Experimental & Applied Acarology, 7 (1989) 313-322
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

Comparison of Flagging, Walking, Trapping, and Collecting from Hosts as Sampling Methods for Northern Deer Ticks, *Ixodes dammini*, and Lone-Star Ticks, *Amblyomma americanum* (Acari: Ixodidae)

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(Accepted 25 July 1988)

ABSTRACT

Ginsberg, H.S. and Ewing, C.P., 1989. Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum* (Acari: Ixodidae). *Exp. Appl. Acarol.*, 7: 313-322.

Ticks were sampled by flagging, collecting from the investigator's clothing (walking samples), trapping with dry-ice bait, and collecting from mammal hosts on Fire Island, NY, U.S.A. The habitat distribution of adult deer ticks, *Ixodes dammini*, was the same in simultaneous collections from the investigator's clothing and from muslin flags. Walking and flagging samples can both be biased by differences between investigators, so the same person should do comparative samples whenever possible. Walking samples probably give a more accurate estimate than flagging samples of the human risk of encountering ticks. However, ticks (such as immature *I. dammini*) that seek hosts in leaf litter and ground-level vegetation are poorly sampled by walking collections. These ticks can be sampled by flagging at ground level.

Dry-ice-baited tick-traps caught far more lone-star ticks, *Amblyomma americanum*, than deer ticks, even in areas where deer ticks predominated in flagging samples. In comparisons of tick mobility in the lab, nymphal *A. americanum* were more mobile than nymphal *I. dammini* in 84% of the trials. Therefore, the trapping bias may result from increased trap encounter due to more rapid movement by *A. americanum*, although greater attraction to carbon dioxide may also play a role. Tick traps are useful for intraspecific between-habitat comparisons.

Early in their seasonal activity period, larval *I. dammini* were better represented in collections from mouse hosts than in flagging samples. Apparently, sampling from favored hosts can detect ticks at low population levels, but often cannot be used to get accurate estimates of pathogen prevalence in questing ticks.

INTRODUCTION

Gray (1985) divided tick-sampling techniques into three major categories: flagging or dragging methods, the use of carbon dioxide-baited tick traps, and

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methods that involve collecting from hosts. A fourth technique, walking samples (walking through tick habitat and collecting ticks from the clothing of the investigators), can be used to sample ticks that attach to humans, and combines features of flagging and collecting from hosts.

Each of these techniques has been used in numerous descriptive studies of tick ecology. For example, *Ixodes dammini*, an important vector of Lyme borreliosis in North America (Spielman et al., 1985), has been studied by flagging (Piesman et al., 1986; Ginsberg and Ewing, 1989), walking (Carey et al., 1980; Schulze et al., 1986), trapping (Ginsberg and Ewing, 1989), and collecting from hosts (Wallis et al., 1978; Piesman and Spielman, 1979; Anderson and Magnarelli, 1980,1984; Carey et al., 1980, 1981; Main et al., 1981, 1982; Bosler et al., 1983, 1984). *Amblyomma americanum*, another potential vector of Lyme disease (Schulze et al., 1984), has similarly been sampled by flagging (Clymer et al., 1970; Semtner and Hair, 1973), walking (Schulze et al., 1986), trapping (Wilson et al., 1972), and collecting from hosts (Patrick and Hair, 1977, 1979; Garris et al., 1979). Several investigators have tried to increase sample sizes and avoid the biases of a single technique by using two or more techniques simultaneously (e.g., Anderson and Magnarelli, 1980; Schulze et al., 1986; Ginsberg and Ewing, 1988). However, these authors did not systematically examine the biases associated with each sampling technique. Sampling biases can influence the interpretation of results, and thus our understanding of tick ecology.

In this study, therefore, a variety of tick-sampling techniques were applied at the same sites. This approach was used to examine the biases of each technique, and to make suggestions for future studies and surveillance programs that involve tick sampling.

MATERIALS AND METHODS

Ticks were sampled at eight sites on Fire Island, a barrier island off the south shore of Long Island, NY. The habitats on Fire Island range from beach grass (*Ammophila breviligulata*) meadows, often intermixed with low shrubby vegetation, to higher scrub and thickets of various ericaceous species and poison ivy (*Rhus radicans*), often tangled with vines of greenbrier (*Smilax rotundifolia*). Maritime forests of American holly (*Ilex opaca*), shadbush (*Amelanchier canadensis*), sassafras (*Sassafras albidum*), black cherry (*Prunus serotina*), and pitch pine (*Pinus rigida*) line the bay side of the secondary-dune area. Forest understory varies in density from site to site, with scattered greenbrier thickets. The vegetation of Fire Island has been described in greater detail by Art (1976) and Stalter et al. (1986).

Three species of ticks that attach to humans are common on Fire Island: northern deer ticks, *Ixodes dammini* Spielman, Clifford, Piesman & Corwin; lone-star ticks, *Amblyomma americanum* (L.); and American dog ticks, *Der-*

macentor variabilis (Say). Four techniques were used to sample ticks on Fire Island from 4 April to 24 November 1986 and from 24 June to 11 July 1987. Techniques were compared on all species when possible, but some comparisons were omitted because sample sizes were too small for statistical analysis or because differences in phenologies precluded direct comparisons.

Walking samples

The investigator (HSG) walked through tick habitat wearing tan cotton clothing, with this trousers tucked into his socks, and collected ticks as he found them on his clothing. Samples were taken along randomly selected transects that ran north from the inland side of the primary dune through the secondary-dune area and maritime forest on Fire Island. A tape-measure was pulled behind the investigator and the distance walked through each habitat type was recorded.

Flagging samples

The terms 'flagging' and 'dragging' are used interchangeably in this paper (Strickland et al., 1976). The technique was modified from that of Clymer et al. (1970). Two flags were made of unbleached cotton muslin stapled to wooden bases (Fig. 1). The small flag was a 30×116 cm (12 in×46 in) piece of muslin, stapled at the center to one end of a 122-cm (48-in) wooden dowel, so that the two ends of the muslin (each 58 cm long) hung like flags from the dowel. The dowel was used to stir up the leaf litter and low vegetation, and the muslin flag was pulled through. The large flag was a 76×112-cm piece of muslin, stapled along the long edge to a wooden base, and dragged behind the investigator by a rope handle attached to the wooden base. The two flags were used simultaneously (to collect nymphs and adults) so that the small flag would sample ticks down in the leaf litter and ground-level vegetation, while the large flag would sample ticks on the litter surface and on higher vegetation, as well as ticks that were moved to the litter surface by the action of the small flag. The small flag alone was used to sample larvae because the time required to search the large flag for the tiny larvae was prohibitive.

Tick traps

The tick traps were modified from the design of Wilson et al. (1972) and Gray (1985). A 1.9-l plastic tub with a tight-fitting lid was mounted on a wooden base (Fig. 2) 1.9 cm thick with edges that formed 45° ramps rising toward the plastic tub. The tub had 4 evenly-spaced holes (4.8 mm diameter) on the sides at the bottom (one facing each edge of the base). Each sample day, 1.1 kg of dry ice was wrapped in paper and placed into the tub. Masking tape was placed at the top of the wooden base, sticky side down, overlapping the top edge of the ramp. Ticks that were attracted to the carbon dioxide climbed the ramp and were trapped by the masking tape, where they were collected the next day.

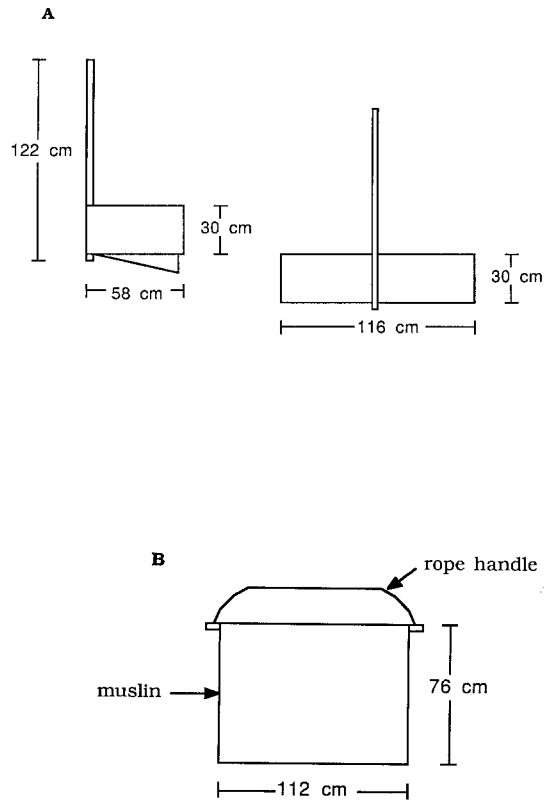

Fig. 1. Tick flags. (A) small flag; (B) large flag.

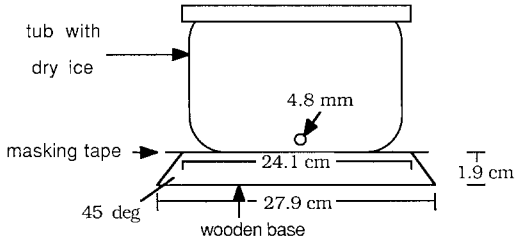

Fig. 2. Tick trap.

Collecting from hosts

White-footed mice, *Peromyscus leucopus* (Rafinesque), were captured in Sherman live traps set in 5×5 grids with 20 m between traps. The traps were baited with a mixture modified from that of Calhoun (1951), which included 2 parts (by weight) beef suet, 2 parts peanut butter, 2 parts raisins, 2 parts oatmeal, and one part paraffin. The suet and paraffin were melted and the ingredients were combined, mixed well, and allowed to cool. Traps were set in

the afternoon and collected the following morning before 0900 h. Mice were returned to the lab where they were anesthetized with methoxyflurane, and the ticks were removed with forceps. After recovering from the anesthesia, the mice were released in the capture area.

Relative mobility of *I. dammini* and *A. americanum* nymphs was compared by conducting tick races in the laboratory. In each race, an *I. dammini* nymph was placed with an *A. americanum* nymph at the center of a 5.2-cm-diameter circle pencilled on a sheet of white paper. The time they took to leave the circle was recorded (each trial was stopped after 1 min). Ten pairs of ticks were each tested five times (total of 50 races). The ticks were collected by flagging on Fire Island on 1 July, and were tested on 2 July 1986.

RESULTS

Flagging vs. walking samples

Adult *I. dammini* were collected by walking randomly placed transects and then flagging in the vicinity of each transect in the fall of 1986 (Table 1). When the total amount of time required for the samples was considered (including setting up, removing ticks from flags, moving between sample sites, etc.), 3.9 ticks h^{-1} were captured during walking samples and 7.7 ticks h^{-1} while flagging (including ticks from the investigator's clothing as well as from flags). The difference was not statistically significant (Wilcoxon matched-pairs signed-ranks test, 2-tailed, $n=8$, $t=9$, $P=0.21$) because of site-to-site variability in the number of ticks collected by each method.

The habitat distribution (woods vs. open habitats) of adult *I. dammini* collected on flags did not differ from those collected from the investigator's clothing during the flagging samples (Fisher exact probability test, $n=42$, $P=1.000$).

TABLE 1

Number of free-living ticks[a] collected by various sampling methods, 1986

Method	Dates	Tick species	Habitat	Number of ticks	Sampling effort
Walking	20 Oct. -25 Nov.	ID adults	high shrubs & woods	20.4 km^{-1}	2.2 km
Flagging	20 Oct. -25 Nov.	ID adults	high shrubs & woods	20.0 h^{-1}	1.5 h
Flagging	1-10 July	ID nymphs	leaf litter	24.5 h^{-1}	14.8 h
Flagging	1-10 July	AA nymphs	leaf litter	4.0 h^{-1}	14.8 h
Tick traps	14-15 July	ID nymphs	leaf litter	1.6 trap $night^{-1}$	20 traps
Tick traps	14-15 July	AA nymphs	leaf litter	2.1 trap $night^{-1}$	20 traps

[a]ID = *Ixodes dammini*; AA = *Amblyomma americanum*.

However, the proportion of samples with just one tick (as opposed to those with more than one) was greater on flags than on the investigator's clothing (Fisher exact probability test, $n=21$, $P=0.0089$). Thus, the techniques apparently did not detect spatial distribution of ticks in a similar manner. However, this difference may have resulted from the greater sample sizes of tick collections from clothing than from flags (Mann-Whitney U test, $n1=9$, $n2=11$, $U=14$, $P=0.004$), rather than from any difference in the way the two techniques detected spatial distribution. Adults of *A. americanum* and *D. variabilis* were also collected in both flagging and walking samples at appropriate times of year. They were omitted from Table 1, however, because they are active in spring and early summer, and so were not collected in the fall when the comparative *I. dammini* samples were taken.

The small flag averaged 1.69 nymphs (SE=0.183) per 5-min sample. Interestingly, this did not differ significantly from the large flag (Wilcoxon matched-pairs signed-ranks test, $n=64$, $t=552.5$, $P>0.40$), which averaged 1.86 nymphs (SE=0.233) sample^{-1}.

Tick traps

Tick traps captured more *A. americanum* than *I. dammini*, even when *I. dammini* predominated in flagging samples from the same general areas (Tables 1 and 2). This could have resulted from differences in host-seeking behavior between the two species. Lone-star ticks actively hunt for hosts (Waladde and Rice, 1982), and may be more likely to encounter traps simply because they move around faster than deer ticks. To test this we ran tick races between the two species by placing a nymph of each species at the center of a 5.2-cm-diameter circle on a sheet of paper in the laboratory. The *A. americanum* nymphs left the circle faster than *I. dammini* nymphs in 84% of the 50 trials (sign test, 1-tailed, $n=10$, $P<0.011$). Lone-star ticks left the circle within 1 min 98% of the time, compared to only 48% for deer ticks; thus lone-star ticks

TABLE 2

Tick species breakdown of samples collected by flagging and carbon dioxide-baited tick traps at three sites on Fire Island, June/July 1986

Sample site	Sampling technique	Numbers of nymphs	
		Amblyomma	*Ixodes*
Watch Hill	flagging	10	90
	tick traps	5	1
Talisman	flagging	8	78
	tick traps	42	33

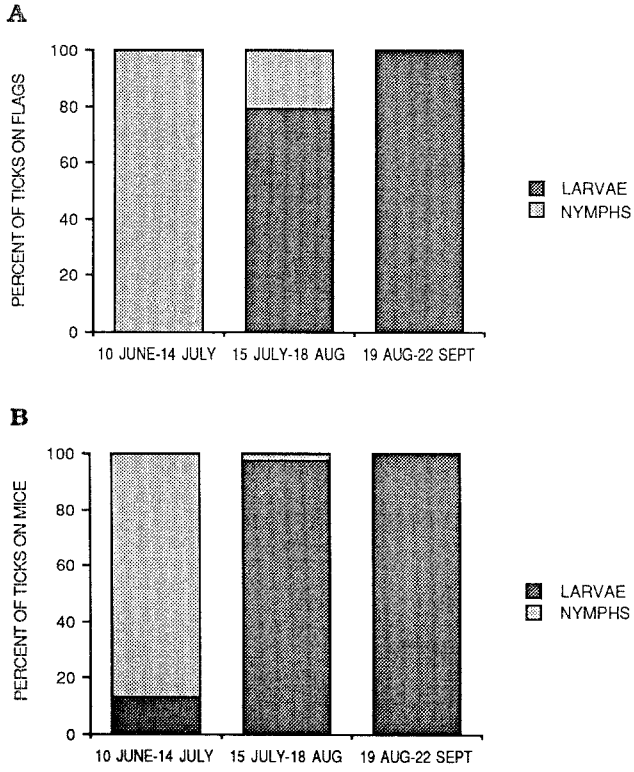

Fig. 3. Percent of larvae and of nymphs in samples of immature ticks from Fire Island, NY. (A) Flagging samples. (B) Ticks collected from white-footed mice.

move more quickly than northern deer ticks. They may also respond more strongly than deer ticks to carbon dioxide, thus enhancing the likelihood of attraction to dry-ice-baited traps. On the other hand, deer ticks may simply be better able to escape from the tape.

Ticks on hosts

Immature *I. dammini* were collected from white-footed mice from 12 June to 11 September 1986. Immature ticks ($n=304$) collected from mice ($n=49$) showed a similar phenology to those collected by flagging ($n=730$), with nymphs most common in late spring/early summer, larvae most common in late summer, and some degree of overlap in midsummer (Fig. 3). However, in the midsummer samples (early in the larval seasonal-activity period) larvae constituted a larger proportion of the samples from mice than of the flagging samples ($\chi^2=29.578$; d.f.$=1$, $n=445$, $P<0.001$).

DISCUSSION

Two commonly-used techniques for sampling free-living adult ticks, walking and flagging, gave comparable numbers and showed similar habitat distribu-

tions of adult *I. dammini* at sample sites on Fire Island. However, during the flag-sampling, more ticks were collected from the investigator's clothing than from the flags. Walking samples have two advantages over flagging samples for assessing the risk of tick bite. First, they sample the expected number of ticks that are encountered by the target organism, humans, rather than by a sheet of material. Second, the investigator is more likely to find and remove ticks on his/her person, and less likely to be bitten. At sites such as Fire Island, NY, where roughly half of the adult *I. dammini* carry Lyme disease spirochetes (Ginsberg and Ewing, 1989) this is not a trivial consideration.

In terms of validity and comparability, both walking and flagging samples can be randomized, and sampling effort can be easily quantified by time or distance sampled. However, comparative walking samples require that the same investigator take the samples in each area (wearing the same clothes) to avoid bias resulting from differences between investigators in attractiveness to ticks. Flagging samples can be standardized by using equal surface areas of the same material at each site (light-colored flannel or corduroy are commonly used materials). However, investigators pulling flags might avoid dense thickets that are difficult to get through and can shred the flagging material. Therefore, flagging samples can be biased by differences in sampling technique between investigators (e.g., in the propensity to avoid greenbrier thickets). Thus, both walking and flagging samples should be standardized, if possible, by having the same investigator(s) sample at each site.

The habitat distribution of a tick species has obvious implications for the efficacy of a given sampling technique. For example, walking samples are useful for adult *I. dammini*, which quest in high vegetation. However, because the nymphs and larvae dwell in leaf litter and ground-level vegetation (Ginsberg and Ewing, 1989), this method does not effectively sample immatures (Schulze et al., 1986), whereas flagging can, if the investigator pulls the flag through leaf litter and ground-level vegetation. Flags can easily be pulled at ground level in woods that are open beneath the canopy, but not in areas with dense shrub layers, either in or out of the woods. Therefore, flagging for ground-level dwellers is probably not equally efficient in different habitats.

Tick traps can be placed down in the leaf litter, and can therefore sample immature lone-star and deer ticks. However, species such as *I. dammini* which do not respond well to carbon-dioxide-baited traps (or which can escape from the traps) are not as well sampled by tick traps as are species such as *A. americanum*, which move around more quickly (and are thus more likely to encounter stationary traps), or which respond more strongly to carbon-dioxide bait. Tick traps have the advantage that they can be placed in either sparsely or thickly vegetated areas with little interference from shrub branches (which impair flagging samples). They are therefore useful for between-habitat comparisons of a single species (Gray, 1985).

The above methods sample free-living ticks, so they can be used to estimate

the prevalence of a given disease agent in tick populations. Researchers studying the prevalence of pathogens in ticks that might attach to humans should sample pathogens from questing ticks rather than from ticks on hosts, if possible, because attached ticks may have picked up pathogens from their current hosts. On the other hand, sampling ticks from hosts has the advantage that favored hosts can be efficient collectors of ticks, and can thus be used to detect ticks at relatively low population levels. On Fire Island, the phenology of larval *I. dammini* on white-footed mice was similar, but not exactly the same, as in flagging samples (Fig. 3). In studies of tick ecology it is important to consider the influence of possible sampling biases on observed tick phenologies and population parameters.

ACKNOWLEDGEMENTS

We thank J.L. Benach, E.M. Bosler, J. Donahue and J.K. Shisler for help with the planning, execution, and interpretation of this research. Constructive comments on early versions of the manuscript were provided by P.A. Buckley, T.L. Schulze, and two anonymous reviewers. Field quarters, laboratory space at the Talisman Laboratory facility, use of microscopes and mouse traps, and logistical support were provided by the staff of Fire Island National Seashore. This is contribution no. 1030 from the Center for Coastal and Environmental Studies at Rutgers - The State University of New Jersey. The research was supported by Cooperative Agreement CX 1600-3-0005 from the North Atlantic Regional Office, National Park Service, to the Rutgers University Cooperative Research Unit, P.A. Buckley, Director.

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