally located in the framed area. A - PVC pipe frame covered with netting. B - Alsynite® panel marked with a grid and coated with Tac Trap®, C - Perforated tubing above panel thru which CO_2 was released.

AI/gm net (Fig. 2) and evaluated at 24 hr intervals until it no longer provided the desired effectiveness (<80%), then retreated.

A sticky trap was centrally located in the new enclosure and the population monitoring "outside" trap was operated as described above.

The relative effectiveness of each treatment of the first 2 series was determined by comparing the trapping rate inside the treated area with that of the untreated area or the "outside" trap. From these data a coefficient of protection from intrusion (CPI) into the treated area was calculated by using the following formula: $\frac{(A-B) \times 100}{A}$, where A is the number trapped inside the untreated control net or the "outside" trap in 24 hr and B is the number trapped in the treated area in 24 hr. A CPI of 80% or more was considered an effective level of repellency while a CPI of less than 80% was considered a failure. A CPI of 80% might not seem sufficient when very high population densities are recorded, but for the purposes of these tests we felt it a useful figure when comparing the residual efficacy of the various treatments.

For the third series (March 1982), human bait was used inside and outside the 12 × 12 m net enclosure treated with deet. The purpose of this series was to determine if a person within the treated space could be protected from bites. Evaluations were made before noon in one set of tests or at dusk in a second set due to changes in biting midge activity that correspond to seasonal variation and temperature (Kline and Roberts 1981). One test subject counted the numbers of midges biting the forearm and hands inside the enclosure and the other did likewise outside the enclosure (ca 20 m distant) for 1 hr each test day. Positions of subjects were reversed for each test. Evaluations were at 1 hr after treatment and weather permitting, each 24 hr period thereafter until failure (<80% CPI), at which time the net enclosures were retreated. In these and the following tests the CPI was based on the numbers of bites recorded on subjects inside and outside the treated net enclosure. Collections of midges biting were made for each series to determine species identity and composition of the population during tests.

For the fourth series (April–May 1982), the net was replaced and the new net was spray-treated with AI3–20830 (diisopentyl malate (DPM) in ethanol solution at the rate of 0.125 or 0.25 gm AI/gm net. This compound is one of several reported by Gouck et al. 1971 as having effective residual repellency (67 days) against the salt marsh mosquito *Aedes taeniorhynchus*

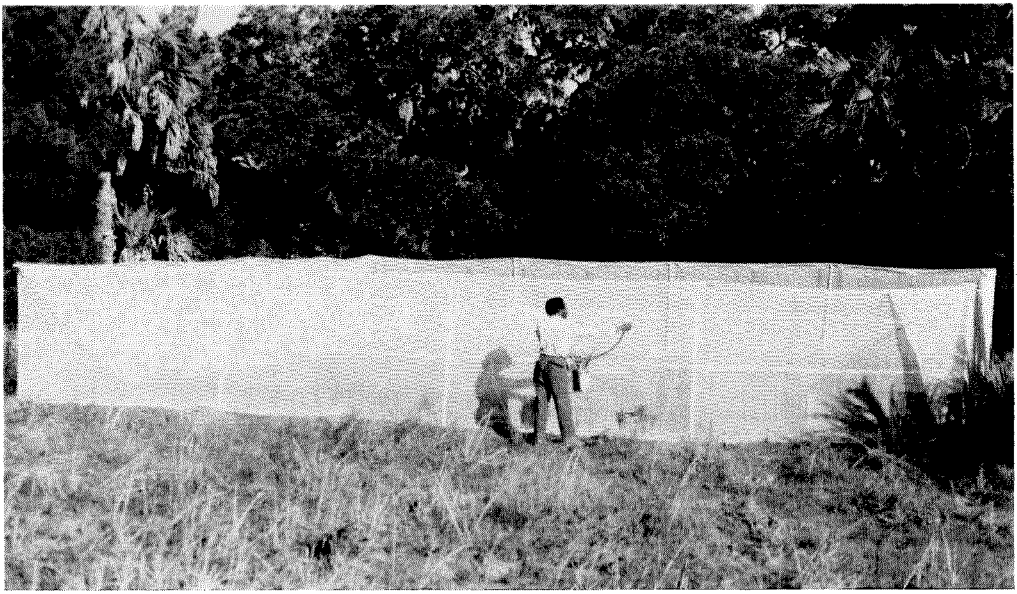

Fig. 2. Netting covered frame in 12 × 12 m configuration being sprayed with the repellent candidate.

Wiedemann at a treatment rate of 0.25 mg/cm^2. Such compounds might provide long lasting area protection by repelling *Culicoides* species as well. Human bait was used inside and outside the net enclosure for each test as in test series 3; all tests in series 4 were in the evening.

For the fifth series (October 1982), the net was sprayed with indalone (*N*-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyrone-2-carboxylate) at 0.25 gm AI/gm net. Human bait was used as in series 3 and 4, however all tests were conducted in the morning.

Weather records were kept for the entire study. It should be noted that weather conditions affected the numbers of midges recorded and the numbers of replicated tests at the various hours after treatment. Wind and/or rain directly affected the activity of the midges in terms of numbers trapped or biting.

RESULTS AND DISCUSSION

Greater numbers of midges were always (13 tests) recorded from the untreated enclosure trap (1239.6 ± 2094.7 SD) than from the "outside" trap (627.1 ± 1341.3 SD). This indicated the untreated net did not provide a physical barrier to biting midge intrusion, also, the standard deviations indicate the variability in midge density from day to day. Further tests with the untreated net would not be necessary because the "outside" trap provided adequate population density data.

Table 1 gives data indicating the effectiveness of the 2 m high deet treated nets of 2 sizes. Based on the trapping data in 24 hr, the deet treatment provided the 72 m^3 space with over a 99% CPI or about 0.05 midges/hr, while the 288 m^3 space was provided with ca a 92% CPI or about 13 midges/hr. Rainfall data from the 2 series (Table 1) were insufficient to determine if this environmental parameter affected the duration of protection.

Table 2 gives the results of the 3rd series of tests using bites recorded on human bait to determine CPI. The means for 8 tests at 1 hr after treatment showed less than 1 bite occurred in 1 hr inside the treated net while 278 bites were recorded outside the net. Thus the treatment gave over a 99% CPI. The residual effectiveness of deet was evaluated in a limited number of tests up to 96 hr (4 day) after treatment. The CPI from bites was 89% or greater for 24 to 96 hr after treatment when no rain fell on the netting. When rainfall occurred in these tests (Table 2) the repellency of the deet treatment was quickly lost. Rainfall and evaporation were probably the major causes for loss in effectiveness. *Culicoides mississippiensis* was the only species present during these tests.

Table 3 gives the results of the 4th and 5th series of tests using biting rates on human sub-

Table 1. Mean numbers of biting midges (*Culicoides* spp) caught in 24 hr by CO_2 baited sticky traps inside net enclosures 24 hr after treatment with deet at 0.25 gm/gm net.

Area of enclosure (m^3)	No. tests	Rainfall (cm)	Mean no. of midges[a] trapped in 24 hours[b]			CPI[c] based on:		Mean no. days effective (80% CPI)
			Deet-treated net	Untreated net	"Outside" net	Untreated net	"Outside" net	
72	3	0.3	1.3(0.05)	556.7(23.2)	344.7 (14.4)	99.8	99.6	2.3
288	4	4.1	314.5(13.1)	—	3853.3(160.5)	—	91.8	1.5

[a] Species present April and May 1981 inside and outside the nets were: 99.8% *C. mississippiensis* while testing the 72 m^3 enclosure and 61% *C. furens*, 14% *C. mississippiensis*, 23% *C. barbosai* and 2% (mostly) *C. floridensis* (May–July 1981) while testing the 288 m^3 enclosure.
[b] Numbers in ( ) are the mean numbers trapped/hr.
[c] Coefficient of protection from intrusion (see text for formula).

Table 2. Spatial repellency of deet-treated netting (0.25 gm/gm net 12 × 12 × 2 m high) as determined by the biting of *Culicoides mississippiensis* on human test subjects during a 1 hr exposure.

Time after treatment (hr)	No. tests	Rainfall (cm)	Mean no. of midges biting in 1 hr[a, b]		CPI[c] inside deet-treated enclosure
			Inside net	Outside net	
1	8	0	0.8	278	99.7
24	3	0	1.7	15	88.7
24	1	0.89	287	972	70.5
48	2	0	1.0	22	95.5
72	1	0	0	74	100.0
96	1	0	7	112	93.8
96	1	0.64	48	54	11.1

[a] Data represent combined AM and PM test results.
[b] When rainfall was recorded the accompanying test data were treated separately for each 24 hr period.
[c] Coefficient of protection from intrusion.

jects to determine the effectiveness of 2 treatment rates of DPM and one of indalone on the netting. The DPM treatment of 0.125 gm AI/gm of netting provided a mean of 95.6% protection in tests 24 hr after each of 2 treatments. At 120 and 144 hours, however (inclement weather and other factors prevented scheduling evaluations at the other times), the treatment failed to provide a CPI greater than 53%. Treatments of 0.25 gm/gm netting provided a CPI of more than 86% up to 5 days (120 hr) after treatment. Two and one-half cm of rain fell on the net before the 120 hr evaluation, however the CPI was 91.3% indicating DPM was somewhat resistant to rain at this treatment rate.

At 168 hr after treatment with DPM the mean CPI of 3 tests was 60.5% ± 8.1 and 1 test at 288 hr after treatment gave a CPI of 66.2%. In 2 of the 4 tests a small amount of rainfall (0.64 and 0.51 cm) occurred, but it did not appear to be the major source of reduced effectiveness.

The indicated resistance to rain would suggest that evaporation was a major cause for loss of effectiveness of DPM after 6 days.

The indalone treatment of 0.25 gm/gm of netting provided poor initial protection 24 hr after treatment (CPI, 46%), with relatively low numbers of biting midges present. At 48 hr, however, and relatively high numbers of midges, 87% protection was recorded. At 72 hr, protection had dropped to 69% and by 96 hr to 60%. The data represent 2 treatments but only the 96 hr data were obtainable for the second treatment because of inactivity of the midges due to cold (<13°C) windy (> 8 mi/hr) weather each day for 3 days after the chemical was applied. No rainfall was recorded during these evaluations.

Midges captured while biting human bait inside or outside the treated net during the tests were only samples of the attacking population. The species composition of these samples varied with the time of the year (Tables 1 and 3). Samples taken from inside the treated net had a higher overall proportion of *C. barbosai* than samples from collections made outside the net, however, a *t* test of the data showed the differences were not statistically significant.

The data suggest an outdoor protective barrier can be created using repellent-treated net-

Table 3. Numbers of *Culicoides* midges biting human subjects in 1 hr inside or outside an enclosure 12 × 12 × 2 m high, at various times after treatment with AI3-20830 (diisopentyl malate (DPM)) or *n*-butyl-6,6-dimethyl-5,6-dihydro-1,4-pyrone-2-carboxylate (Indalone) at the rates of 0.125 or 0.25 gm AI/gm net.

Time after treatment (hr)	Rainfall (cm)	No. midges biting/hr Inside net	Outside net	CPI[a] treated enclosure
0.125 gm DPM/gm net[b]				
24	0	0	279	100
24	0	8	90	91.1
120	trace	48	56	14.3
144	2.3	482	1027	53.1
144	0	399	465	14.2
0.25 gm DPM/gm net[c]				
1	0	0	929	100
24	0	1	200	99.5
48	0	95	691	86.3
120	2.5	45	517	91.3
168	0	34	112	69.6
168	0.64	298	648	54.0
168	0.51	185	438	57.8
288	0	237	702	66.2
0.25 gm Indalone/gm net (1 test)[d]				
24	0	25	46	45.7
48	0	138	1069	87.1
72	0	308	985	68.7
96	0	58	146	60.3

[a] Coefficient of protection from intrusion.
[b] Species biting:61% *C. mississippiensis* and 39% *C. barbosai* (April 1982).
[c] Species biting:25% *C. mississipiensis*, 72% *C. barbosai* and 3% *C. furens* (May 1982).
[d] Species biting:92% *C. mississipiensis*, and 8% *C. barbosai* (October 1982).

ting for large area protection (12 × 12 × 2 m) of people from the attack of biting midges. The repellent-treated enclosure concept appears to have both civilian and military application in area protection against biting midges. This area treatment technique may offer some relief from the attack of other flying insect pests as well.

ACKNOWLEDGMENTS

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ERRATA

The following corrections should be made in the paper, "A genetic-sexing strain based on malathion resistance for *Culex tarsalis*," by P. T. McDonald and S. M. Asman, published in *Mosquito News*, volume 42, pages 531–536 (1982).

p. 534, right hand column, lines 10–11. Change "spermatogonial metaphase" to "spermatocyte metaphase I".

p. 535. Change caption for Fig. 2 to: "Spermatocyte metaphase I cytology of sex-sorting translocation strains, etc."