

PAPER

PATHOLOGY/BIOLOGY

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DEET (N,N-Diethyl-meta-toluamide) Induced Delay of Blowfly Landing and Oviposition Rates on Treated Pig Carrion (*Sus scrofa* L.)

ABSTRACT: The question of whether the insect repellent N,N-Diethyl-meta-toluamide (DEET) affected fly attraction, oviposition, and larval development was investigated; in part, to determine whether the common habit of wearing DEET as a repellent could affect the rate of human decomposition. Experiments using pig surrogates of human decedents were carried out in a rural environment. Dead piglets were sprayed with DEET, and fly behavior, colonization levels, and maggot development were compared with those in nonsprayed controls. Piglets treated with DEET experienced significant delays in fly visitation and oviposition and delayed appearance of each larval instar, as well as reduced total larval numbers ($p < 0.01$ for all variables), with subsequently reduced decomposition ($p < 0.05$). Such changes in fly behavior and larval population development would significantly impact the estimation of the period following the death from entomological evidence in decedents wearing DEET at the time of their death.

KEYWORDS: forensic science, forensic entomology, insect repellents, N,N-Diethyl-meta-toluamide, Calliphoridae, Sarcophagidae, oviposition, decomposition

Humans commonly employ insect repellents to defend against biting insects. N,N-Diethyl-meta-toluamide (DEET) appears most frequently as the active ingredient in topical repellents (1). Originally developed as a mosquito repellent (2), DEET successfully repels several genera of mosquitoes (3–6). DEET affects many other blood-seeking invertebrates as well, including sand flies (7), assassin bugs (8), Simuliid flies (9), Tsetse flies (10), face flies (11), ticks (12,13), and even leeches (14). That DEET also affects fruit flies suggests an invertebrate-repelling mechanism not limited to the prevention of blood feeding (15,16). DEET may also affect the blowflies seeking to lay eggs on the remains of homicide victims. Indeed, the common household repellent Citronella oil protects dead rats from Calliphorids (17). Thus, the effects of DEET on blowfly behavior may alter their role in decomposition and confound the significance of insect evidence in homicide investigations.

The extensive previous research on mosquito repellency might suggest how DEET would function in this new context. DEET could block odors and other factors produced by human skin that mosquitoes find attractive (18,19), or otherwise alter mosquito perception of these behavioral modifiers (20). The strength of these hypotheses faded following the discovery of DEET-sensitive olfactory receptor neurons that directly detect DEET and ultimately elicit avoidance behavior in mosquitoes (21). DEET also affects mosquito behavior in fundamentally different ways from toxicants that cause it to be classified as a “repellent” (22). If this repellency

hypothesis prevails, the behavior of carrion-feeding flies, such as Calliphoridae and Sarcophagidae, should also be influenced by DEET. For example, one would expect to find reduced fly landing rates on a decedent, which had been wearing DEET at the time of death, together with corollary delays in oviposition and maggot population development.

A controversial but key assumption of forensic entomology posits that blowflies locate and colonize an exposed body soon after death. DEET on the decedent would theoretically extend this interval, delaying onset of insect infestations. The duration of maggot infestation on a body is a type of minimum postmortem interval (PMI—period between homicide and finding the victim) and is used to estimate time of death or other parameters in homicide investigations (23,24). The length of the largest maggots then approximates this minimum PMI, because maggots appear to grow in a continuous manner; consequently, maggot length or instar becomes an indicator of maggot age. Temperature, however, regulates the rate of growth and development of immature insects. Thus, to determine this minimum PMI, one compares the lengths or instar of the largest maggots collected from a decedent to published growth rate studies carried out at temperatures prevalent at the scene prior to discovery. Failure to account for a repellent-induced delay in the laying of the first eggs would lead to an underestimation of the minimum PMI with consequences for the investigation (25). If a person should die covered with DEET, as could be expected in a hiker, camper, hunter, or soldier (26), the period prior to maggot infestation would be extended, truncating the estimates of the minimum PMI.

In a more general behavioral context, the effects of DEET on blowfly behavior would improve the understanding of how DEET works (27,28) by further testing the theory of DEET repellency. In

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an applied sense, if DEET alters the landing and oviposition behavior of the first scavenger flies that visit a decedent, then DEET contamination has great significance for forensic sciences, with implications in cases past and future (29). Therefore, if DEET repels blowflies or affects oviposition, would the speed of development in maggot populations be delayed or reduced on a DEET-treated decedent?

It may be that DEET delays scavenger fly landing and oviposition behavior on pig carrion. Accordingly, we selected a rural study site and set out dead piglets, half of which were topically treated with a commercially available form of 100% DEET. From each pig, we recorded visual changes in decomposition state, timing of fly landing, egg-laying rate, and the appearance of first- and second-stage larvae. When third-instar larvae began to wander, we extracted all the remaining insects and assessed population numbers. We then measured the lengths of the oldest cohort of maggots. In particular, we sought the evidence of changes in scavenger fly behavior postponing colonization by maggots and decomposition in DEET-treated carrion.

Methods

At the University of California, Davis, Putah Creek Riparian Reserve Experimental Ecosystem (38° 31'52.89"N 121°48'28.01"W), we selected nine locations 25 m apart along a wire fence under the shade of a row of Monterey Pines (*Pinus radiata* D. Don). Local winds prevail on an axis perpendicular to this fence (30). An Onset Corporation Hobo U12-008 Data logger with two TMC6-HC air/water/soil temperature sensors recorded temperatures. We purchased a total of 18 frozen piglets (*Sus scrofa* L.) from the Animal Science Department, UC Davis Swine Research Facility, assigned them numbers, recorded their thawed weights, and divided them into two groups of nine. Each group constituted one of the two field experiments (Experiment 1—August 23 to 26, 2010; Experiment 2—September 13 to 17, 2010) in which we set one pig out at each location along the fence. We caged each pig individually in a previously weighed (Calibrated Berkely FS15 Hanging Fishing Scales), c. 2-cm mesh chicken wire cage, for protection from vertebrate scavengers. We set each cage on a 50 × 100 cm window-screen tray with c. 1-cm vertical walls at the edges to temporarily corral wandering third-instar larvae. Alternate pigs were sprayed with c. 0.2 oz of 98.11% liquid DEET (Repel brand, Bridgeton, MO), beginning and ending the line with non-sprayed pigs. The quantity of liquid was enough to ensure the entire surface of each pig was exposed to DEET. Great care was taken to ensure that control pigs did not become contaminated with DEET spray. Thus, each experiment comprised five control and four treated pigs.

Each experiment started at 7:30 AM daily, and we recorded the observations until 7:30 PM. Sunrise occurred at about 6:00 AM over this period. We photographed the changes in decomposition (Nikon Coolpix 5700; Nikon, Shinjuku, Tokyo, Japan) including color, bloat, and tissue loss, and recorded the number of flies and egg masses on each pig at 2-h intervals (31). We additionally recorded the first appearance of each larval instar and the time when egg masses became so numerous and dense as to be no longer individually distinguishable. When wandering, the third-instar maggots appeared on the window-screen tray under any one pig, and we carefully weighed it in its cage over the tray and later subtracted this final weight from the initial weight to determine the proportion of weight loss sustained. At the end of the first experiment, we carefully submerged each caged pig and the insects that had fallen onto the screen into 10 L of boiling water to fix and

remove all crawling insects and maggots from the carcass. We removed the pig and sieved the remaining fluid through 10, 20, and 40 size mesh (2.00, 0.853, and 0.422 mm, respectively) and transferred the maggots to 70% ethanol. We later measured the total volume of maggots using a 500-mL graduated cylinder. This volume was used as an estimate of total maggot population.

We identified maggots from the pigs and adult flies reared from small cohorts of maggots obtained prior to boiling the pigs. Following the identification (32,33), we sampled the 10 largest sarcophagids (when present) and calliphorids from the maggot sample and assigned these samples code numbers. These coded maggots were presented to one of us who was not present when sampling. This investigator, who estimated the maggot age, was thusly blinded as to which pig each sample came from. A Dino-Lite Digital Microscope (AnMo Electronics Corp., New Taipei City, Taiwan) calibrated for measurements taken at <80×, employing powers up to c. 10×, and connected to a netbook computer using Dino-Capture (Ver. 2) software (AnMo Electronics Corp.) was used to measure maggot lengths compiled with Microsoft Excel version 2010 (Redmond, WA). These lengths were combined with the temperature data and published fly life cycle and development tables to estimate and compare the apparent range of ages of these maggots (34).

Data from both trials were combined. If we did not directly observe an event on a given pig (e.g., the first egg mass appeared overnight), that pig was not included in the statistical analysis for that event. All the variables examined were tested for normality using the Shapiro–Wilks test (35). We compared the total maggot volume of the two treatment groups, DEET and control pigs, using Fisher's *F*-test followed by two-sample *t*-tests (Microsoft Excel). All other variables were compared using a one-sided Mann–Whitney *U*-test (36, p. 376).

Results

Although the temperature range did not differ between the two experiments, cooler conditions prevailed during the period of the second experiment (Table 1). The green bottle fly *Lucilia sericata* (Meigen) (Calliphoridae) and the flesh fly *Blaesoxipha plinthopyga* (Wiedemann) (Sarcophagidae) predominated on these pigs; we identified no other species. To the eye, rates and changes in the decomposition of each pig differed strikingly between DEET-treated and control pigs. Maggots reduced the untreated pigs to skeletons and tattered skin in 4 days (Fig. 1a,b), a state corresponding to the final stages of decay where only skin and hard tissues remain (31). In contrast, DEET-treated pigs remained physically intact, with unbroken skin and bloating characteristic of the putrefaction or bloat stage of decomposition (Fig. 1c,d), the second stage of decay when external signs of decomposition first become apparent (31). Visual evaluation of the general decomposition state of pigs at day 4 of the second experiment suggested a PMI for control pigs of about 1 week but only 2–3 days for those treated with DEET. Control pigs lost on average nearly half of their weight, twice as

TABLE 1—Temperatures (°C) during the first (August) and second (September) experiments.

Statistic	Experiment 1	Experiment 2
Mean	81.88	68.83
Median	80.47	70.78
Range	47.06	42.58
Minimum	60.43	44.81
Maximum	107.50	87.39

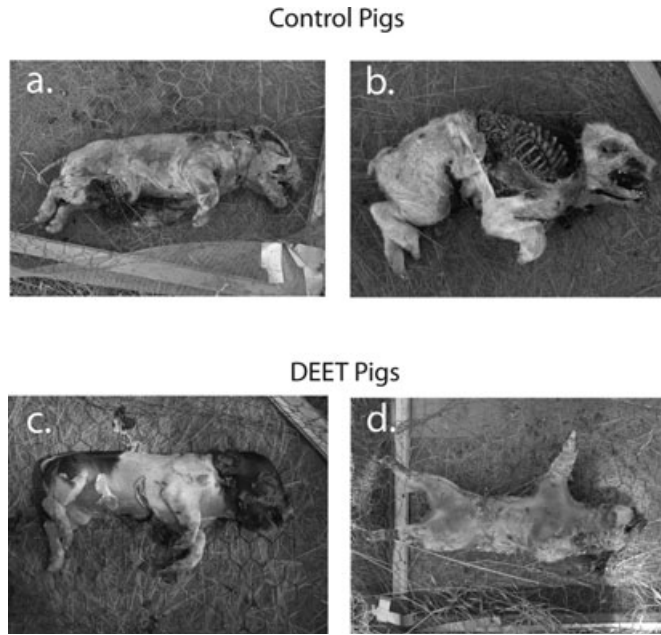


FIG. 1—Photographs of four pigs from the second trial, 4 days into the experiment (September 17, 2010). The untreated pigs (top) are badly damaged, skeletonized, and contain more maggots than the *N,N*-Diethyl-meta-toluamide (DEET) pigs (bottom). An exemplar of the effect of DEET is Pig D, which was still in the bloat stage of decomposition as no maggots had cut holes in the flesh that would otherwise have allowed gases to escape.

TABLE 2—Means and standard deviations (SD) of fly behavior, maggot development, and pig decomposition values.

Variable	Mean Values \pm SD	
	Control	DEET
Time to first fly landing (h)	1.78 \pm 1.86	12.75 \pm 8.61
Time to first egg mass (h)	5.11 \pm 1.05	29.29 \pm 12.87
Time to uncountable # egg masses (h)	17.22 \pm 8.33	73.00 \pm 15.75
Time to first 1° instar (h)	24.78 \pm 7.01	55.50 \pm 19.05
Time to first 2° instar (h)	47.89 \pm 15.58	74.50 \pm 23.28
Time to first 3° instar (h)	76.67 \pm 21.81	N.A.
Total weight lost (kg)	0.501 \pm 0.31	0.268 \pm 0.21
Proportion of carcass lost by weight	0.482 \pm 0.29	0.220 \pm 0.15
Total volume of maggots (mL)*	475.0 \pm 193.6	27.5 \pm 17.08
<i>Blaesoxipha plinthopyga</i> (Wiedemann)		
Maggot length (mm) [†]	16.64 \pm 1.67	13.58 \pm 2.82
<i>Lucilia sericata</i> (Meigen)		
Maggot length (mm) [†]	13.54 \pm 1.10	10.46 \pm 2.30

DEET, *N,N*-Diethyl-meta-toluamide.

*Only the first trial of eight pigs was included in the maggot volume.

[†]Maggot length means were based on the 10 largest individuals of each family from each pig, all combined. In each case, these maggots were third-instar larvae.

much as DEET-treated pigs (Table 2) and a significantly larger proportion of predecomposition weight ($U = 17$, $n_1 = 9$, $n_2 = 8$, $p_1 < 0.05$).

Clear differences existed between DEET-treated and untreated pigs in the timing of appearance and behavior of scavenger insects (Table 2). Flies visited all control pigs within the first 4 h of pig deposition, while DEET pigs remained untouched by flies for a significantly longer period of time, nearly 24 h ($U = 68$, $n_1 = 9$, $n_2 = 8$, $p_1 < 0.01$). While the number of flies on the surface of control pigs was often too great to accurately count, the number of

flies on DEET pigs never exceeded one dozen throughout the entire experiment. DEET-treated pigs experienced significant delays in appearance of the first egg mass ($U = 63$, $n_1 = 9$, $n_2 = 7$, $p_1 < 0.01$), when egg masses became too numerous to count ($U = 72$, $n_1 = 9$, $n_2 = 8$, $p_1 < 0.01$), and appearance of first-instar ($U = 67$, $n_1 = 9$, $n_2 = 8$, $p_1 < 0.01$) and second-instar larvae ($U = 59$, $n_1 = 9$, $n_2 = 8$, $p_1 < 0.01$). In DEET-treated pigs, conversion to third-instar larvae occurred in cryptic locations that would have required extensive disturbance of the pigs to see, and thus, we could not consistently make the observations of that event. In control pigs, however, not enough flesh remained to conceal third instars, which appeared about the same time as the oldest DEET-treated pig larvae reached second instar (Table 2). Other insects characteristic of the carrion-seeking community, such as carrion beetles and yellow jackets, infested five of the eight control pigs, but no DEET-treated pigs.

The volume of maggots removed from each pig in the first trial differed significantly between control and DEET-treated pigs, ($t(3) = 4.60$, $p < 0.05$), often by an order of magnitude or more (Fig. 2). The average lengths of the largest, final-instar maggots from DEET-treated pigs were significantly shorter than those from control pigs (Table 2), both for *B. plinthopyga* ($U = 482$, $n_1 = 58$, $n_2 = 50$, $p_1 < 0.01$) and for *L. sericata* ($U = 1036$, $n_1 = 100$, $n_2 = 80$, $p_1 < 0.01$).

Discussion

Our observations highlight the various forms of delay. Not only did blowflies visit DEET-treated pigs later than they did control pigs, but also the duration between visitation and egg deposition, and nonquantifiable egg mass number, lengthened on DEET-treated pigs. This suggests that DEET made the pigs less appealing as oviposition sites long after the flies had detected the corpses' presences. DEET, therefore, delays scavenger fly behavior, forestalling colonization by maggots, and ultimately, decomposition in DEET-treated carrion. Comparison of *L. sericata* maggot lengths with growth rate reference data at 28°C (37) aged the DEET-treated pigs' maggots at roughly 1 day old and control pigs' maggots at roughly 2 days, suggesting a difference in the period of infestation similar to the observed 24 h average delay in oviposition (Table 2).



FIG. 2—Photograph of the volume of maggots collected from a *N,N*-Diethyl-meta-toluamide-treated pig (left) and a control pig (right) in 1 L jars, showing the dramatic difference in maggot count and stage of development after 4 days. Maggots are preserved in 70% ethanol.

Combined with the apparent difference in pig decomposition stage and the qualitative difference in proportion of carcass lost, the data suggest that DEET might cause the underestimations of the period of infestation by up to several days. This could have important consequences for criminal cases incorporating entomological evidence.

Understanding the roles of repellents on insect behavior thus has importance beyond preventing bites on the living. Should investigators suspect that DEET has been applied to a decedent pre- or post-mortem, chemical analysis of the skin or clothing could confirm the suspicion and entomological estimates of period of infestation be altered accordingly. Consider the hypothetical case where an assailant covers the victim's body with DEET for the purpose of affecting the forensic data. If the body is not immediately discovered, the entomologist's eventual report will underestimate the period of infestation by a factor of several days. If the assailant has an alibi for this incorrectly estimated time of death, the criminal may go free. Confirming the presence of DEET would prevent this scenario by allowing the forensic entomologist to factor in the effect of the repellent when making PMI estimates. Because the perturbation of fly behavior early on leads to changes in maggot populations days later, DEET-induced effects are likely to outlast the presence of the chemical's residue on the skin. Thus, pre- or post-mortem insect repellent use may be suspected in cases where the entomological data deviate from other forensic measures of PMI.

That DEET affected the behavior of scavenger flies supports the repellent hypothesis, not an odor-masking mode of action (21). If DEET prevented mosquito bites by blocking reception of CO₂ and other compounds that attract mosquitoes and other blood feeders to sources of blood, then it should not have any effect on cold remains that produce such compounds in insignificant amounts. That blowflies and incidental insects, as reported here, avoided DEET-treated pigs for the same 12-h average time that DEET repels mosquitoes from living humans (38) suggests that DEET elicits broad-spectrum insect-repellent activity by means of a mechanism unrelated to the surface to which it is applied. The physiology of how blowflies detect DEET and why they avoid it remains unknown. But it is likely to combine those processes found in mosquitoes and other Nematocera (21) with detritivore-specific mechanisms such as gustatory receptors, leading to suppressed feeding or oviposition (15).

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