Efficacy of Dry Ice-Baited Traps for Sampling *Amblyomma americanum* (Acari: Ixodidae) Varies With Life Stage But Not Habitat

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ABSTRACT The carbon dioxide-baited trap is the most common and effective method for sampling vector life-stage *Amblyomma americanum* (L.) (Acari: Ixodidae), although confounding environmental variables are rarely considered. A mark–recapture experiment was designed to compare recapture proportions of *A. americanum* nymphs and adults between two habitat types: old field and oak–hickory forest. Powdered fluorescent dye was used to mark *A. americanum* ticks released in 1-m increments from carbon dioxide-baited traps. Adults were recaptured in significantly higher proportion than nymphs, but habitat type had no significant effect on recapture proportions. Tick abundance is an important parameter in the estimation of human risk of exposure to tick-borne disease and the influence of life stage on capture rates should be considered when calculating entomological risk.

KEY WORDS *Amblyomma americanum*, mark–recapture, carbon dioxide trap sampling, tick-borne disease
and the difference in efficacy of the traps between two primary habitat types: open fields and closed canopy forests. Our primary goals are to 1) evaluate the influence of habitat type on the effective sampling area of CO₂ traps, 2) evaluate sampling bias between nymphal and adult life stages, and 3) suggest the 24-h trap period as a standard method of ecological sampling to compile larger, collaborative data sets. Because larval "A. americanum" ticks are neither captured in significant numbers by CO₂ traps (Koch and McNew 1982), nor as likely to vector pathogens to humans (Paddock and Yabsley 2007), they were not considered in our experiment. This study systematically compares the proportions of marked wild nymphs and adults recaptured across two primary habitats for "A. americanum", testing the effective range of CO₂ traps in 1-m increments.

Materials and Methods

Location. Tyson Research Center is an 809-ha biological field station located on the northeastern end of the Ozark Highlands Plateau, 40.234 m (25 miles) southwest of St. Louis, MO (38°31' N, 90°33' W). Oak–hickory forests on steep ridges and an herbaceous understory dominate 85% of the site. Dominant tree species on protected slopes include "Cornus florida" (West.) Schelle (flowering dogwood), "Quercus alba" L. (white oak), and "Quercus celutina" Lamb. (black oak), whereas more exposed south-facing slopes tend to be dominated by "Juniperus virginiana" Taylor (eastern red cedar) and "Quercus muehlenbergii" Engelsh. (chinquapin oak). Several disturbed areas have turned into typical old field habitats (~9 ha), dominated by an array of native and invasive grasses and forbs, including "Festuca arundinacea" Schreb. (tall fescue), "Bromus inermis" Leyss. (smooth brome), "Elymus virginicus" L. (Virginia wild rye), "Lespedeza cuneata" Dum. Cours. (smooth brome), "Eupatorium rugosum" (L.) King & H. E. Robins. (boneset).

Tick Collection and Handling. All "A. americanum" ticks used in the mark–recapture experiment were collected in forest-edge habitat by using drag cloth sampling to avoid any harm to the ticks. Experimental ticks were captured between 9:00 and 11:00 a.m. and were held in collecting jars prepared with a damp layer of plaster of Paris to prevent desiccation. Approximately 0.1 g of DayGlo Axx series fluorescent powdered dye (DayGlo Color Corporation, Cleveland, OH) in one of five distinct colors was added to experimental release jars as per Solberg et al. (1992). There were two 0.5-cm holes drilled near the bottom of each side of the beverage cooler through which gaseous CO₂ could emit. Double-sided 3M carpet tape was used to cover the topside perimeter of the plywood base and 1 ± 0.09 kg of block form dry ice was added to each trap as CO₂ bait, an amount that proved sufficient to persist for a 24-h trap period by using the hard-sided beverage coolers. The CO₂ signal attracted host-seeking ticks, which then became ensnared in the double-sided tape.

Mark–Recapture Experiments. All mark–recapture experiments were established on level ground between 11:00 am and 1:00 p.m. and were collected 24 h later. During two rounds of preliminary observations, no nymphal or adult "A. americanum" ticks were recaptured from 6 to 10 m. Thus, for each mark–recapture experiment, 10 nymph and 10 adult "A. americanum" were released incrementally from 1 to 5 m from a single CO₂ trap, with a different color of fluorescent dye corresponding to each distance. We conducted 16 mark–recapture trap experiments in two habitat types (n is number of replicates): old fields (n = 8) and oak–hickory forests (n = 8). Each experiment consisted of 50 nymphs and 50 adult "A. americanum", requiring 1,600 individual ticks in total. All traps were paired temporally across field and forest habitat. Traps were set between 17 May and 18 July 2009, and experiments were performed on days with no precipitation and low wind speeds (mean ± SD, 1.44 ± 0.84 km/h). Ticks were consistently released in the same cardinal direction on each day and slopes at study sites were <5°.

Vegetation Surveys. To quantify the potential effect of differences in vegetation structure between the two habitat types on tick recapture rates, we ran three 20-m transects in each habitat type (old field and oak–hickory forest) proximate to locations where mark–recapture experiments were conducted. We used the line-transect method, which entailed two poles connected by a 5-m line that ran 25 cm above the ground. The number of contacts with the line by green vegetation was recorded along each transect and used for statistical analysis.

Statistical Analysis. Mark–recapture experiments were analyzed using a permutable multivariate analysis of variance (ANOVA) with a nested design (PERMANOVA, Anderson 2001, McArdle and Anderson 2001). PERMANOVA makes no particular assumptions regarding the distributions of original variables because all P values are obtained by permutation. This analysis evaluated the influence of habitat type, release distance, and life stage on the proportion of ticks recaptured per trap and was performed with type III sums of squares and 9,999 unrestricted permutations of the raw data by using correct permutable units. Pairwise posteriori comparisons among release distances were also made by 9,999 permutations. Vegetation surveys were analyzed by an F-test to compare variances and then a Welch two-sample t-test. Analyses were based on the number of live vegetation contacts 25 cm above the ground along the transects.
and were conducted in the R programming environment.

Results and Discussion

We found a significant effect of life stage (F = 341.61, df = 1, P < 0.001) and release distance (F = 3215.49, df = 8, P < 0.001), but not habitat type (F = 3.61, df = 10, P = 0.074), on the proportion of A. americanum recaptured (Fig. 1). The mean proportion of adults and nymphs recaptured declined with distance in both field and forest habitats. Adults were recaptured in greater proportions than nymphs at all five distance categories; no nymphs were recaptured at distances of 4 or 5 m. Permutational a posteriori pairwise comparisons indicated significant differences in the proportion of nymphs and adults recaptured between all distance categories (t value range, 6.50–18.73; P < 0.001 in all cases). Effect sizes consistently increased with differences in distance category comparisons, with the strongest difference occurring between distances of 1 and 5 m in both the forest and field habitats.

The old field sites used in our study consist of dense vegetation composed of tall grasses that are significantly denser than the understory of the deciduous forest study sites. Adults were recaptured in greater proportions than nymphs at all five distance categories; no nymphs were recaptured at distances of 4 or 5 m. Permutational a posteriori pairwise comparisons indicated significant differences in the proportion of nymphs and adults recaptured between all distance categories (t value range, 6.50–18.73; P < 0.001 in all cases). Effect sizes consistently increased with differences in distance category comparisons, with the strongest difference occurring between distances of 1 and 5 m in both the forest and field habitats.

The signifi cantly lower recapture rates for nymphs are most likely a result of their constrained mobility relative to adults because of their small size. Nymphs also may be more susceptible to desiccation, and this may further decrease their mobility in direct sunlight and open habitat, although microscale habitat distributions of A. americanum suggest that it is relatively desiccation tolerant (Schulze et al. 2002). However, in field surveys using CO2 traps, nymphs can be far more abundant than adults during periods when both life stages are active. Our results suggest that nymphs are not able to travel as far as adults in response to a CO2 attractant; therefore, the area over which nymphs are captured is smaller. Thus, actual nymph relative abundance may be proportionally higher than represented from field trap data, and attempts to quantify tick abundance via CO2 traps should take into account the different size areas over which the two life stages are sampled.

Several other studies have used mark–recapture approaches to estimate the efficacy of CO2 traps for sampling A. americanum in forest habitats (Wilson et al. 1972, Koch and McNew 1982, Koch 1987, Solberg et al. 1992). Several reports have suggested that CO2 traps are often rebaited and sampled for up to a 7-d period. However, both our recapture rates of marked ticks and the area over which we found CO2 traps to be effective were consistent with the results from the first 24-h period of these other studies. For broad-scale field studies, the inefficiency and expense of repeatedly rebaiting CO2 traps at numerous sites may render this approach infeasible. However, the consistency of the results from the first 24-h time period between our study and others suggests that this is a reliable sample period for comparing the abundances of nymphal and adult A. americanum between study sites and longer sampling periods may be unwarranted.

A crucial step in developing widely applicable disease models that can be catered to multiple spatial scales (local, regional, and national) is to apply an axiomatic sampling method for vector abundance parameters, so data sets between regions can be standardized. Considering the increasing diversity and medical importance of pathogens vectored by A. americanum, it is important to promote standardized sampling approaches so that variation in disease cycles between regions may be understood. Our recapture data obtained in this study were comparable to other 24-h mark–recapture experiments performed in forest habitats, and we hypothesize that the effects of life stage and capture distance will be repeatable throughout the range of A. americanum. Considering that, under most circumstances, CO2 traps provide a relative estimate of tick abundance, increased standardization of sampling methods will facilitate comparisons between studies. Therefore, we encourage future studies to use the method of CO2 traps baited with sufficient quantities of dry ice for 24-h trap periods to standardize future studies and promote the compilation of larger data sets.

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