Journal of Chemical Ecology, Vol. 31, No. 1, January 2005 (© 2005)
DOI: 10.1007/s10886-005-0974-4

ANOINTING CHEMICALS AND ECTOPARASITES: EFFECTS OF BENZOQUINONES FROM MILLIPEDES ON THE LONE STAR TICK, *Amblyomma americanum*

J. F. CARROLL,[1,*] M. KRAMER,[2] P. J. WELDON,[3] and R. G. ROBBINS[4]

[1]*USDA, ARS, Animal Parasitic Diseases Laboratory*
Beltsville Agricultural Research Center
Beltsville, Maryland 20705, USA

[2]*USDA, ARS, Biometrical Consulting Service*
Beltsville Agricultural Research Center
Beltsville, Maryland 20705, USA

[3]*Wildlife Conservation Society, Science Resource*
Center, 2300 Southern Boulevard, Bronx
New York 10460, USA

[4]*Armed Forces Pest Management Board*
Walter Reed Army Medical Center, Washington
District of Columbia 20307, USA

(Received December 31, 2003; accepted August 17, 2004)

Abstract—Many mammals and birds roll on or rub themselves with millipedes that discharge benzoquinones. Chemicals transferred from millipedes onto the integument of anointing animals are thought to deter ectoparasites. We tested the lone star tick, *Amblyomma americanum* (L.), for responses to three widespread components of millipede defensive secretions, 1,4-benzoquinone; 2-methyl-1,4-benzoquinone (toluquinone); and 2-methoxy-3-methyl-1,4-benzoquinone (MMB). In toxicity tests, ticks were confined for 1 hr in filter-paper packets treated with serial dilutions of each of the benzoquinones or the commercial acaricide permethrin. Ticks were least affected by toluquinone, and most affected by permethrin. Of the benzoquinones, only MMB showed repellent activity. Behavioral assays were more sensitive than mortality for measuring the effects of the benzoquinones. Latencies for ticks to right themselves and to climb were greater with all compounds, even at the lowest concentrations, than with controls. Ticks exposed to low concentrations of benzoquinones appeared to recover over time, whereas those exposed to high concentrations exhibited behavioral abnormalities 1–3 mo later. Our results indicate that benzoquinones appropriated via anointing may reduce the tick loads of free-ranging animals,

* To whom correspondence should be addressed. E-mail: jcarroll@anri.barc.usda.gov

although key questions remain on the amounts of these compounds available to and effectively appropriated by anointing animals.

Key Words—Benzoquinones, ectoparasites, ticks, anointing, repellent, toxicant.

INTRODUCTION

A number of vertebrates rub on, or roll in, selected plants, arthropods, and other scent-bearing materials. Chemicals topically appropriated by self-anointing are known or suspected to deter predators, ectoparasites, or microbial pathogens (summarized by Weldon, 2004). Millipedes are used for self-anointing by a variety of birds and mammals. Free-ranging birds in the New and Old Worlds have been observed rubbing these myriapods against their plumage (reviewed in Parkes et al., 2003). Among mammals known to self-anoint with millipedes are Malagasy lemurs (Overdorff, 1993; Birkinshaw, 1999) and Central and South American cebid monkeys (Baker, 1996; Valderrama et al., 2000; Zito et al., 2003). Most identified millipedes used in self-anointing belong to the orders Julida (Harper in Cramp, 1981; Harrup, 1992), Spirobolida (Clunie, 1976; Overdorff, 1993; Zito et al., 2003), or Spirostreptida (Overdorff, 1993; Birkinshaw, 1999; Valderrama et al., 2000). These taxa, which collectively occur throughout temperate and tropical regions worldwide, comprise the "quinone millipedes," so called because they characteristically secrete benzoquinones from their numerous paired segmental glands (Eisner et al., 1978).

Valderrama et al. (2000) reported that the spirostreptidan millipede *Orthoporus dorsovittatus* was used in self-anointing by free-ranging wedge-capped capuchin monkeys, *Cebus olivaceus*, in Venezuela. They hypothesized that mosquitoes are repelled by benzoquinones appropriated from this millipede. To evaluate this hypothesis, Weldon et al. (2003) presented female yellow fever mosquitoes, *Aedes aegypti*, with the two secretory compounds isolated from *O. dorsovittatus*, 2-methyl-1,4-benzoquinone (toluquinone) and 2-methoxy-3-methyl-1,4-benzoquinone (MMB), on nylon-reinforced silicone membranes placed over wells filled with human blood. Mosquitoes landed and fed less frequently, and flew more frequently (a possible indication of repellency), in the presence of membranes treated with benzoquinones than with controls.

Studies with a variety of other insects demonstrate that benzoquinones deter feeding (Loconti and Roth, 1953; Ogden, 1969; Mondal and Port, 1984), act as topical irritants (Ogden, 1969; Peschke and Eisner, 1987), or are toxic (Kanehisa, 1969). Some benzoquinones also have been shown to be noxious or toxic to vertebrates, and to inhibit microbial growth (De Rosa et al., 1994). However, apart from the recent findings with mosquitoes (Weldon et al., 2003), no studies have tested the effects of these compounds on the ectoparasites of tetrapods.

Ixodid (hard) ticks are important pests of a wide range of vertebrates, including mammals and birds (Sonenshine, 1993). Heavy infestations of these

ectoparasites can debilitate or kill even large mammals (Strickland et al., 1976), but tick-borne diseases caused by bacteria (e.g., rickettsiae, spirochetes), viruses, and protozoa may pose an even greater danger to wildlife (Strickland et al., 1976; Sonenshine, 1993). In the New World tropics and subtropics, wounds caused by tick bites can serve as entry sites for flesh-eating and potentially deadly screwworms, *Cochliomyia hominivorax* (Strickland et al., 1976). Ticks, therefore, exert significant selective pressure on host species.

We report here on the behavioral responses and morbidity of the lone star tick, *Amblyomma americanum* (L.), to three compounds that occur widely in the defensive secretions of millipedes, 1,4-benzoquinone; toluquinone; and MMB (Eisner et al., 1978) and, for comparative purposes, to permethrin, a commercially available synthetic pyrethroid used to repel and kill ticks (summarized by Sonenshine, 1993). *A. americanum* ranges from the south-central and southeastern United States northward along the Atlantic seaboard to New England (Keirans and Durden, 1998; Guglielmone et al., 2003). In order to complete its life cycle, *A. americanum* must find a host three times and remain attached for several days each time (Strickland et al., 1976). The genus *Amblyomma* is predominantly tropical and subtropical in distribution (Hoogstraal, 1973; Hoogstraal and Aeschlimann, 1982), and includes species known to parasitize the monkeys *C. capucinus* and *C. olivaceus* (Fairchild et al., 1966; Jones et al., 1972), which are known to self-anoint with benzoquinones. Various *Amblyomma* species feed on birds (Strickland et al., 1976).

METHODS AND MATERIALS

Subjects. Nymphs of *A. americanum* were obtained from a colony at the United States Department of Agriculture, Agricultural Research Service, Knipling-Bushland US Livestock Insects Laboratory, Kerrville, TX. They were maintained at 23–24°C, ≈99% RH, and on a photoperiod of 16:8 hr (L:D). At the time of testing, ticks had been in the nymphal stage 5–8 wk.

Chemicals. 1,4-Benzoquinone and toluquinone were obtained commercially (Sigma-Aldrich, St. Louis, MO). MMB was synthesized, as described by Weldon et al. (2003). Permethrin was obtained from FMC Corporation (Philadelphia, PA). Solutions of 7.8, 31.0, 125, and 500 mM in acetone were prepared for each compound; an additional solution of 1.9 mM was prepared for permethrin. This dilution series was chosen, on the basis of results from preliminary studies, to include threshold concentrations; the lower dilution of permethrin was included because this compound appeared to be more potent than the benzoquinones we used. Solutions were kept refrigerated (5°C) and removed only for bioassay.

Repellent Bioassay. Repellency tests were conducted on a plastic Petri dish (5.5 cm diam, 1.5 cm high) containing a flat layer of modeling clay 0.5 cm deep, and glued centrally within a larger (9 cm diam, 1.3 cm high) dish. Water (≈0.5 cm deep) was added to the space between the concentric walls of the two Petri dishes, creating a moat to contain ticks. A 2 ×10-cm rectangle was marked with a lead

pencil on a 3 × 11-cm rectangular piece of filter paper (Whatman No. 4), 1 cm from a long and short edge. The filter paper was placed into a glass Petri dish (15 cm diam, 2 cm high), and 165 μl of either solvent (acetone) or test solution were evenly applied by a pipettor to the 2 × 10-cm rectangular area. After drying for 10–15 min, the paper was rolled crosswise and taped together to form a short cylinder (≈3.2 cm diam, 3 cm high). The treated portion of the filter paper formed a continuous band around the inside surface of the cylinder. The untreated edge of the filter paper cylinder was pushed ≈3 mm into the central area of the modeling clay, allowing a ≈7 mm high band of untreated filter paper surmounted by the 2-cm high band of treated filter paper to project above the clay.

Three groups of 10 ticks were tested with acetone and a 500 mM solution of each compound. MMB also was tested as a 125 mM solution. A different filter paper cylinder was used for each group of ticks. Ten active and ostensibly healthy nymphs were placed into a Teflon-lined plastic test tube (9 cm deep). The ticks were dumped from the tube onto the clay in the center of the filter paper cylinder. The water moat confined ticks that climbed out of the filter paper cylinder. The locations of ticks were recorded at 15 min after they were released. Ticks on the clay or on the untreated lower portion of filter paper inside the cylinder were considered repelled.

Exposure Packets. The process by which ixodid ticks find attachment sites and begin to feed generally takes several hours. Thus, in the absence of strong repellency, ticks would have extended exposure to benzoquinones on pelage or plumage, which we attempted to simulate to evaluate its possible physiological consequences on ticks. A 5 × 6-cm rectangle of Whatman No. 4 filter paper was marked with a lead pencil with lines 0.5 cm from each side. The filter paper was placed into a glass Petri dish and 165 μl of solvent or one of the test solutions (7.8, 31.0, 125, and 500 mM, and for permethrin, 1.9 mM) were applied evenly with a pipettor to the 4 × 5-cm rectangle bordered by the pencil lines. After the filter paper had dried for 10–15 min, it was folded crosswise. A bulldog clip (5.3 cm wide) closed each of two margins of the folded filter paper. Ten active nymphs were placed into the cavity formed by the folded paper, and another bulldog clip was attached to the open end of the packet to enclose the ticks. The clips were affixed slightly more than 0.5 cm from the edges of the paper, so that the confined ticks could only contact the treated area of the filter paper. The packet holding the ticks was kept for 1 hr in a glass desiccator jar (≈2 l) containing water (below the shelf) to maintain high humidity (>95% RH). The jars were rinsed with water after each exposure. Five replicates of 10 ticks each were prepared for each concentration–compound combination.

Righting and Climbing Responses. The packet was removed from the desiccator, opened, and the 10 nymphs were removed singly with forceps. Each tick was placed on its dorsum on clay within an encircling cylinder of untreated filter paper similar to that used for the repellent test. As with the repellent test, the clay arena was situated in a moated Petri dish. To prevent ticks from using the forceps

as leverage to right themselves as they were released, they were grasped so that their legs did not contact the forceps. The number of ticks that were right-side-up, and the number of ticks that had climbed to the rim of the filter paper cylinder or beyond, were recorded at 0.5, 1, 5, 10, and 15 min after release. After 15 min, the ticks were placed into a plastic vial with holes in the cap and returned to a regime of 23–24°C, 99% RH and a photoperiod of 16:8 hr (L:D). We refer to these trials below as 0-hr trials because they immediately followed the 1-hr exposure to one of the solutions. To evaluate how long the exposure to the benzoquinones and permethrin affected the nymphs, the behavioral tests were repeated with the ticks that were used in the last three trials at 24 hr (referred to as 24-hr trials) and again 1–3 mo after exposure (1–3-mo trials). Only the ticks exposed to the acetone (control) and 125 mM solutions were used in the 1–3-mo trials.

Toxicity. At 24 hr after exposure in filter paper packets, the survivorship of ticks was determined by observing the ticks in a moated Petri dish. Ticks moving only in an uncoordinated manner and a distance of <5 mm were considered moribund. Ticks that failed to move after 5 min in the Petri dish, even when exhaled upon or prodded with forceps, were considered dead. The behavioral test was repeated 24 hr after exposure with ticks in the last three replicates. In the analysis, we combined dead and moribund ticks because ticks in neither category would pose a risk to hosts. Ticks that were alive 24 hr after exposure were again examined for toxic effects 1–3 mo later. The criteria for mortality used in the toxicity tests were applied to these ticks to determine whether they should be excluded from the trial.

Statistical Analysis. We used logistic regression (Proc Genmod in SAS, 1999) to analyze the repellency of the compounds at 500 mM at 15 min. Since MMB repelled all ticks, we subtracted 0.5 from one of the scores (changing 10/10 to 9.5/10) to obtain meaningful parameter estimates. We estimated four one-degree-of-freedom contrasts with the acetone control.

Data on the number of ticks that were dead or moribund (incapacitated) 24 hr following the 1-hr exposure were modeled as dose–response relationships by using the arcsine-transformed proportion of dead and moribund ticks as the dependent variable and the square root of concentration as the independent variable. The square-root transformation of concentration yielded a straight-line relationship for all compounds except permethrin. We modeled permethrin only at the lowest concentrations, and set the percent of incapacitated ticks to 100% for concentrations >7.8 mM. Since the bioactivity of the benzoquinone solutions appeared to deteriorate over time, and the first trial on each group of 10 ticks was conducted from 24 to 104 days after the solutions were prepared, we also included the age of solution in the model for incapacitated ticks and for the models of behavioral change (discussed subsequently). We used the log of age − log (66) (66 days was the mean solution age) to reduce colinearity with the intercept term. We used the Proc Mixed routine in SAS (1999) for this analysis.

The behavioral data were not independent because each group of 10 ticks was observed five times within a concentration–compound combination on two different criteria and, for some groups, on three trials (0 hr, 24 hr, 1–3 mo following the initial 1-hr exposure). We found that the relationship between the arcsine-transformed proportion of ticks exhibiting the behavior (excluding incapacitated ticks) and the log of time was approximately linear. Therefore, for each of the six behavior-trial combinations (two behaviors, three trial times), we estimated a slope and an intercept (and their standard errors) for each concentration-compound combination. In effect, we modeled each group of 10 ticks with a straight line that relates the proportion (transformed scale) exhibiting the behavioral measure to observation time (transformed), yielding six lines (two behaviors, three trials) per group. For the righting behavior, we used all five observation time points to construct the lines, but for climbing behavior we used only the last four time points because the proportion for the first time point was zero for all groups.

We then created one degree-of-freedom contrasts (as t-values) between the control groups, which were modeled in the same way for each of the treatment groups. Each pair of contrasts compared the control intercept and control slope with the intercept and slope from one of the treatments. The magnitude and sign of the pair of contrasts indicate how the treatments differed from controls, with absolute values less than about two indicating no statistical difference. A positive value for an intercept contrast indicated that controls were more active at the earliest observation time. A positive value for a slope contrast indicated that, over the 15 min period, the proportion of ticks exhibiting the behavior in the treatment group increasingly lagged behind that of the control, i.e., differences between the treatment and control groups increased over time.

A multiple comparison adjustment was not required when comparing treatment effects within one of the three trials (0 hr, 24 hr, and 1–3 mo) and within one of the two behavioral measures. However, analyses of the two behaviors were correlated (ticks cannot climb if they have not first righted), as were analyses over the three trials (repeated measures of some ticks). Rather than present a recondite analysis with the data modeled jointly using a complicated covariance structure (to incorporate the time series structure and other potential dependencies), we present unadjusted t-values for the slopes and intercepts, which readers can interpret conservatively, and focus attention on their patterns.

Not all contrasts with controls could be made for two reasons. Because only live ticks were used to calculate these values for the 24-hr and 1–3-mo trials, there were some treatment combinations with no live ticks, and, thus, no possible estimate. In other cases, especially for higher concentrations, no living ticks exhibited the behavior for the entire 15 min observation period. In that case, a flat line of zero was estimated (with no variance), so a t-value based on a comparison with the control would be inappropriate. Model fitting was performed by using the R software (Ihaka and Gentleman, 1996), output was parsed, and t-values calculated using Perl programs. Other statistical comparisons were performed with standard t-tests.

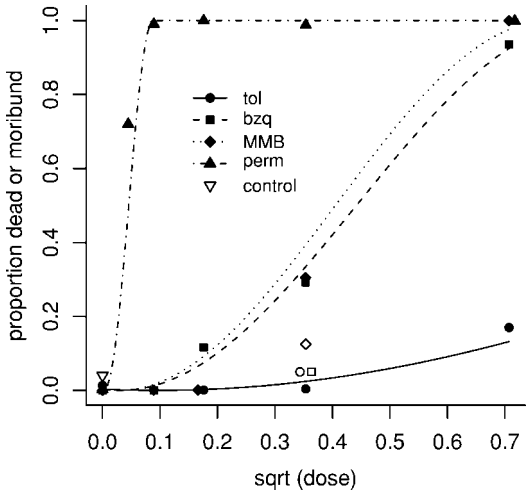

FIG. 1. Dose–response curves for incapacitation (morbidity and mortality) of *A. americanum* nymphs 24 hr after 1-hr exposure to benzoquinones or permethrin. The open symbols represent values for ticks tested 1–3 mo later. The compounds indicated are toluquinone (tol); 1,4-benzoquinone (bzq); 2-methoxy-3-methyl-1,4-benzoquinone (MMB); and permethrin (perm).

RESULTS

Repellency. Of the benzoquinones, only MMB repelled *A. americanum* nymphs ($\chi^2 = 17.56$, $P < 0.001$, 1 *df*). MMB repelled all nymphs at 500 mM (25.0 mM/cm^2), but none at the 125 mM (6.3 mM/cm^2) treatment. At 500 mM, permethrin repelled 63% of the nymphs, significantly more than the control ($\chi^2 = 14.94$, $P = 0.001$, 1 *df*).

Toxicity. Dose–response curves for incapacitation of *A. americanum* nymphs, adjusted to the mean solution age of 66 days and back-transformed to the original proportion scale, are shown (Figure 1). The model (on the transformed scale) is $t(p_i) = 0.982 + \beta_i\sqrt{\text{dose}} - 0.248 \log(d)$, where $t(p)$ gives the expected value of the arcsine-transformed proportion of incapacitated ticks, i indexes compounds, β_i estimates (standard errors) are 1.911 (0.189) for 1,4-benzoquinone, 2.084 (0.189) for MMB, 0.609 (0.189) for toluquinone, and 18.482 (1.482) for permethrin (only modeled for concentrations <7.8 mM), and d +66 is the number of days since the solution was prepared. The proportions of ticks incapacitated 1–3 mo after they were exposed to the 125 mM 1,4-benzoquinone treatment and acetone control also are depicted as open symbols in Figure 1.

Toluquinone was less toxic than either 1,4-benzoquinone or MMB ($P < 0.001$, *t*-test, 84 *df*), with the latter two statistically indistinguishable ($P = 0.74$, *t*-test, 84 *df*). Only 2% of ticks exposed to 7.8 mM permethrin were alive and

active 24 hr after exposure. At this concentration, the other compounds had little or no effect. For 1,4-benzoquinone and MMB, the proportion of ticks incapacitated decreased 1–3 mo later, as some of the ticks moribund at 24 hr recovered. All ticks classified as moribund at 24 hr after exposure to permethrin died within 1–3 mo. The proportion incapacitated 1–3 mo later changed little for toluquinone.

Behavioral Responses—Righting. In general, slope differences between control and treatment groups were not as great as intercept differences (Figure 2). Thus, the proportion of ticks righting at the beginning of testing differed between the control and treatment groups, but righting rates following the initial observation were roughly the same for the remainder of the observations. In general, these contrasts mirrored the toxicity statistics, with larger t-values for higher doses within a compound and the smallest t-values for toluquinone, the least toxic compound. Many of the intercept t-values were quite large and all were >3 for the first trial (0 hr), even for toluquinone, indicating an effect of all compounds at all doses

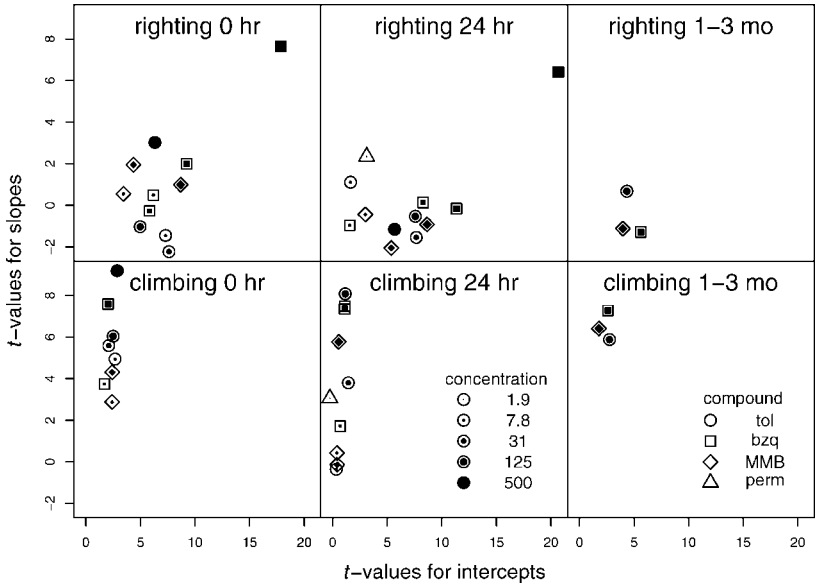

FIG. 2. Contrasts (as t-values) between treatment and control groups for the righting and climbing responses and for three trial times for the behavioral tests were calculated from regressing the arcsine-transformed proportion of ticks exhibiting the behavior on the log of time since the test started. Each point is associated with an intercept contrast (on abscissa) and slope contrast (ordinate). Increased filling of symbols indicates higher concentrations (mM). The greater the t-value differs from zero, the greater the behavioral difference between the treated and control group, with significant differences greater than about two. Not all treatment/dose combinations are given (see text).

on the righting behavior. One day later, some ticks appeared to have recovered at the lower concentrations, where the intercept *t*-values had moved toward 0 for all compounds, even permethrin. However, for higher concentrations, *t*-values for intercepts increased, indicating that the effect on ticks at these concentrations is more pronounced after 24 hr than immediately after exposure. There is evidence of continued recovery for the trials conducted 1–3 mo later: intercept *t*-values for compounds at these concentrations decreased relative to righting at 24 hr.

Behavioral Responses—Climbing. In contrast to righting, *t*-values were largest for slope contrasts (Figure 2). This is readily understood since intercepts, the estimates for 0 min, should be near 0 because all individuals, regardless of treatment, first had to right. However, intercept *t*-values did shrink from values close to 2.5 in the 0 hr trials to close to 0 in the 24-hr trials. Thus, earlier small, but consistent, differences in initial observations nearly disappeared 24 hr later.

In contrast to the intercept *t*-values, the slope *t*-values were large for almost all compounds at all concentrations. A clear concentration effect is evident, as the *t*-value order closely follows that of concentration. This indicates that the latency (or inability) to climb becomes more obvious over the 15-min observation period and depends on concentration. After 24 hr, there appears to be some return to control levels for low concentrations, as in the righting data. However, even after 1–3 mo, large lags in the climbing behavior of the ticks receiving the 125 mM concentration were evident. These individuals appeared to have suffered long-lasting effects from exposure to the compounds months earlier. With few exceptions, the differences between compounds were not as pronounced as differences between doses for both kinds of behavioral data. The highest concentrations generally are not depicted in Fig. 2 for either behavioral response because most ticks at these concentrations were incapacitated, and these contrasts could only be made for ticks that were not dead or moribund.

DISCUSSION

The phenomenon of anointing is unique among allelochemical interactions because organisms may respond in nature to chemicals from species with which they ordinarily do not interact (Weldon, 2004). Millipedes rarely and inconsequentially encounter tetrapod ectoparasites, but the responses of such ectoparasites to millipede chemicals may attain ecological relevance through the vehicle of avian and mammalian anointing. Our study is predicated on the assumption that ticks encounter benzoquinones deposited on the plumage or pelage of anointing animals.

The preprandial behaviors of ixodid ticks differ from those of mosquitoes and most other biting flies in ways that dispose ticks to receive greater exposure to anointing chemicals. Ixodids are highly selective in identifying feeding sites

(Waladde and Rice, 1982), wandering extensively on the skin before locating a suitable site of attachment (Kemp et al., 1982). Ticks crawling through the pelage or plumage of a host would be surrounded by anointed surfaces, encountering chemicals by contact or, in the case of volatiles, as fumigants. After penetration of their host's integument, many ticks secrete a hardened encasement around their feeding appendage, the hypostome, and proceed to engorge over a few days. Thus, the 1-hr confinement of ticks in treated filter paper packets during our bioassays was not excessively protracted in simulating chemical exposure to an anointed host.

A repellent, by definition, elicits movement away from a chemical source (Dethier et al., 1960). Our repellency bioassay allowed ticks to either crawl away from a treated area or fall off a vertical surface to avoid contact with repellent treatments. Only MMB at the highest dose we used (500 mM) prevented ticks from crawling across treated filter papers; the next lower dose (125 mM) of this compound had no detectable effect. These results are worth noting in light of numerous reports indicating that insects are irritated or repelled by benzoquinones (e.g., Ogden, 1969; Peschke and Eisner, 1987). Nonetheless, MMB was significantly more effective in preventing *A. americanum* from climbing out of treated filter paper cylinders than was permethrin, a synthetic pyrethroid marketed as a tick repellent and acaricide. Several ticks dropped from the permethrin-treated filter paper, whereas the ticks tended not to crawl on the MMB-treated filter paper. The repellency of permethrin may be due as much to its fast-acting toxicity requiring contact with treated surfaces as to actual avoidance (summarized in Sonenshine, 1993).

The morbidity/mortality of ticks in response to the compounds we used was greatest for permethrin, which was effective even at 1.9 mM. Morbidity/mortality in response to benzoquinones was manifest only at intermediate and high doses (125 and 500 mM), but ticks exposed even to the most dilute concentrations of these chemicals exhibited persistent impairments in righting and climbing abilities. The clearest behavioral difference in response to the three benzoquinones we tested, and among the doses of each, was manifest with respect to climbing. Climbing ability, an integral part of host acquisition by many ixodid ticks (Lees and Milne, 1951; Sonenshine, 1993), was found impaired upon retesting ticks more than 1 mo after exposure in our tests. Climbing inhibition would seriously impair a tick's ability to contact a suitable host, and may be a manifestation of a more basic behavioral or physiological impairment. The toxicities of toluquinone and other benzoquinones also have been demonstrated with insects (Kanehisa, 1969). Mosquitoes (*Ae. aegypti*) exposed to toluquinone and MMB on feeding membranes sometimes became immobilized and died, especially with MMB (Weldon et al., 2003).

The benzoquinones were not as toxic to ticks as permethrin. However, ticks exposed for 1 hr to benzoquinones even at low concentrations behaved abnormally, and intermediate concentrations clearly were toxic. Other than MMB, these

compounds do not seem to repel ticks, suggesting that, for protection against ticks, anointing animals must apply sufficient quantities of compound to disorient or physically disable potential tick parasites. Tests of climbing produced a clearer pattern than righting for detecting the behavioral effects of the compounds and their concentration effects. The climbing test also better showed concentrations at which effects were temporary and at which they were long-lasting. Both measures demonstrated that even low concentrations adversely affected these two behaviors important for successful host attachment.

The toxicities of benzoquinones demonstrated in our study of *A. americanum* indicate the potential for protection against ticks afforded by anointing with these compounds. Key questions remain, however, on the quantities of benzoquinones available to and effectively appropriated by free-ranging animals. Estimates of the quantities of benzoquinones produced by millipedes range up to 350 mg per individual for some tropical species (Fairhurst, 1993). These compounds may be appropriated from other sources, as well. Laughingthrushes (*Garrulax* spp.) from Fiume, Italy (currently Rijeka, Croatia), for example, are reported to anoint with tenebrionid beetles of the genus *Blaps* (Callegari, 1955), which may defensively discharge benzoquinones (Blum, 1981).

Because attachment and commencement of feeding in ixodid ticks takes hours and completion of feeding is a matter of several days, the exposure that ticks receive to chemicals applied to the integument of their hosts is protracted. Repeated anointment may be necessary to maintain the efficacy of topically applied compounds due to their volatility and/or degradation. We detected a reduced efficacy of the benzoquinones used in our tests over time, even though solutions were stored in cold, light-free conditions; this prompted us to include a time covariate for solution age when modeling the data. Quantitative studies of the transfer and retention of anointing chemicals, and of their efficacy in deterring ectoparasites on hosts, are needed.

Acknowledgments—We thank J. M. Pound and C. Santos, USDA, ARS Knipling-Bushland US Livestock Insects Research Laboratory, Kerrville, TX for supplying *A. americanum* nymphs, and J. E. Oliver and A. Weatherwax, USDA, ARS, Chemicals Affecting Insect Behavior Laboratory, Beltsville, MD for performing syntheses.

REFERENCES

BAKER, M. 1996. Fur rubbing: Use of medicinal plants by capuchin monkeys (*Cebus capucinus*). *Am. J. Primatol*. 38:263–270.

BIRKINSHAW, C. R. 1999. Use of millipedes by black lemurs to anoint their bodies. *Folia Primatol*. 70:170–171.

BLUM, M. S. 1981. Chemical Defenses of Arthropods. Academic Press, New York.

CALLEGARI, E. 1955. La "Mirmecosimpatia" è un fenomeno esteso anche ad altri gruppi di insetti. *Riv. Ital. Orn*. 25:147–148.

CLUNIE, F. 1976. Jungle mynah "anting" with millipede. *Notornis* 23:77.

CRAMP, S. 1981. Handbook of the Birds of Europe, the Middle East, and North America: The Birds of the Palearctic. Vol. V. Oxford University Press, Oxford. pp. 596–616.

DE ROSA S., GIULIO, A., and IODICE, C. 1994. Biological effects of prenylated hydroquinones: Structure–activity relationship studies in antimicrobial, brine shrimp, and fish lethality assays. *J. Nat. Prod.* 57:1711–1716.

DETHIER, V. G., BROWNE, L. B., and SMITH, C. N. 1960. The designation of chemicals in terms of the responses they elicit from insects. *J. Econ. Entomol.* 53:134–136.

EISNER, T., ALSOP, D., HICKS, K., and MEINWALD, J. 1978. Defensive secretions of millipeds, pp. 41–72, *in* S. Bertini (ed.). Handbook of Experimental Pharmacology, Vol. 48. Springer-Verlag, New York.

FAIRCHILD, G. B., KOHLS, G. M., and TIPTON, V. J. 1966. The ticks of Panama (Acarina: Ixodoidea), pp. 167–219 *in* R. L. Wenzel and V. J. Tipton (eds.). Ectoparasites of Panama. Field Museum of Natural History, Chicago.

FAIRHURST, C. P. 1993. Poisonous exudates of millipedes. *Anal. Proc.* 30:429–430.

GUGLIELMONE, A. A., ESTRADA-PEÑA, A., KEIRANS, J. E., and ROBBINS, R. G. 2003. Ticks (Acari: Ixodidae) of the Neotropical Zoogeographic Region. Special Publication, International Consortium on Ticks and Tick-Borne Diseases, Atalanta Houten, The Netherlands, 173 pp.

HARRUP, B. 1992. Robin 'anting' with millipede. *British Birds* 85:189.

HOOGSTRAAL H. 1973. Acarina (ticks), pp. 89–103, *in* A. J. Gibbs (ed.). Viruses and Invertebrates. North Holland Publishing Co., Amsterdam and London.

HOOGSTRAAL, H. and AESCHLIMANN, A. 1982. Tick-host specificity. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft/Bulletin de la Société Entomologique Suisse* 55(1–2):5–32.

IHAKA, R. and GENTLEMAN, R. 1996. R: A language for data analysis and graphics. *J. Comput. Graph. Stat.* 5:299–314.

JONES, E. K., CLIFFORD, C. M., KEIRANS, J. E., and KOHLS, G. M. 1972. The ticks of Venezuela (Acarina: Ixodoidea) with a key to the species of *Amblyomma* in the Western Hemisphere. *Brigham Young Univ. Sci. Bull. Biol. Ser.* 17:1–40.

KANEHISA, K. 1969. Quinone secretion by rust-red flour beetles, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), and their toxicity to other insects. *Nogaku Kenkyu* 53:5–14 (In Japanese).

KEIRANS, J. E. and DURDEN, L. A. 1998. Illustrated key to nymphs of the tick genus *Amblyomma* (Acari: Ixodidae) found in the United States. *J. Med. Entomol.* 35:489–495.

KEMP, D. H., STONE, B. F., and BINNINGTON, K. C. 1982. Tick attachment and feeding: Role of the mouthparts, feeding apparatus, salivary gland secretions and the host response, pp. 119–168, *in* F. D. Obenchain and R. Galun (eds.). Physiology of Ticks. Pergamon Press, New York.

LEES, A. D. and MILNE, A. 1951. The seasonal and diurnal activities of individual sheep ticks (*Ixodes ricinus* L.). *Parasitology* 41:189–209.

LOCONTI, J. D. and ROTH, L. M. 1953. Composition of the odorous secretion of *Tribolium castaneum*. *Ann. Entomol. Soc. Am.* 46:281–289.

MONDAL, K. A. M. S. H. and PORT, G. R. 1984. Repellent effect of synthetic methylquinone on larvae of *Tribolium castaneum* Herbst. *Int. Pest Control.* 26:68–71.

OGDEN, J. C. 1969. Effect of components of conditioned medium on behavior in *Tribolium confusum*. *Physiol. Zool.* 42:266–274.

OVERDORFF, D. J. 1993. Similarities, differences, and seasonal patterns in the diets of *Eulemur fulvus rufus* and *Eulemur rubiventer* in the Ranomafan National Park, Madagascar. *Int. J. Primatol.* 14:721–753.

PARKES, K. C., WELDON, P. J., and HOFFMAN, R. L. 2003. Polydesmidan millipede used in self-anointing by a strong-billed woodcreeper (*Xiphocolaptes promeropirhynchus*) from Belize. *Ornithol. Neotrop.* 14:285–286.

PESCHKE, K. and EISNER, T. 1987. Defensive secretion of the tenebrionid beetle, *Blaps mucronata*: Physical and chemical determinants of effectiveness. *J. Comp. Physiol. A* 161:377–388.

SAS INSTITUTE, INC. 1999. SAS/STAT User's Guide, Version 8. SAS Institute, Inc., Cary, NC, 3884 pp.

SONENSHINE, D. E. 1993. Biology of Ticks. Vol. 2. Oxford University Press, New York, 465 pp.

STRICKLAND, R. K., GERRISH, R. R., HOURRIGAN, J. L., and SCHUBERT, G. O. 1976. Ticks of veterinary importance. APHIS, US Dept. Agric. Hdbk. No. 485, pp. 122.

VALDERRAMA, X., ROBINSON, J. G., ATTYGALLE, A. B., and EISNER, T. 2000. Seasonal anointment with millipedes in a wild primate: A chemical defense against insects? *J. Chem. Ecol.* 26:2781–2790.

WALADDE, S. M. and RICE, M. J. 1982. The sensory basis for tick feeding behavior, pp. 71–118, *in* F. D. Obenchain and R. Galun (eds.). Physiology of Ticks. Pergamon Press, New York.

WELDON, P. J. 2004. Defensive anointing: Extended chemical phenotype and unorthodox ecology. *Chemoecology* 14:1–4.

WELDON, P. J., ALDRICH, J. R., KLUN, J. A., OLIVER, J. E., and DEBBOUN, M. 2003. Benzoquinones from millipedes deter mosquitoes and elicit self-anointing in capuchin monkeys (*Cebus* spp.). *Naturwissenschaften* 90:301–304.

ZITO, M., EVANS, S., and WELDON, P. J. 2003. Owl monkeys (*Aotus* spp.) self-anoint with plants and millipedes. *Folia Primatol.* 74:159–161.