CHEMICAL TREATMENT OF WIDE-MESH NET CLOTHING FOR PERSONAL PROTECTION AGAINST BLOOD-FEEDING ARTHROPODS

C. E. SCHRECK, D. E. WEIDHAAS, NELSON SMITH AND K. H. POSEY

Insects Affecting Man Research Laboratory, Agr. Res. Serv., USDA, Gainesville, Florida 32604

ABSTRACT. Studies were conducted with resmethrin-impregnated netting to determine its potential as a clothing impregnant for protecting human hosts from blood-feeding arthropods. Tests were conducted in a laboratory olfactometer with *Aedes aegypti* (L.) and *Anopheles albimanus* (Wiedemann). Outdoor cage experiments were conducted with these 2 species and the stable fly, *Stomoxys calcitrans* (L.). Field tests were conducted with *Ae. taeniorhychus* (Wiedemann) and the deer fly, *Chrysops atlanticus* Pechuman. Resmethrin showed no repellent activity (i.e., alternating approach to an attractive host) against these species but did show very rapid intoxication and behavioral changes resulting in complete or high level protection from biting as well as kill of attacking insects. There was no evidence of vapor toxicity in these tests. In olfactometer tests *Ae. aegypti* and *An. quadrimaculatus* were not repelled but died from momentary contact with treated netting. In cage tests these 2 species and stable flies were killed by momentary contact while attempting to feed on hosts. In field tests, protection was obtained against a deer fly and *Ae. taeniorhynchus* and the numbers of attacking insects was reduced to low levels.

The application of repellents to skin and/or clothing has been for many years a recommended and accepted method of protecting individuals against mosquitoes, ticks, mites, black flies, and other blood-feeding arthropods (Smith and Burnett 1948, Smith and Cole 1951, Smith 1958, and Travis and Morton 1946). However, repellents applied to skin lose effectiveness through abrasion, evaporation, and absorption and have limitations such as an "oily" feel; many are plasticizers, have a relatively short protection period, and require a high dose applied to all exposed areas. Repellents applied to clothing have certain advantages that include: (1) clothing can be treated in advance of need and exposure; (2) proper storage provides much longer protection time per treatment; (3) oily feeling is eliminated because the repellent is not applied directly to skin; and (4) effectiveness against arthropods that crawl or move over a surface before biting such as ticks, mites, black flies, and sand flies. In fact, netting and net jackets treated with a repellent (deet: *N,N*-diethyl-*m*-toluamide) were effective against a number of species of flying, blood-feeding insects (Gouck et al. 1967 and Grothaus et al. 1976); also, the lightweight, open-mesh material was more comfortable, and less repellent was required for treatment.

However, whatever the method of using repellents, their effectiveness diminishes with time. Meanwhile increasing numbers of the blood feeding arthropods tend to congregate around the host and finally begin feeding. In addition, repellents generally have little or no effect on the number of arthropods in the area other than providing temporary protection from annoyance and bites. The one exception has been trombiculid mites (Cross and Fye 1948; Travis and Smith 1950). These mites were actually killed by contact with the treated skin or clothing though the effect was the same, temporary protection against biting. A similar effect probably occurs with small biting insects such as sand flies which are trapped in the repellent deposit.

It was therefore of interest when we observed that resmethrin (5 - benzyl - 3 - furyl) methyl *cis-*, *trans-*(±) - 2, 2 - dimenthyl - 3 - (2 - methylpropenyl) cyclopropanecarboxylate) did not appear to be repellent; i.e., it did not cause nymphal

Amblyomma americanum (L.) to orient away from or avoid the host. Ticks readily climbed a treated cloth patch and, apparently as a result, were moribund within a very short time. Observations with biting insects such as mosquitoes, stable flies and tabanids suggested that little or no repellency occurred, but intoxication, knockdown, and mortality occurred very quickly and after only momentary contact with resmethrin. Indeed, pests attempting to bite were affected so rapidly that few or no bites were recorded. Thus, treatment of clothing with this material might be more effective in protecting humans against biting arthropods than clothing repellents. In addition, the use of such a material would remove the attacking arthropod from the local population.

Consequently, we decided to investigate the possibility that a synthetic pyrethroid would protect individuals from annoyance and biting when the material was impregnated in netting. The tests would be made against mosquitoes, stable flies, or tabanids in a laboratory olfactometer, in outdoor test cages, and in field situations. We would also study the degree of protection afforded human and animal hosts by the treatment and attempt to determine the fate of insects coming in contact with the treatment. Resmethrin was chosen as the test material, despite the fact that some synthetic pyrethroids are more stable to light decomposition, because it appeared to have little or no repellency, was readily available, and was safe for our intended use. The material used in these studies was provided by S. B. Penick and Co., Orange, N.J. It was impregnated into light weight net jackets (Grothaus et al. 1976) or net yard goods by the absorption of acetone solutions at a rate of 0.0625 g of resmethrin to 1 g of netting (approximately 4.3 g/m^2). This dose is only about 1/5 the dose of repellent use in standard clothing treatments.

OLFACTOMETER TESTS WITH *Aedes aegypti* (L.) AND *Anopheles quadrimaculatus* SAY. Treated nets were tested against laboratory-reared 6- to 7-day-old female *Ae. aegypti* or *An. quadrimaculatus* in an olfactometer (Schreck et al. 1967) by the method of Gouck et al. (1967). Thus, females were placed in a response chamber and exposed to an airstream coming from the port in the olfactometer. A piece of treated or untreated netting was placed in the port so attracted mosquitoes entering the port and its trap had to pass through either treated or untreated netting and thereby made momentary contact with it. This experiment was replicated 6 times with each type of netting and with each species; approximately 115 adult females of each species were used in each replicate.

The repellency (prevented orientation and approach to the host) and toxicity (vapor or contact) of resmethrin demonstrated in the olfactometer are presented in Table 1. Large percentages (no significant differences) of both species responded through both the treated and untreated net. Also, the total numbers responding were in the range for previous tests in which no treated net was involved (Schreck et al. 1970). There was thus no evidence that resmethrin repelled the mosquitoes. Mosquitoes trapped by untreated netting showed no mortality in 24 hr; likewise mosquitoes not responding within the time of the experiment to the attractive airstream (thus not in contact with the netting) showed no mortality (*Ae. aegypti*) or 9% mortality (*An. quadrimaculatus*) after 24 hr. There was thus no evidence of vapor toxicity. However, mosquitoes (of both species) that passed through the treated netting showed over 90% mortality at 4 hr and most that touched the netting, even momentarily, were knocked down or showed symptoms of exposure within 10 min. Resmethrin therefore did not alter approach to a host, and the vapor was not toxic; but momentary contact caused rapid change of behavior and high mortality.

FIELD TESTS WITH *Ae. taeniorhynchus* (WIEDEMANN). The olfactometer tests did not permit contact of female mosquitoes with the human host as would occur in the field. Therefore we conducted field tests at South New Smyrna Beach, Florida, in

Table 1. Repellency and vapor and contact toxicity of resmethrin-treated netting to *Ae. aegypti* and *An. quadrimaculatus* in an olfactometer (aveg. 6 tests).

	% Responding through				% Mortality of mosquitoes passing through treated netting after			
	Untreated net		Treated net		4 hr		24 hr	
Species	Avg.	Range	Avg.	Range	Avg.	Range	Avg.	Range
Ae. aegypti	60	26–96	86	75–92	92	80–99	93	82–98
An. quadrimaculatus	73	50–96	54	32–69	97	90–100	97	93–100

an area between the Atlantic Ocean and the Intercostal Waterway. Here *Ae. taeniorhynchus* readily bite in the daytime and are present in moderately high numbers. We were primarily interested in whether it was possible to measure any reduction in the number of biting mosquitoes in the area after the mosquitoes made contact with hosts wearing pyrethroid-treated jackets. Additionally, we planned to observe the degree of protection against biting afforded by the jackets on initial entry into a mosquito-infested area. The test was replicated 4 times. In each test, 2 pairs of subjects made pretreatment determinations of populations at each of 2 heavily vegetated sites (areas of 90 x 60 m; 100 x 67 yd) on 2 trails belonging to the Florida State Park Service. A determination consisted of counting the total *Ae. taeniorhynchus* that landed on the upper halves of the bodies of each pair of standing subjects within 20 sec. (The lower half of the bodies (waist to shoes) was treated with deet to keep the biting pressure on the upper part of the body.) The 4 subjects then immediately returned to a buffer zone outside of the test area, put on white T-shirts and resmethrin-treated net jackets, and returned to the test area. There they walked and squatted intermittently for 1 hr to attract mosquitoes and keep the biting pressure on the jackets. As soon as the hour was over, the subjects returned to the buffer zone, removed the jackets, and went back to the designated areas where they immediately made posttreatment determinations and 1 hr later made additional posttreatment determinations. Similar counts were made approximately 2 miles from the test site to determine whether natural fluctuations in the population occurred during the test period, but these subjects did not wear treated jackets.

Table 2 shows the results obtained as the averages of the 16 counts (4 replicates of 4 subjects) made at each interval. The reductions in the number of mosquitoes biting immediately after the 1-hr exposure (91%) and then 1 hr later (73%) were high when one considers that the only "treatment" of the 5400 m² (approximately 1.4 acres) area was 4 people wearing treated net jackets and walking through the area. The subjects walking in the area with

Table 2. Effect on *Ae. taeniorhynchus* of resmethrin-treated net jackets (avg. of 16 counts).

	Area with no treatment			Treated area		
Time of counts	Range of count	Average count	% Change	Range of count	Average count	% Change
Pretreatment	8–52	18.8		13–75	31.1	
Immediate postreatment	2–48	22.6	+17	1–9	2.7	–91
1 hr posttreatment				4–28	8.3	–73

treated jackets observed that the mosquitoes readily attacked and landed. However, the majority did not bite through the treated netting and the T-shirts though some bites were received. The combination of the protection provided by the jackets though not complete, and the reduction in number of mosquitoes was highly significant. Again there was no evidence of any repellency, and protection resulted from the contact of the mosquitoes with the pyrethroid.

OUTDOOR CAGE TESTS WITH *Ae. aegypti* AND *An. quadrimaculatus*. In the field tests, it was difficult to determine the fate of the mosquitoes after contact because they could recover and fly away, cease seeking hosts temporarily, or die. From olfactometer tests, one would assume they died. Therefore, we conducted outdoor cage experiments to determine the survival of *Ae. aegypti* and *An. quadrimaculatus* in the presence of a host with a treated net covering.

An outdoor screen cage with dimensions of 7.3 x 7.3 x 2.7 m (24 x 24 x 9 ft) and 2.7 m (9 ft) high was sectioned into 4 separate test units 3.7 x 3.7 m (12 x 12 ft). Four rabbits, each housed with food and water in 61 x 61 cm (2 x 2 ft) cages, were placed one per unit. The cages were covered with chicken wire to allow mosquitoes easy access to the rabbits. A 15.2 x 15.2-cm (6 x 6 in.) patch on the lower back of each rabbit was shaved and covered (not the head and feet) with a 25 x 31 cm (9 x 12 in.) net (treated or untreated) that was secured by tying under the rabbit's stomach and over its shoulders. Also, each unit was provided with cotton moistened with 10 percent sucrose solution and a moistened dark colored cloth, tent-like shelter for the adult mosquitoes.

Approximately 300 female and 150 male *Ae. aeygpti* and 200 female and 200 male *An. quadrimaculatus* were released as pupae in each section. Three days later when females began to take blood meals, counts of the total number of females in the cage were started. Counts were made at 8:15 am and 4:00 pm daily for 5 days. The test was repeated on another occasion so that tests in 8 separate units were involved; in 3 of these replicates, rabbits were covered with untreated netting and in 5 they were covered with treated netting. Since the netting did not cover areas where hair is short and mosquitoes can feed readily, the females were not restricted to feeding through the net to obtain a blood meal but had a choice of feeding on the treated or untreated parts of the rabbit.

The results are reported in Table 3. In the untreated units, the average number of female *Ae. aegypti* counted (3 replicates) was 255 on day 3 (approximately 85% of the total introduced into the cage) so the calculated average daily survival from introduction to day 3 was 0.92. For *An. quadrimaculatus*, it was 99, approximately 50% of the total introduced for a calculated average daily survival of 0.70. (The average daily survival (S_a) was calculated from $S_a = \sqrt[X]{N_1/N_0}$ where N_0 is the number of insects present on a given day, N_1 is the number on some subsequent day, and X is the number of days.) From day 3 to 7 survival of both species in the untreated cages continued to be high: The number of *Ae. aegypti* decreased from 255 to 212, an average daily survival of 0.95, which approximated the 0.92 average survival per day of the first 3 days; and the number of *An. quadrimaculatus* decreased from 99 to 43, an average daily survival of 0.81.

In the treated units, the number of females present on day 3 was approximately 40–50% lower for both species, and the calculated average daily survivals were 0.66 and 0.53 for *Ae. aegypti* and *An. quadrimaculatus*, respectively. From day 3 to 7, the average daily survival was calculated as 0.56 for *Ae. aegypti* and 0.43 for *An. quadrimaculatus*. Thus, the females apparently contacted the netting before the first counts were made on day 3, but the killing effect was greater after 3 days when more females were seeking blood meals.

Thus, the netting had a significant effect on the survival of both species: only 1% of the *An. quadrimaculatus* and 4% of the *Ae. aegypti* survived to day 7 when treated netting was used, while 22 and

Table 3. Effect on 2 species of female mosquitoes in outdoor cages when rabbits were covered with net material untreated or treated with resmethrin.

	Untreated population (avg. 3 tests)						Treated population (avg. 5 tests)					
	Ae. aegyti			*An. quadrimaculatus*			*Ae. aegypti*			*An. quadrimaculatus*		
Day	Range of popu-lation	Average popu-lation	% Change	Range of popu-lation	Average popu-lation	% Change	Range of popu-lation	Average popu-lation	% Change	Range of popu-lation	Average popu-lation	% Change
3	190–353	255	-15	69–167	99	-51	82–192	131	-56	16–100	57	-72
4	152–308	227	-24	63–118	87	-57	56–99	82	-73	2–40	19	-91
5	171–309	231	-23	45–99	66	-67	18–57	36	-88	2–16	7	-97
6	149–300	215	-28	3–85	53	-74	6–43	20	-93	0–9	4	-98
7	161–292	212	-29	1–67	43	-78	4–22	13	-96	0–5	2	-99

Table 4. Effect on stable flies, *S. calcitrans*, released into 2 cages housing a male calf in each wearing net covering treated or not treated with resmethrin (average of 4 tests).

		Untreated netting			Treated netting					
					Range of population		Average population		% Change	
Time of counts	Length of exposure (hours)	Range of population	Average population	% Change	Series 1	Series 2	Series 1	Series 2	Series 1	Series 2
0815	0	35–115	80.5		17–239	35–57	105.0	43.3		
1300	5	16–137	89.5	+10	4–133	2–32	41.5	11.8	-61	-73
1600	8	51–143	109.3	+26	12–75	1–14	33.5	6.0	-68	-86
0815	24	18–117	58.3	−28	0–41	0–3	15.8	1.0	-85	-98
1300	29	33–142	93.0	+13	1–29	0–2	11.0	0.8	-90	-98
1600	32	50–105	73.5	−9	4–14		8.8		-92	

71%, respectively, survived in the untreated units. This increased mortality (in addition to that in the check) occurred even though the mosquito could feed on the rabbits other than through the treated netting. On occasion, females of both species were observed to get partial blood meals by feeding through the net. However, they were affected sufficiently so they did not get a complete blood meal.

OUTDOOR CAGE TESTS WITH *Stomoxys calcitrans* (L.). A similar test was conducted with stable flies in another outdoor cage. Two male Holstein calves were confined with appropriate food and water in 2 halves of an outdoor screen cage 5.5 wide x 7.3 long x 2.4 m high (18 x 24 x 8 ft) divided by a screened partition. One thousand laboratory reared 2-day-old *S. calcitrans* were released in each half of the cage and allowed to feed on the calves for 24 hr. After 24 hr, 1 untreated and 1 resmethrin-treated net 1.2 x 1.2 m (4 x 4ft) were tied to each of the calves so they covered them from the neck to the tail and on the sides down to the knee of the forelegs. The hindquarters and legs were only partially covered. Initially it was impossible to make total cage counts of all surviving flies so counts were made on panels in 4 corners of each half of the cage to determine relative numbers. Counts were made at 8:15 am, 1 pm, and 4 pm for each of the 2 days the nets were being worn.

The results are shown in Table 4. The population in the untreated check cage remained relatively unchanged until the last day of the test when heavy rains made counting difficult. In the treated side containing the calf wearing treated netting, the count was reduced by 92% in 32 hr, and after 32 hr, only 35 live stable flies were counted. The test was repeated without the untreated check (one of the calves was unavailable for concurrent testing) and showed a 98% reduction in counts in 29 hr.

FIELD TESTS WITH THE DEER FLY, *Chrysops atlanticus,* PECHUMAN. Field tests to determine the effect of treated net jackets were conducted on forest lands owned by the International Paper Company near Richmond Hill, Georgia. The sites selected were intersecting logging roads in 2 locations approximately 3.2 km (2 miles) apart. Four persons participated in each of 2 tests at each of the 2 sites. Markers were placed at 91.4 m (100-yd) intervals for 457 m (500 yd) in each direction, north and south, from the center of the intersection at each site. The first 91.4 m (100 yd) in each direction was used as a buffer zone. First the four subjects, 2 in each direction, walked the 457 m (500 yd) up and back at a slow pace and about 9.1 m (10 yd) apart, pausing at each of 8 points to make pretreatment counts. The subjects alternated counts, one taking points 1–4 and the other points 5–8. A 5-min pause was taken before the return trip. From the pretreatment counts we calculated the average number of flies actually landing and attempting to bite at any point along the test road. After the return to the buffer zone and a 15-min pause, 2 subjects wearing treated net jackets walked in one direction and 2 wearing untreated net jackets walked in the other. At each of the 8 stopping points, deer flies that landed on the jackets were collected with an aspirator (alternated as before), but no more than 12 were collected on each route to avoid affecting the population counts. The collected flies were put in holding cages, provided with 10% sucrose solution on cotton, and held for observation. As soon as the subjects returned to the buffer zone, they removed the jackets and immediately returned to the test area to make a posttreatment count in the same way as the pretreatment count. Also, additional posttreatment counts were made at 1 and 6 hr.

The results are shown in Table 5. In the untreated area the number of *C. atlanticus* attacking and attempting to bite averaged 6.1/count. Walking through an area without the treated jacket reduced the rate of attack considerably (57–70%); walking through an area with the treated net jacket reduced the attack rate much more; 94–95% within the 1st hour and 87% after 6 hr. All the flies collected after momen-

Table 5. Effect on deer flies, *C. atlanticus,* at 2 test sites for 2 days when the net jackets were worn with or without treatments of resmethrin (average of 4 counts except where noted).

Time of counts	Counts					
	Untreated jacket			Treated jacket		
	Range of counts	Average count	% Change	Range of counts	Average count	% Change
Pretreatment	4.9–8.3	6.1		3.4–11.5	7.7	
Immediate posttreatment	0.4–3.4	1.9	–69	0–1.1	0.4	–95
1 hr posttreatment	.6–5.4	2.6	–57	0–1.1	.5	–94
6 hr.posttreatment[a]	.8–2.8	1.8	–70	.9–1.0	1.0	–87

[a] Average of 2 counts.

tary contact with the treated jackets died; mortality of flies collected from untreated jackets ranged from 0 to 50% Only 1 deer fly bite was sustained by 1 of the 4 subjects when they were wearing the treated jackets. The treated jackets thus protected the subjects from the bites and also influenced the number attacking subsequently.

DISCUSSION. Results of our studies indicated that the concept of using safe, nonrepellent toxicants on clothing for protection against blood-feeding arthropods has potential and also many ramifications. Those wearing treated jackets obtained a high degree of protection from mosquitoes and a deer fly though protection was not total. In addition, the toxicity of resmethrin was manifested so immediately that the numbers of blood feeding insects present was reduced. Thus our original premise that toxicants such as the synthetic pyrethroids might be replacements for clothing repellents seems valid. In the case of arthropods such as deer flies, sand flies, ticks, mites, and black flies that land and crawl before biting, the protection is almost complete, and the attacking blood feeders are killed. In the case of mosquitoes, some biting may occur, but the attacking mosquitoes do not survive. At this time we do not know what the effect would be if large numbers of people wore treated jackets in a limited area though large numbers of biting insects could be killed while affording protection to individuals. For example, it would be interesting to study the effect on biting arthropods of a platoon of about 60 men moving into an area of high arthropod density. The toxic effect of the clothing might reduce or rid an area of disease vectors and pests meanwhile having little or no environmental impact since only those species in search of a blood meal would be affected.

The method should be tested against many of the world's major disease vectors in laboratory and field studies to determine its full value. Preliminary tests with *Glossina morsitans* (Westwood and *G. pallidipes* Austen in the field in Africa, showed momentary tarsal contact of these species with resmethrin-treated netting quickly caused abnormal behavior and resulted in death. Such a method might be an effective means of reducing populations of tsetse flies in areas of Africa where it would be feasible for inhabitants to wear treated clothing and where animals might be trained to wear treated covers or would receive topical skin applications.

Over the years many types of traps, baits, and attractants have been described to lure blood-sucking arthropods to a site where they could be killed, but few, if any, have been as effective as the host itself. This method uses the host as the attractant while protecting it at the same time. However, the ideal protectant has not been found. Preferably it should be nonrepellent, extremely rapid in its action, safe, long-lasting, and resistant to weathering, washing and light-induced chemical breakdown. Some of the new synthetic pyrethroids may have these properties.

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THE USE OF TACHOMETERS TO IMPROVE THE ACCURACY OF PESTICIDE APPLICATIONS BY GROUND EQUIPMENT

ALLAN R. PFUNTNER

Northwest Mosquito Abatement District, 6851 Granite Hill Dr., Riverside, Ca. 92509

ABSTRACT. A combination of a vehicular tachometer and a common stop-watch constitutes a system with which pesticides can be applied more accurately and efficiently when using ground-based spray equipment. The method also provides a useful means of determining the size of the area treated, concurrent with the actual spraying operation.

The accuracy with which pesticides are applied is of utmost concern to vector control personnel. Inaccurate applications may produce undesirable results or possibly damage the environment (Mulhern 1976, Womeldorf and Peck 1975). In addition, such applications are a waste of time and money.

Many of the chemical control measures pursued by the personnel of the Northwest Mosquito Abatement District employ the use of the Potts Mist Blower (Potts Mist Blower, Crawford, Miss.). This device consists of a blower unit driven by a gasoline engine. The air flow produced is routed through a housing and out a tubular opening about 6 inches (15 centimeters) in diameter. Mounted at the aperture of this tube are nozzles which dispense liquid pesticide at a desired rate. The exit-