

Similarity in Responses of Laboratory-Reared and Field-Collected Lone Star Tick (*Acari: Ixodidae*) Nymphs to Repellents¹

J. F. Carroll,² J. A. Klun,³ and M. Kramer⁴

U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, Maryland 20705 USA

J. Entomol. Sci. 43(4): 426-430 (October 2008)

Key Words *Amblyomma americanum*, nymphs, behavioral bioassays, dose response

Ticks and tick-borne diseases that affect humans are a growing source of concern in the U.S. and elsewhere in the world (Gratz 1999, Annu. Rev. Entomol. 44: 51-75). The lone star tick, *Amblyomma americanum* (L.), is increasingly a problem in the southeastern, south-central and mid-Atlantic states because it readily bites humans, is expanding its range, and has been implicated in the transmission of ehrlichial pathogens (Childs and Paddock 2003, Annu. Rev. Entomol. 48: 307-337). Although progress has been made recently in the development of area-wide tick control technologies (Pound et al. 2000, J. Med. Entomol. 37: 588-594), repellents remain the primary method of personal protection against tick bites (CDC 2002 Lyme disease. Department of Health and Human Services, Centers for Disease Control and Prevention, Ft. Collins, CO, 12 p).

The ultimate test of a repellent is its efficacy in the field. However, in conducting a successful field test using multiple concentrations of multiple repellents intended for use on human skin, one is faced with several challenges. It is difficult to recruit sufficient volunteers, who may be required to walk through tick habitats and expose their skin to thorns, poisonous plants, and biting arthropods to contact ticks. On a local scale, ticks tend to follow a clumped distribution (overdispersed data) that complicates data analysis and may result in some repellent-concentration combinations being exposed to few ticks. Wet vegetation hampers host acquisition by ticks, so weather postponements requiring volunteer rescheduling are a potential complication. Not surprisingly, much of the efficacy data for tick repellents have been generated in the laboratory. The reluctance to extrapolate from these laboratory efficacy data to the field stems from valid concerns, not the least of which involves a lack of

comparative efficacy data for laboratory-reared and field ticks. By virtue of their relatively small size, laboratory colonies may be subject to the dynamics of population genetics (e.g., genetic drift) not associated with large natural populations (Whitlock 2000, Evolution 54: 1855-1861). Selection pressures on laboratory and field populations also differ. Generally, laboratory-maintained ticks are reared under conditions to minimize development time and stress to the ticks. However, periodic introduction of new field-collected ticks may alleviate genetic problems. To determine if rearing conditions affect responses in laboratory dose-response trials, we conducted an experiment to compare the responses of laboratory-reared and field-collected *A. americanum* nymphs to 2 repellents, Deet (N,N-diethyl-3-methylbenzamide) and racemic 2-methylpiperidinyl-3-cyclohexene-1-carboxamide (AI3-37220).

Ticks. Appetent nymphs of *A. americanum* were obtained from laboratory colonies maintained at USDA, ARS, Knippling-Bushland Livestock Insects Research Laboratory (K-BUSLIRL), Kerrville, TX and Oklahoma State Univ. (OSU). The OSU colony of *A. americanum* was established in 1976 from ticks collected in Cherokee Co., OK. Every 2 yrs, female ticks collected at Stillwater, Payne Co., OK, have been introduced into the colony, which had ~15,000 adults. The K-BUSLIRL colony was established from *A. americanum* collected in Kerr Co., TX. The nymphs used in this study from the K-BUSLIRL colony were generation F13. No field-collected ticks had been introduced into the K-BUSLIRL colony, and it had ~2,000 adults. Once at our facilities, the colony-reared ticks were kept at 22-23°C, 97% RH and a photoperiod of 16:8 h L:D. Host-seeking nymphs (~600) were collected in June and July 2006, at the USGS Patuxent Wildlife Research Center, Laurel, MD. The field-collected ticks were kept at 21-24°C, 97% RH and ambient photoperiod. Nymphs from the Oklahoma State University and USDA, ARS, K-BUSLIRL colonies were tested 8-9 wk and 14-15 wk, respectively, after they had fed as larvae. Field-collected ticks were tested 2-3.5 wk after they were collected.

Repellents. AI3-37220 was obtained from Morflex (Greensboro, NC), and Deet was purchased from Sigma Aldrich (St. Louis, MO). Gas chromatographic analyses of the compounds showed that they were 99% chemically pure. Stock solutions of the compounds were prepared gravimetrically using 95% ethanol as solvent, and diluted volumetrically to obtain solutions required for dose x response bioassays.

Bioassay. An in vitro bioassay described by Carroll et al. (2004, J. Med. Entomol. 41: 249-254) was used to ascertain tick responses to the repellents. Briefly, using a lead pencil, 2 lines were drawn on a 4 x 7 cm strip of Whatman No. 4 filter paper, each line 1 cm from a narrow end of the paper. The test solution (165 µL) was applied evenly by pipettor to the middle 20 cm² of the filter paper. The paper was allowed to dry for 10-15 min before testing. A vial containing ticks was opened in the inner of 2 Petri dishes with water forming a moat between their sides. A bulldog clip was affixed to the upper untreated end of the paper. The filter paper was held vertically and ticks were allowed to crawl onto the lower untreated end of the filter paper. Once the tenth tick was on the filter paper, the paper was suspended vertically, over a moated Petri dish from an adjustable multi holder. Tick locations were recorded 1, 3, 5 and 10 min after the tenth tick crawled onto the paper. Ticks that dropped from the paper and those that were in the lower untreated portion of the filter paper at 10 min after having climbed on it were considered repelled. Repellents in ethanol solutions were applied to the filter paper in concentrations of 0, 0.2, 0.39, 0.79, 1.57, 3.14 and 6.28 µmoles/cm² paper. Three groups of 10 nymphs from each colony and field source were tested against each concentration of each repellent. In most instances, a complete replicate

¹Received 24 December 2007; accepted for publication 17 February 2008.

²Address inquiries (email: john.carroll@ars.usda.gov). Animal Parasitic Diseases Laboratory.

³Invasive Insects Biocontrol and Behavior Laboratory.

⁴Biometrical Consulting Service.

was tested on 1 d. When a second day was needed, a control (zero concentration) was among the samples tested each day.

Statistical Analysis. The data were analyzed in a generalized linear model framework, where, for each group of ticks tested, the number of ticks repelled is modeled as a binomial response variable (this is sometimes described as a logistic regression). The independent variables modeled as affecting the response variable were the square root of the repellent concentration, the repellent compound, a variable describing the source of the ticks (broken down to testing date categories), and two-way interactions between compound and concentration, and between tick source and concentration.

Data for the first replicate of Laurel field-collected nymphs were excluded from the modeling because they were clearly inconsistent with the other 2 Laurel replicates, as well as all the replicates using laboratory-reared ticks. We attribute the inconsistencies seen in the responses of the first Laurel replicate to experimental technique. We investigated but found no other replicate (date tested) effect, and ticks were grouped by their source (Laurel, OK, and TX) only for the model results reported. The analysis was conducted using the R statistical software (R Development Core Team, 2006, Vienna, Austria).

For all nymphs except the first replicate of Laurel ticks, the slope of the dose-response curves from all 3 populations of *A. americanum* was steeper for AI3-37220 than for Deet ($P = 0.002$), that is, AI3-37220 was a more effective repellent than Deet at the same concentration. Responses of the ticks from different sources, other than for an overall difference in the way ticks responded to the 2 compounds, were statistically indistinguishable (the P values of contrasts of slope coefficients with Laurel field-collected ticks were both >0.5), thus a compound by tick source interaction term was not needed for the model. Ticks from all sources except the first replicate of Laurel ticks had similar dose-response curves. Figure 1 gives curves for Deet and AI3-37220 on the back-transformed proportion scale, with approximate 95% confidence intervals for the means at the concentrations used.

For the first replicate of Laurel ticks, the proportions of ticks repelled by Deet for the concentrations of 0, 0.02, 0.039, 0.079, 0.157, 0.314 and 0.628 $\mu\text{mole}/\text{cm}^2$ filter paper were 0.3, 0.3, 0, 0.7, 0.6, 0.9 and 1.0, respectively, and for AI3-37220 the proportions repelled were 0, 0.1, 0.8, 0.5, 0.4, 1.0 and 1.0. This group of ticks had a dose-response curve (not illustrated) that differed from all other sources of ticks in 2 ways: a higher intercept (more ticks "repelled" at zero and low concentrations) but also a flatter slope (less effect of increasing concentration), so at high concentrations, ticks in this group tended to be among the least repelled. The slope of the curve was slightly higher for AI3-37220, but not significantly so ($P = 0.48$).

Although we suspect that experimental technique was the source of the odd data for the first replicate with Laurel ticks, we do not know whether the Laurel ticks on the first date of testing differed from the other groups because of testing conditions (that is, if any other group of ticks had been tested that day, results would be similar) or if that group of ticks differed in some fundamental way from subsequent replicates tested from Laurel and the laboratory sources.

The similarity in responses to each of 2 repellents by nymphs from the 2 laboratory colonies and 1 field population indicates that it is reasonable to extrapolate with some confidence from results of repellent tests using laboratory-reared ticks as to how field-collected ticks will respond. Other factors associated with field conditions, such as heavy perspiration and abrasive removal of repellent from skin by contact with

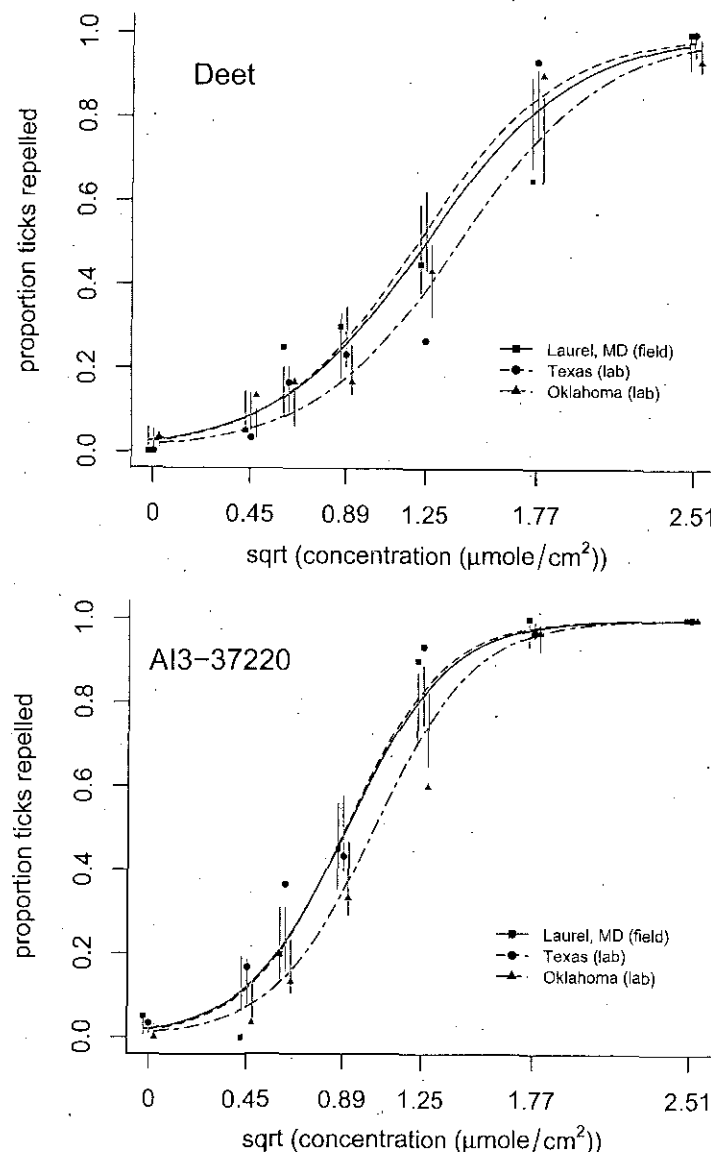


Fig. 1. Proportion repelled of field-collected *A. americanum* nymphs and nymphs from two laboratory colonies to the increasing concentrations of the repellents Deet and AI3-37220 ($n = 30$ ticks for each dot symbol). Curves represent best fit dose-response lines (on the logit scale), back-transformed to the proportion scale. Vertical bars give an approximate 95% confidence interval of the estimated mean at each concentration used. Ticks were considered repelled if they dropped from the filter paper strip or were on the lower untreated portion 10 min after climbing on the strip. Sqrt = square root.

clothing and various surfaces, can influence repellent effectiveness. However, if necessary, such conditions can be simulated indoors.

Uniformity tends to be a distinguishing feature of laboratory-reared organisms, and ticks are no exception. A cohort of ticks reared in a laboratory is generally descended from the same few founder females, maintained under the same temperature, RH and photoperiod (all favorable for development) and placed on hosts at the same time. In contrast, field-collected ticks differ among themselves in chronological and physiological age, species of host fed on, and the microclimatic conditions to which each has been exposed.

Although it is preferable to compare laboratory-reared and field-collected nymphs of the same age directly in nearly simultaneous bioassays, the natural phenology of *A. americanum* in MD precluded such a comparison. The field-collected *A. americanum* from MD sought hosts in May-July, but fed most recently as larvae in August or September of the previous year. In contrast, laboratory colonies are maintained under temperature and photoperiod regimes that minimize developmental time (months shorter than field ticks). The laboratory-reared nymphs in our study were tested 2-3.5 mo since feeding, whereas the field-collected nymphs had overwintered and not fed in ~9-10 mo. Age may influence ticks' response to repellents, as nymphs of *I. scapularis* that were 10-11 wk since their last molt tended to be slightly more sensitive to Deet and AI3-37220 than nymphs that were 4-6 wk post molt (Carroll et al. 2004). However, age did not seem to be a factor in results reported here. Our data show that *A. americanum* nymphs reared under typical laboratory regimes responded to repellents similarly to field-collected ticks and support the validity of using laboratory-reared ticks in bioassays to approximate the responses of field populations.

Acknowledgments

The authors thank J. M. Pound, USDA, ARS, Knipling-Bushland, U.S. Livestock Insects Laboratory, Kerrville, TX for supplying *A. americanum* nymphs. We are grateful to A. Abrams and D. Zapotok, USDA, ARS, Animal Parasitic Diseases Laboratory, Beltsville Agricultural Research Center, Beltsville, MD for their invaluable assistance in collecting ticks and conducting bioassays.

NOTE

Relative Collection Efficiency of the Keep-It-Simple-Sam Cotton Fleahoppers (Hemiptera: Miridae) in Cotton¹

Charles P.-C. Suh²

USDA, ARS, Areawide Pest Management Research Unit, College Station, Texas 77845 USA

J. Entomol. Sci. 43(4): 431-433 (October 2008)

Key Words cotton fleahopper, *Pseudatomoscelis seriatus*, sampling, Keep Sampler

Reassessment of action thresholds to initiate treatment of the cotton fleahopper *Pseudatomoscelis seriatus* (Reuter), has recently been identified as a priority by extension and research entomologists in Texas. Imperative to this mission is the ability to accurately and efficiently estimate fleahopper levels in cotton, *Gossypium hirsutum* L. The standard method for quantifying fleahopper abundance involves direct counts of adults and/or nymphs on plant examination method). This sampling method, however, typically requires a considerable level of experience and keen vision to obtain accurate counts. Consequently, there is a need among researchers to identify or develop alternative methods for fleahoppers in cotton. Beerwinkle et al. (1997, pp. 1330-1333 Beltwide Cotton Conf.) introduced a hand-held pneumatic device, the Keep Sampler (KISS), as a portable tool that could be used to efficiently sample fleahoppers in various row crops. However, the potential use of the KISS for sampling cotton fleahoppers in cotton has not been examined. Presented here are estimates of the collection efficiency of the KISS for fleahopper adults and nymphs relative to the whole-plant examination method, and some observations and disadvantages of using the KISS.

Commercial cotton fields located in the Brazos River Bottom production area of Texas were sampled for cotton fleahoppers in 2005 (4 fields) and 2006 (5 fields) using both the KISS and whole-plant examination method. Fields were sampled weekly during the initial 3 wks of squaring except when rain or insecticide application prevented sampling. All fields were sampled between 0830 and 1400 h (in the same order on each sampling date to minimize potential time-of-day effects). Additionally, node counts and plant height measurements were taken weekly on 50 plants in each field to provide supporting information.

Within each field, sampling was confined to a 0.4-ha area partitioned

¹Received 07 December 2007; accepted for publication 18 February 2008.

²Address inquiries (email: suh@usda-apmru.tamu.edu).