Comparison of novel and conventional methods of trapping ixodid ticks in the southeastern U.S.A.

S. E. MAYS1, A. E. HOUSTON1,2 and R. T. TROUT FRYXELL1

1Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN, U.S.A. and 2Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, TN, U.S.A.

Abstract. Tick-borne disease surveillance and research rely on resource-effective methods for tick collection. This study compared the respective performances of several trapping methods in a mixed grassland–forest habitat in western Tennessee. To test for temporal differences in effectiveness, sites were sampled monthly (April–August 2013) using dry ice, dragging, flagging, sweep netting, carbon dioxide (CO2) dragging and CO2 flagging methods. To evaluate the effect of habitat on method effectiveness, four methods (dragging, CO2 dragging, CO2 flagging and dry ice) were compared in four habitat types (bottomland deciduous, upland deciduous, coniferous and grassland) in June 2014. In the temporal comparison, ticks were found to be most abundant in April and May, and there was a significant sampling period and method interaction, such that method effectiveness varied across sampling period. Sweep netting was significantly less effective than the other methods. In the habitat comparison, dry ice trap collections represented the most effective method in upland deciduous and coniferous habitats. Flagging using CO2 was significantly less effective than CO2 dragging and dragging in bottomland deciduous habitats. The success of the various collection methods did not differ significantly within grassland habitats. Overall, dry ice trapping and dragging were the most effective methods for tick collection across time and habitat.

Key words. Amblyomma americanum, Amblyomma maculatum, Dermacentor variabilis, Ixodes scapularis, carbon dioxide, dragging, flagging, questing, trapping.

Introduction

Ticks are significant pests that transmit pathogens affecting both humans and animals worldwide, and are the primary vectors of arthropod-borne disease in the U.S.A. (Parola & Raoult, 2001). Several tick species commonly encountered in the southeast of the country may contribute to human disease cases. These include species such as the lone star tick Amblyomma americanum (Linnaeus) (Ixodida: Ixodidae), the Gulf Coast tick Amblyomma maculatum Koch, the range of which is currently expanding in areas of the southeast, the American dog tick Dermacentor variabilis (Say) (Ixodida: Ixodidae), and the black-legged tick Ixodes scapularis Say (Ixodida: Ixodidae) (Stromdahl & Hickling, 2012). These tick species are associated with agents for various diseases of concern, including anaplasmosis, borreliosis, ehrlichiosis and rickettsiosis. The collection of questing ticks is one of the best ways of representing the risk for human encounter with ticks and tick-borne pathogens (Reye et al., 2012). A number of methods are employed to collect questing ticks, but these methods may vary in the number of ticks collected, in tick species specificity or diversity, and other biases (Ginsberg & Ewing, 1989; Schulze et al., 1997; Petry et al., 2010).

Commonly used methods for collecting tick species include trapping with dry ice, dragging and flagging. Sweep netting is a method of arthropod collection commonly employed by entomologists. Dry ice trapping uses carbon dioxide (CO2) (given off when the dry ice sublimes) to attract actively host-seeking ticks. Because this method is stationary, it is not as restricted by vegetation type and density as methods such as sweep netting.
(Kinsinger & Allan, 2011); however, not all species or life stages of tick are equally attracted (Holscher et al., 1980; Ginsberg & Ewing, 1989; Schulze et al., 1997; Cohnstaedt et al., 2012). Additionally, variables such as wind direction and wind speed make it difficult to determine the actual area being sampled (Adeyeye & Butler, 1991; Cohnstaedt et al., 2012). Dragging involves moving a piece of flannel or cotton cloth across vegetation behind an observer and allowing ticks to attach to the cloth as it passes. The area or distance sampled can be more easily quantified in dragging than in dry ice trapping, and dragging is more representative of the risk for human encounter with host-seeking ticks (Armed Forces Pest Management Board, 2012); however, dragging can be more easily inhibited by vegetation than dry ice trapping, and the number of ticks collected with this method may vary by species (Ginsberg & Ewing, 1989). Whereas dragging tends to sample upper vegetation layers, such as in grassland habitat types; however, it can be impeded by dense vegetation such as blackberry and greenbrier (Sentermer & Hair, 1975).

Tick questing behaviour involves responses to multiple stimuli, such as movement, CO₂, light and temperature (Gherman et al., 2012), but many collection methods function by targeting only one of these responses, such as dragging (movement) or dry ice trapping (CO₂). Gherman et al. (2012) attempted to increase the efficiency of tick collection by combining the stimulus of movement and CO₂ using a traditional flag reinforced with CO₂ dispersed throughout the body of the flag. This combination yielded significantly more *Ixodes ricinus* ticks, but not more *Dermacentor marginatus*, than traditional flagging in woody-edge habitat in Romania (Gherman et al., 2012).

Knowledge about the diversity of species collected with the various methods can help improve sampling and surveillance procedures and thus enhance estimates of disease exposure risks, evaluation of pathogen prevalence, estimates of relative tick densities, comparison of habitat use, and monitoring of changes in populations. The purpose of this project was to examine the effectiveness of several conventional and novel methods for the collection of questing ticks, and to investigate whether they vary by tick species, temporally and by habitat type. This study was conducted in two parts: a temporal comparison was carried out in April–August 2013, and a habitat comparison in June 2014.

**Materials and methods**

**Study site**

This study was carried out at the Ames Plantation Research and Education Center. Ames is located in southwest Tennessee and operates as a 7446-ha University of Tennessee research and education centre devoted to forestry and wildlife ecological research, livestock development, forage and row crop research, and archaeological research. Various occurrences of tick-borne disease cases have been reported at Ames, and previous studies have identified human and animal pathogens in *A. americanum* (Hendricks, 2013), *A. maculatum* (Mays et al., 2016), and *I. scapularis* (Mays et al., 2014) collected at Ames.

**Tick collection methods**

Six methods were selected for comparison: four conventional methods (dry ice trapping, dragging, flagging and sweep netting), and two novel methods integrating a conventional method with the use of CO₂ (CO₂ dragging and CO₂ flagging) (Gherman et al., 2012; Niebuhr et al., 2013). Dry ice traps consisted of a small cooler filled with dry ice [~3.5 lbs (1.6 kg)] placed on a 1-m² white cloth. The dry ice traps operated overnight and were collected the next morning to maximize the time the trap was active. Ticks on the cloth were removed with forceps and placed into a vial of 80% ethanol. The tick drags were constructed of 1-m² pieces of light-coloured corduroy sewn onto a dowel rod of 30 mm in diameter and 122 cm in length. A rope was attached to either end of the dowel rod so that it could be dragged behind the sampler. The flag was a 60 x 80-cm rectangle constructed of the same corduroy material as the drag. The shaft of the flag was a hollow PVC pipe of 130 cm in length and 20 mm in diameter. The sweep net was made from canvas, with a net hoop 38 cm in diameter and a handle measuring 61 cm in length (BioQuip Products, Inc., Rancho Dominguez, CA, U.S.A.). The CO₂ drag was constructed as the conventional drag, but included vinyl tubing of 4.76 mm in diameter along the dowel rod, inside the drag. The end of the hose was attached to a 5-lb (2.3-kg) tank of compressed CO₂ carried in a backpack. The tubing was punctured every 10 cm with a 22-gauge needle to allow for the release of CO₂. The CO₂ flag was constructed based upon a design by Gherman et al. (2012), similarly to the conventional flag. Thin vinyl tubing (1 cm inner diameter) was run throughout the body of the flag in a serpentine pattern and through the PVC shaft, and attached to a tank of CO₂. The tubing was punctured every 10 cm with a 22-gauge needle to allow for the release of CO₂ throughout the body of the flag. One side of the flag was left unsewn and secured with Velcro to allow access to the tubing (Fig. 1). All collections were stored in 80% ethanol and ticks were identified to life stage, sex and species (Cooley & Kohls, 1944; Keirans & Litwak, 1989; Keirans & Durden, 1998) in the Medical and Veterinary Entomology Laboratory at the University of Tennessee.

**Environmental data collection**

At each site, temperature (°C) and relative humidity (RH) (%) were measured using a Kestrel 3500 weather meter (Nielsen Kellerman Co., Boothwyn, PA, U.S.A.), held at knee height near the dry ice trap. Temperature and RH were measured and compared [analysis of variance (ANOVA) with a Tukey’s mean comparison] by collection month (in the temporal study) and habitat type (in the habitat study) to identify differences by month and habitat. Precipitation data for the Ames Plantation were collected by Ames Plantation personnel.

© 2016 The Authors. *Medical and Veterinary Entomology* published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, *Medical and Veterinary Entomology*, 30, 123–134
Temporal study

Twenty sites were selected and classified as either grassland (n=14) or woodland (n=6) habitat types. Sampling was designed to ensure a diverse species collection (A. americanum, A. maculatum and D. variabilis). Sites were selected by choosing 10 sites at which a minimum of three species had been collected during previous sampling efforts (Hendricks, 2013), and then choosing for each of the 10 an additional site of complementary habitat type at which three species had not been collected. These sites were sampled monthly from April to August in 2013. Each site contained six 20 x 20-m plots, which were sampled in six 20-m segments (Fig. 2). All six methods were compared in this study. Traps were randomly assigned to a plot at each site upon each sampling trip (Fig. 2). For all methods except dry ice trapping, ticks were collected at the end of each 20-m segment and stored in 80% ethanol vials. Dry ice traps were placed in the centres of their assigned plots and operated overnight; ticks were collected the following morning. For statistical comparisons, total tick counts were analysed and data for each species were analysed individually in SAS Version 9.4 (SAS Institute, Cary, NC, U.S.A.) using a PROC GLIMMIX procedure with a Poisson distribution. Tukey-Kramer adjustment for multiple comparisons was used for means separation. Because of the high number of A. americanum nymphs collected, A. americanum adults and nymphs were analysed separately. Adult A. americanum data, A. maculatum data and D. variabilis data were rank-transformed for analyses. Total tick collection data, A. americanum nymph and total data were not transformed (i.e. raw counts were used).

Habitat study

For the habitat comparison, 76 sites were selected and classified as grassland (n=19), coniferous (n=14), bottomland deciduous (n=14) or upland deciduous (n=29) habitat types. Collections were carried out in June 2014. Each site contained three 100-m parallel transects positioned 10 m apart (Fig. 3). For this study, four methods were selected from those used in the 2013 temporal comparison: dragging; dry ice trapping; CO₂ dragging, and CO₂ flagging. One method was randomly assigned to each transect and the dry ice trap was placed in the centre of the middle transect. Traps were checked and ticks collected every 20 m along the 100-m transects. The dry ice trap remained overnight and ticks were collected from the cloth the following morning. For statistical comparisons, raw total tick collection data, total A. americanum data and A. americanum nymph data were used. Adult A. americanum data, A. maculatum data, D. variabilis data and I. scapularis data were rank-transformed. Means were compared in SAS Version 9.4 using a PROC GLIMMIX procedure with a Poisson distribution and Tukey-Kramer adjustment for multiple comparisons for means separation.

Results

Environmental data

During the temporal study from April to August 2013, temperatures ranged from 18.3 °C to 35.1 °C [mean ± standard error of the mean (SEM) 29.34 ± 0.33 °C] at trap set-up, and from 18.9 °C to 33.8 °C (mean ± SEM 26.10 ± 0.93 °C) at trap collection. Relative humidity ranged from 36.7% to 97.1% (mean ± SEM 71.52 ± 1.23%) at trap set-up, and from 56.3% to 100% (mean ± SEM 78.47 ± 0.93%) at trap collection. During the habitat comparison in June 2014, temperatures ranged from 22.2 °C to 36.2 °C (mean ± SEM 29.39 ± 0.35 °C) at trap set-up, and from 21.4 °C to 32.4 °C (mean ± SEM 25.10 ± 0.27 °C) at trap collection. Relative humidity ranged from 48.6% to 100% (mean ± SEM 76.02 ± 1.18%) at trap set-up, and from 71.0% to 100% (mean ± SEM 84.91 ± 0.61%) at trap collection. June 2014 experienced greater precipitation than normal (29.82 cm). The mean ± SEM rainfall in June at Ames Plantation for 2000–2014 was 11.43 ± 1.83 cm. Environmental variables for each month in the temporal study and for each habitat in the habitat study are presented in Table 1. During the temporal study, April was significantly cooler than the other months (F = 12.348; d.f. = 4, 96; P < 0.0001), and RH was lowest in April and May (F = 8.5899; d.f. = 4, 96; P < 0.0001). During the habitat study, grassland sites had significantly warmer...
Fig. 2. (A) Map of sites at Ames Plantation and (B) example of plot design for 2013 temporal comparison of tick trapping methods. Six plots were created within each site to test each trapping method. For dry ice traps, the trap was placed in the centre of the plot. For all other active trapping methods, the dashed line indicates collection transects within each plot and stars indicate each 20-m segment.

Temporal study

A total of 2106 ticks were collected, consisting of three species: 1795 *A. americanum* (455 adults and 1340 nymphs); 237 *D. variabilis* (231 adults and six nymphs), and 74 *A. maculatum* (adults). Each method collected individuals of all three tick species. For overall tick collection using traditional tick trapping methods, the mean ± SEM number of ticks collected per site per sampling period was 4.80 ± 1.08 by dry ice trapping, 5.47 ± 1.53 by dragging, 2.81 ± 0.62 by flagging and 1.21 ± 0.36 by sweep netting. Using the two novel methods, the mean ± SEM number of ticks collected per site per sampling period was 3.82 ± 0.59 by CO₂ dragging and 2.95 ± 0.41 by CO₂ flagging.

For overall tick collection, there was a significant sampling period effect \( (F = 58.99; \text{d.f.} = 4,462; P < 0.0001) \), such that significantly more ticks were collected in May (probably associated with high numbers of *A. americanum* collections) than in any other month except April, and significantly fewer ticks were collected in July and August (probably associated with decreasing *A. americanum* collections) than in any other month (Fig. 4A). Sweep netting was significantly less effective than all the other methods \( (F = 5.75; \text{d.f.} = 5,114; P < 0.0001) \). There were no differences between the other methods (Fig. 4B). There was a significant trapping method by sampling period effect.
Comparison of tick trapping methods

(F = 17.59; d.f. = 20,462; P < 0.0001) for overall tick collection, such that the differences between trapping methods varied across the sampling periods (Fig. 4C). In April, there were no significant differences among any of the trapping methods. In May, all methods except dragging were significantly more effective than sweep netting. In June, only dragging differed significantly from sweep netting, with dragging being significantly more effective. In July, only CO₂ dragging and CO₂ flagging were significantly more effective than sweep netting. In August, dragging was significantly more effective than all other methods except CO₂ dragging.

For collection of A. americanum, there was a significant sampling period effect (F = 54.64; d.f. = 4,462; P < 0.0001), with the number of ticks collected declining across the sampling periods. Significantly more A. americanum were collected in April and May, with June collections significantly lower than those in April, but not May, and July and August collections significantly lower than those in June. Trapping methods varied significantly (F = 3.64; d.f. = 5,114; P = 0.0043), with sweep netting being less effective than all other methods. There was also a significant trap by sampling period effect (F = 18.14; d.f. = 20,462; P < 0.0001), such that the difference between the trapping methods varied across the sampling periods (Fig. 4D). In April, tick numbers did not differ among trapping methods. In May, all methods except dragging were significantly more effective than sweep netting. In both June and July, there were no significant differences among trapping methods. In August, dragging was significantly more effective than flagging, CO₂ flagging and sweep netting, although not significantly different from dry ice trapping and CO₂ dragging.

© 2016 The Authors. Medical and Veterinary Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, Medical and Veterinary Entomology, 30, 123–134
Dividing the *A. americanum* collections into adult and nymph life stages for analysis yielded similar results and showed a significant effect of sampling period for the collection of adults (*F* = 55.87; d.f. = 4, 462; *P* < 0.0001) and nymphs (*F* = 31.91; d.f. = 4, 462; *P* < 0.0001). Adult *A. americanum* collections in April did not differ from collections in May or June, although significantly more adults were collected in May than in June. Adult collections were lowest in July and August. Collections of nymphal *A. americanum* were significantly higher in April, May and June than in July and August. There was no significant trap by sampling period effect for adult *A. americanum* (*F* = 1.39; d.f. = 20, 462; *P* = 0.1204) (Fig. 4E); however, there was a significant trap by sampling period effect (*F* = 17.97; d.f. = 20, 462; *P* < 0.0001) for the collection of *A. americanum* nymphs (Fig. 4F). In April, there were no significant differences among any of the trapping methods. In May, all methods except dragging were more efficient than sweep netting. Methods did not differ in efficiency in June and July. In August, dragging was significantly more effective than sweep netting, but did not differ from CO₂ dragging, dry ice trapping, flagging or CO₂ flagging.

For collection of *D. variabilis*, there was a significant sampling period effect (*F* = 146.21; d.f. = 4, 462; *P* < 0.0001), with significantly more *D. variabilis* collected in April and July, followed by June. Collections in May and August were significantly lower than in all other months. There was a significant trap by sampling period effect (*F* = 5.65; d.f. = 5, 114; *P* < 0.0001), with sweep netting being less effective than all other methods except flagging. There was also a significant trap by sampling period effect (*F* = 38.56; d.f. = 20, 462; *P* < 0.0001), such that the difference between the trapping methods varied across sampling periods (Fig. 4G). In April, dragging was more effective than dry ice trapping and sweep netting, although it did not differ from CO₂ dragging or CO₂ flagging. In May, there were no significant differences between trapping methods. In June, both dragging and CO₂ dragging were significantly more effective than flagging and sweep netting, but were not better than dry ice trapping and CO₂ flagging; no *D. variabilis* were collected with the sweep net. In August, there were no significant differences among trapping methods.

Numbers of nymphal *D. variabilis* and *A. maculatum* were insufficient to allow for a sampling period or trapping method comparison. Mean ± SEM data for *A. maculatum* are presented in Fig. 4H.

### Habitat study

A total of 5040 ticks were collected, consisting of four species: 4893 *A. americanum* (727 adults and 4166 nymphs); 128 *D. variabilis* (adults); 12 *A. maculatum* (adults), and seven *I. scapularis* (nymphs). A total of 271 ticks were collected from the 19 grassland sites (mean ± SEM 14.26 ± 1.32 ticks per site), 2664 from the 29 upland deciduous sites (91.86 ± 19.9 ticks per site), 411 from the 14 bottomland deciduous sites (29.36 ± 3.48 ticks per site), and 1694 from the 14 coniferous sites (121 ± 17.59 ticks per site). *Amblyomma americanum* and *D. variabilis* were collected with all methods and in all habitats, whereas *A. maculatum* was collected with all four methods but only in grassland sites and *I. scapularis* was collected only by CO₂ dragging in deciduous upland and bottomland sites.

There was a significant trapping method effect in upland deciduous habitat (*F* = 9.65; d.f. = 3, 100; *P* < 0.0001), bottomland deciduous habitat (*F* = 3.56; d.f. = 3, 40; *P* = 0.023), and coniferous habitat (*F* = 11.53; d.f. = 3, 56; *P* < 0.0001). Trapping methods in grassland habitat did not differ (*F* = 1.79; d.f. = 3, 44; *P* = 0.163). In both upland deciduous and coniferous habitats, dry ice trapping was significantly more effective than dragging, CO₂ dragging and CO₂ flagging. In bottomland deciduous habitat, the mean numbers of ticks per site collected by dry ice trapping, dragging and CO₂ dragging did not differ; however, CO₂ flagging was significantly less effective than dragging and CO₂ dragging (Fig. 5A).

There was a significant trapping method effect for collection of *A. americanum* in upland deciduous habitat (*F* = 8.85; d.f. = 3, 100; *P* < 0.0001), bottomland deciduous habitat (*F* = 3.92; d.f. = 3, 40; *P* = 0.0153), and coniferous habitat
Fig. 4. Mean ± standard error of the mean (SEM) values for 2013 temporal comparison of trapping methods for tick collection at Ames Plantation by (A) sampling period, (B) method, (C) tick collection by method by month, (D) Amblyomma americanum by method by month, (E) A. americanum adults by method by month, (F) A. americanum nymphs by method by month, (G) Dermacentor variabilis by method by month, and (H) Amblyomma maculatum by method. Letters indicate significant differences. For all method by month graphs, means were compared within month.

© 2016 The Authors. Medical and Veterinary Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society,
Medical and Veterinary Entomology, 30, 123–134
Fig. 5. Mean ± standard error of the mean (SEM) values for 2014 habitat comparison of trapping methods for tick collection at Ames Plantation for (A) tick collection methods by habitat, (B) *Amblyomma americanum* by method by habitat, (C) *A. americanum* adults by method by habitat, (D) *A. americanum* nymphs by method by habitat, (E) *Dermacentor variabilis* by method by habitat, (F) *Amblyomma maculatum* by method by habitat, and (G) *Ixodes scapularis* by method by habitat. Letters indicate significant differences. For all method by habitat graphs, means were compared within habitat.
Table 2. Recommended trapping methods for tick collection by species, life stage, month and habitat based upon results of temporal and habitat comparisons.

| Tick species            | Life stage | Month       | Habitat                               | Recommended method |
|-------------------------|------------|-------------|---------------------------------------|--------------------|
| *Amblyomma americanum*  | Nymphs     | April–June  | Upland deciduous, coniferous          | Dry ice            |
|                         | Adults     | April, May  | Upland deciduous, coniferous          | Dry ice > drag     |
|                         | Total      | April, May  | Upland deciduous, coniferous          | Dry ice            |
| *Dermacentor variabilis*| Adults     | April, July | Upland deciduous, coniferous          | Dry ice = drag     |
| *Amblyomma maculatum*   | Adults     | June        | Grassland                            | CO₂ flag*          |
| *Ixodes scapularis*     | Nymphs     |             | Upland deciduous                     | CO₂ drag*          |
| Total ticks             |            | April–June  | Upland deciduous, coniferous, grassland | Dry ice, drag      |

*Trends suggest that these methods may be effective, although not enough individuals of these species were collected to detect a significant difference in trapping methods.

\(F = 10.31; \text{d.f.} = 3.56; P < 0.0001\). There was no significant trapping effect for collection of *Amblyomma americanum* in grassland habitat \(F = 1.63; \text{d.f.} = 3.44; P = 0.1956\). In both upland deciduous and coniferous habitats, dry ice trapping was significantly more effective than all other methods. In bottomland deciduous habitat, CO₂ flagging was significantly less effective than dragging and CO₂ dragging (Fig.5B). Dividing and analysing the two species, temporally and by habitat type were examined. Results indicated that specific strategies are needed to collect different species and life stages (Table 2). Although all tick species were collected with all the methods evaluated, some methods were clearly better than others because they collected only ticks (avoided non-targets), were easy to handle or operate, and were best at different times of the year or in a specific habitat. Novel methods (CO₂ dragging and CO₂ flagging) demonstrated performance comparable with that of their conventional counterparts (dragging and flagging); there were no significant differences between each novel method and its conventional counterpart for any species, in any time period or in any habitat. Although trapping methods for *Amblyomma maculatum* and *Ixodes scapularis* could not be compared in this study, the differences in efficiency between sampling methods for various life stages and habitat types of *Amblyomma americanum* and *I. scapularis* (Ginsberg & Ewing, 1989; Schulze et al., 1997) and the apparently decreased responsiveness of *I. scapularis* to dry ice-baited traps in comparison with both *Amblyomma americanum* and *Dermacentor andersoni* (Ginsberg & Ewing, 1989; Falco & Fish, 1991) suggest that methods vary in effectiveness depending on the species targeted. Careful selection of sampling methods with consideration to target species, as well as the timing and location of collections, is necessary when designing experiments. When several tick species are targeted, the integration of multiple methods is necessary to ensure that representative samples of all species present are collected (Rynkiewicz & Clay, 2014), particularly in studies that involve measurements of species diversity and relative abundance. Alternatively, if time and resources are limited, the dry ice trap may be the best method of collecting all ticks in different habitats. Failure to account for potential differences in species and life stages collected with a specific method may result in biased estimations of relative abundance in comparisons of multiple tick species (Schulze et al., 1997).

The most abundant ticks in the study were *Amblyomma americanum* and *Dermacentor variabilis*, which allowed for detailed trapping comparisons. Upland deciduous and coniferous sites had abundant *A. americanum* populations early in the season (April and May) that were easily collected with dry ice traps. *Dermacentor variabilis* adult populations peaked in April and July in upland deciduous and coniferous habitats, and both dry ice trapping and dragging were sufficient for collecting. These results are similar to those of previous studies. Both Solberg et al. (1992) and Petry et al. (2010) found dry ice trapping to be more
effective than dragging for the collection of *A. americanum* in forested habitats. Petry *et al.* (2010) found no significant difference between dry ice trapping and dragging for the collection of adult *D. variabilis* in either woodland or grassland habitat types in Missouri. Another study in the midwestern U.S.A. collected *D. variabilis* with both dragging and dry ice trapping and counted higher numbers of *D. variabilis* in woodland habitat than in grassland habitat (Rynkiewicz & Clay, 2014). This slight variation in collection method by the different species may result because *D. variabilis* does not quest as aggressively as *A. americanum* (Petry *et al.*, 2010).

All seven *I. scapularis* nymphs were collected in both deciduous habitat types using only the CO₂ drag. Populations of questing *I. scapularis* are notoriously difficult to collect in Tennessee (Rosen *et al.*, 2012), probably because of their decreased abundance in comparison with northeast populations (Dennis *et al.*, 1998; Brownstein *et al.*, 2003; Ginsberg *et al.*, 2014), the use of different hosts by immature stages (Apperson *et al.*, 1993), and/or differences in questing behaviour (Arnsne *et al.*, 2015). Although the present collections did not support trap comparisons, another study found no significant difference between traditional flagging and dragging for the collection of *I. scapularis* nymphs (Rulison *et al.*, 2013). Gherman *et al.* (2012) collected significantly more *I. ricinus* ticks during spring collections in Romania using a CO₂ flag similar to that used in this study than with a conventional flag. Although the addition of CO₂ to the drag method in this study may have contributed to the increased collection of *I. scapularis*, the small collection number prevents an accurate comparison. Falco & Fish (1991) indicated that dry ice trapping was less effective for *I. scapularis* than for other species, including *A. americanum*, and attributed this to decreased mobility and less aggressive host-seeking behaviour in comparison with *A. americanum*. Ginsberg & Ewing (1989) found that dry ice trapping and flagging collected disproportionately numbers of *A. americanum* and *I. scapularis*, and Schulze *et al.* (1997) also reported that dry ice trapping was more effective for the collection of *A. americanum* than for *I. scapularis*. A study comparing dry ice trapping and dragging, however, collected greater numbers of *I. scapularis* with the dry ice trap than with dragging despite the apparently decreased mobility of *I. scapularis* in comparison with other species (Solberg *et al.*, 1992).

The inability to detect a difference in sampling methods for *A. maculatum* is attributable to the fact that few specimens were collected. This species has only recently been found in Tennessee and does not occur in such densities as *A. americanum* or *D. variabilis*. These data support the idea that *A. maculatum* prefers open grassland habitat (Teel *et al.*, 1998, 2010; Goddard & Varela-Stokes, 2009). Grassland habitats have significantly warmer and dryer environmental conditions than the other habitats, which may help *A. maculatum* resist desiccation, increase questing time, and/or provide suitable habitat for potential hosts. The present investigators have been more successful in collecting *A. maculatum* adults from cattle and immatures from small mammals at the same field site (Pompo *et al.*, 2016). It appears necessary to create collection devices that mimic *A. maculatum* hosts. Recently, Portugal & Goddard (2015) developed another novel method to collect questing immature *A. maculatum* using a swab-like device that samples underbrush and animal burrows.

Because the dry ice-baited traps were the most consistent across habitat types, this method is the most appropriate when sampling areas that may undergo changes in vegetation, such as those subject to periodic prescribed fires, or when comparing habitat types. Kinsinger & Allan (2011) found no significant differences in the proportion of ticks recaptured on dry ice traps in a mark–recapture study carried out in grasslands and deciduous forests, which further suggests that this method may be consistent in the proportions of ticks collected across habitat type. Although different species may respond differently to the dry ice trap depending upon questing behaviour (Ginsberg & Ewing, 1989; Falco & Fish, 1991), it may still be a more efficient method for tick collection than other alternatives (Solberg *et al.*, 1992).

The use of effective trapping methods is critical for accurate estimations and comparisons of tick presence and abundance, as well as for the surveillance of pathogen presence and prevalence. In addition to the effectiveness of each method, practicality must also be considered. Although the novel CO₂-reinforced methods were in most instances comparable with their conventional counterparts, the downfalls of the methods may outweigh any potential benefit. The added weight and bulk of the CO₂ tank carried in a backpack causes increased difficulty when sampling in areas of dense vegetation (e.g. dense woodland undergrowth) or rough terrain (e.g. steep slope). The need to ensure that gas is flowing correctly through the tubing involves additional maintenance when using CO₂-reinforced methods, and the exposed portions of hoses running from the tank to the collection material must be protected from snagging or puncturing by vegetation. The CO₂-reinforced methods are also more expensive than their traditional counterparts.

Dry ice trapping was very effective for collecting ticks; however, the amount of dry ice necessary for large-scale trapping efforts can be difficult and expensive to obtain, and challenging to transport to collection sites. When available, dry ice trapping is very efficient for tick collection. In most instances, the use of dry ice trapping reduces the amount of time spent at each site, which can decrease the amount of time for which collectors are exposed to potentially infected ticks; however, when large numbers of ticks are collected on the cloth (at some sites several hundred ticks were collected on the dry ice trap), the time required to remove the ticks becomes comparable with the time required to complete other sampling methods. A slight change in methodology, such as by placing the cloth in a sealable bag, storing the bag and contents (cloth and ticks) in a freezer, and removing the ticks at another location, may be necessary in situations in which high tick densities occur or in areas with high pathogen prevalence where human exposure is a health concern.

In almost all situations, dragging was among the most effective methods for tick collection. Dragging is simple, gives results that are comparable with those of other studies, is less costly than dry ice trapping, and less costly and requires less maintenance than CO₂ dragging. When dry ice is not available, dragging is a suitable replacement. This study examined the effectiveness of several traps under different environmental conditions, but it is also necessary to conduct a cost analysis to determine the most cost-effective trapping method.

Knowledge of the most appropriate methods for collection based upon the targeted tick species, time of year and targeted
habitats is important in designing and carrying out protocols for tick and tick-borne pathogen surveillance and monitoring, as well as for estimates of relative tick densities and habitat use when the use of the most accurate and representative method is critical. In view of the differences shown here, the target tick species, as well as the habitat type, should be considered in the process of selecting a method for any of these purposes.

Acknowledgements

The authors thank the Board of Trustees and the employees of Ames Plantation, particularly Larry Teague and James Morrow for their assistance with tick collection. The authors would also like to thank University of Tennessee (UT) Medical and Veterinary Entomology Laboratory members Dave Paulsen, Chelsea Casteel, Brian Hendricks, Megan Long, Drew Mallinak, Megan Noseda, Kim Pompo, Casey Wesselman and Cassie Urquhart for assistance in collecting, identifying and processing specimens. Ann Reed and Xiaocun Sun at the UT Institute of Agriculture Research Computing Support deserve thanks for their assistance with statistics, and Drs Ernest Bernard and Graham Hickling and the reviewers of this paper are acknowledged for their critical review. This project was funded by the Department of Entomology and Plant Pathology, UT, the American Kennel Club Canine Health Foundation (01864-A), the U.S. Department of Agriculture Tennessee Hatch Project (TEN00433), and the Ames Plantation Research and Education Center.

References

Adeyeye, O.A. & Butler, J.F. (1991) Field evaluation of carbon dioxide baits for sampling Ornithodoros turicata (Acari: Argasidae) in gopher tortoise burrows. Journal of Medical Entomology, 28, 45–48.

Arpino, C.S., Levine, J.F., Evans, T.L., Braswell, A. & Heller, J. (1993) Relative utilization of reptiles and rodents as hosts by immature Ixodes scapularis (Acari: Ixodidae) in the coastal plain of North Carolina, U.S.A. Experimental and Applied Acarology, 17, 719–731.

Brownstein, J.S., Holford, T.R. & Fish, D. (2003) A climate-based model predicts the spatial distribution of the Lyme disease vector Ixodes scapularis (Acari: Ixodidae) in the United States. Environmental Health Perspectives, 111, 1152–1157.

Cohnstaedt, L.W., Rochon, K., Duehl, A.J. et al. (2012) Arthropod surveillance programs: basic components, strategies, and analysis. Annals of the Entomological Society of America, 105, 135–149.

Cooley, R.A. & Kohls, G.M. (1944) The genus Amblyomma (Ixodidae) in the United States. Journal of Parasitology, 30, 77–111.

Dennis, D.T., Nekomoto, T.S., Victor, J.C., Paul, W.S. & Piesman, J. (1998) Reported distribution of Ixodes scapularis and Ixodes pacificus (Acari: Ixodidae) in the United States. Journal of Medical Entomology, 35, 629–638.

Falco, R.C. & Fish, D. (1991) Horizontal movement of adult Ixodes dammini (Acari: Ixodidae) attracted to CO2-baited traps. Journal of Medical Entomology, 28, 726–729.

Gherman, C.M., Mihalca, A.D., Dumitrache, M.O. et al. (2012) CO2 flagging – an improved method for the collection of questing ticks. Parasites & Vectors, 5, 125–131.

Ginsberg, H.S. & 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). Experimental and Applied Acarology, 7, 313–322.

Ginsberg, H.S., Rulison, E.L., Azevedo, A. et al. (2014) Comparison of survival patterns of northern and southern genotypes of the North American tick Ixodes scapularis (Acari: Ixodidae) under northern and southern conditions. Parasites & Vectors, 7, 394–404.

Goddard, J. & Varela-Stokes, A. (2009) The discovery and pursuit of American Boutonneuse fever: a new spotted fever group rickettsiosis. Mid south Entomologist, 2, 47–52.

Hendricks, B.M. (2013) Identification and characterization of peak activity, environmental variables, and bacterial pathogens in A. americanum L. at Ames Plantation, west Tennessee. Master’s Thesis. University of Tennessee, Knoxville, TN.

Holscher, K.H., Gearhart, H.L. & Barker, R.W. (1980) Electrophysiological response of three tick species to carbon dioxide in the laboratory and field. Annals of the Entomological Society of America, 73, 288–292.

Keirans, J.E. & Durden, L.A. (1998) Illustrated key to nymphs of the tick genus Amblyomma (Acari: Ixodidae) found in the United States. Journal of Medical Entomology, 35, 489–495.

Keirans, J.E. & Litwak, T.R. (1989) Pictorial key to the adults of hard ticks, family Ixodidae (Ixodida: Ixodoidea), east of the Mississippi river. Journal of Medical Entomology, 26, 435–448.

Kinsinger, B.J. & Allan, B.F. (2011) Efficacy of dry ice-baited traps for sampling Amblyomma americanum (Acari: Ixodidae) varies with life stage but not habitat. Journal of Medical Entomology, 48, 708–711.

Mays, S.E., Houston, A.E., Trout Fryxell, R.T. (2016) Specifying pathogen associations of Amblyomma maculatum (Acari: Ixodidae) in western Tennessee. Journal of Medical Entomology, in press.

Mays, S.E., Hendricks, B.M., Paulsen, D.J., Houston, A.E. & Trout Fryxell, R.T. (2014) Prevalence of five tick-borne bacterial genera in adult Ixodes scapularis removed from white-tailed deer in western Tennessee. Parasites & Vectors, 7, 473–479.

Niebuhr, C.N., Breeden, J.B., Lambert, B.D., Eyres, A.I., Haefele, H.J. & Kattes, D.H. (2011) Efficacy of dry ice-baited traps for sampling Amblyomma maculatum (Acari: Ixodidae) in western Tennessee. Journal of Medical Entomology, 48, 897–928.

Parola, P. & Raoult, D. (2001) Ticks and tick-borne bacterial diseases in humans: an emerging infectious threat. Clinical Infectious Diseases, 32, 897–928.

Petry, W.K., Foré, S.A., Fielden, L.J. & Kim, H. (2010) A quantitative comparison of two sample methods for collecting Amblyomma americanum and Dermacentor variabilis (Acari: Ixodidae) in Missouri. Experimental and Applied Acarology, 52, 427–438.

Pompo, K., Mays, S., Wesselman, C., Paulsen, D.J., Trout Fryxell, R.T. (2016) Survey of ticks collected from Tennessee cattle and their parasites for Anaplasma and Ehrlichia species. Journal of Parasitology, 102, 54–59. doi: 10.1645/15-814.

© 2016 The Authors. Medical and Veterinary Entomology published by John Wiley & Sons Ltd on behalf of Royal Entomological Society, Medical and Veterinary Entomology, 30, 123–134.

Comparison of tick trapping methods 133
Portugal, J.S. III & Goddard, J. (2015) Collections of immature *Amblyomma maculatum* Koch (Acari: Ixodidae) from Mississippi, U.S.A. *Systematic and Applied Acarology*, **20**, 20–24.

Reye, A.L., Arinola, O.G., Hübschen, J.M. & Muller, C.P. (2012) Pathogen prevalence in ticks collected from the vegetation and livestock in Nigeria. *Applied and Environmental Microbiology*, **78**, 2562–2568.

Rosen, M.E., Hamer, S.A., Gerhardt, R.R. *et al.* (2012) *Borrelia burgdorferi* not detected in widespread *Ixodes scapularis* (Acari: Ixodidae) collected from white-tailed deer in Tennessee. *Journal of Medical Entomology*, **49**, 1473–1780.

Rulison, E.L., Kuczaj, I., Pang, G., Hickling, G.J., Tsao, J.I. & Ginsberg, H.S. (2013) Flagging versus dragging as sampling methods for nymphal *Ixodes scapularis* (Acari: Ixodidae). *Journal of Vector Ecology*, **38**, 163–167.

Rynkiewicz, E.C. & Clay, K. (2014) Tick community composition in Midwestern U.S. habitats in relation to sampling method and environmental conditions. *Experimental and Applied Acarology*, **64**, 109–119.

Schulze, T.L., Jordan, R.A. & Hung, R.W. (1997) Biases associated with several sampling methods used to estimate abundance of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae). *Journal of Medical Entomology*, **34**, 615–623.

Semtner, P.J. & Hair, J.A. (1975) Evaluation of CO₂-baited traps for survey of *Amblyomma maculatum* Koch and *Dermacentor variabilis* Say (Acarina: Ixodidae). *Journal of Medical Entomology*, **12**, 137–138.

Solberg, V.B., Neidhardt, K., Sardelis, M.R., Hildebrandt, C., Hoffman, F.J. & Boobar, L.R. (1992) Quantitative evaluation of sampling methods for *Ixodes dammini* and *Amblyomma americanum* (Acari: Ixodidae). *Journal of Medical Entomology*, **29**, 451–456.

Stromdahl, E.Y. & Hickling, G.J. (2012) Beyond Lyme: etiology of tick-borne human diseases with emphasis on the south-eastern United States. *Zoonoses and Public Health*, **59**, 48–64.

Teel, P.D., Hopkins, S.W., Donahue, W.A. & Strey, O.F. (1998) Population dynamics of immature *Amblyomma maculatum* (Acari: Ixodidae) and other ectoparasites on meadowlarks and northern bobwhite quail resident to the coastal prairie of Texas. *Journal of Medical Entomology*, **35**, 483–488.

Teel, P.D., Ketchum, H.R., Mock, D.E., Wright, R.E. & Stray, O.F. (2010) The Gulf coast tick: a review of the life history, ecology, distribution, and emergence as an arthropod of medical and veterinary importance. *Journal of Medical Entomology*, **45**, 707–722.

Accepted 5 September 2015
First published online 23 January 2016