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Patience is not always a virtue: effects of terrain complexity on the host-seeking behaviour of adult blacklegged ticks, *Ixodes scapularis*, in the presence of a stationary host

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Abstract. Blacklegged ticks, *Ixodes scapularis* Say (Acari: Ixodidae), are the primary vectors of Lyme disease in the U.S.A. In this study, adult ticks were observed on public trails exhibiting increasing levels of terrain complexity with a potential host nearby. The goal of this study was to (a) examine the extent to which adult ticks may actively search (vs. sit-and-wait) for a nearby host, (b) determine whether or not ticks could locate the position of the host in natural conditions and (c) determine the role of terrain complexity on the distances ticks travelled in a short period of time (30 min). Results indicate that, when a potential stationary host is within 50 cm, ticks will utilize an active-search strategy. The majority of ticks moved in the direction of the host in natural conditions. Finally, ticks in a less complex terrain were more active and travelled greater horizontal distances than ticks in a more complex terrain. In conclusion, the use of an active-search approach would likely increase the foraging success of ticks, especially in terrains with minimal complexity, near host animals that have stopped to rest or feed, reinforcing that humans should be vigilant about checking for ticks after being outdoors.

Key words. Blacklegged tick, Lyme disease, sit-and-wait, vector.

Introduction

Lyme disease and other tick-borne diseases, such as babesiosis, anaplasmosis, Rocky Mountain spotted fever and more, pose a significant health risk in the northeastern U.S.A. *Ixodes scapularis* ticks are the primary vectors for the Lyme disease-causing bacteria, *Borrelia burgdorferi*, which is transmitted when saliva from an infected tick enters a host during feeding. Humans are increasingly at risk of tick exposure in built environments traditionally considered ‘safe’, such as school campuses, public parks, backyards and gardens (Roome *et al.*, 2018). Understanding tick host-seeking behaviour can help us understand how to minimize the risk of tick exposure and, thus, infection.

There is a degree of unpredictability that predators/parasites must cope with in terms of when and where to find prey/hosts (Green, 1984). Those that utilize a ‘sit-and-wait’ strategy minimize the energy spent during foraging; however, they must be adapted to prolonged periods of starvation (Gibbs, 2019). The alternative is to use an ‘active-search’ strategy, but there is a cost associated with energy required to locate, move toward and capture prey/hosts. It is known that ticks seek hosts by questing – they climb up vegetation (stems/leaves) and sit with front legs extended and wait to grab onto a host passing by. However, it is also known that ticks are willing to migrate horizontally (or actively search), when stimulated by the presence of host cues. In general, host-seeking behaviour in ticks differs among species, with ticks displaying varying degrees of sit-and-wait and active-search behaviour (Rechav, 1979). In addition, host-seeking behaviour in ticks differs among life stages, in that the immatures (larvae and nymphs) of many

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species are more likely to sit-and-wait for passing hosts, whereas adults are more active and disperse greater distances (Smittle *et al.*, 1967; Rechav, 1979; ElGhali & Hassan, 2010; Portugal & Goddard, 2016). Studies have quantified the vertical movement and questing height of ticks (Mejlon & Jaenson, 1997; Vail & Smith, 2002; Lane *et al.*, 2009) and horizontal dispersal patterns of ticks over periods of days to weeks (Falco & Fish, 1991; Goddard, 1993; Lane *et al.*, 2009; Romanenko *et al.*, 2016); however, less is known about the distances adult ticks are willing and able to travel *in the short term* and when a host is in close proximity.

It is also not clear how the complexity of the terrain may influence the horizontal distance ticks are able to travel. Ticks are found in a variety of habitats, including bare ground/rocks, deciduous/coniferous leaf litter and grasses/vegetation. The obstacles separating the tick from the host may influence the tick's host-seeking behaviour. In an environment with complex terrain (e.g. dense grasses), the cost of actively searching may be too great (in terms of energy wasted) if the likelihood of reaching the host is low. In contrast, an environment with minimal obstacles and little opportunity for vertical climb (e.g. bare ground, rocks, leaf litter) may facilitate the incorporation of an active-search strategy. Thus, although adult *I. scapularis* ticks are understood to travel horizontally, the pace of horizontal movement in the short term and the impact of terrain complexity on horizontal and vertical movement are less known.

Host cues that activate and/or attract ticks include odours such as exhaled carbon dioxide (Falco & Fish, 1991; Kahl, 1996; Gherman *et al.*, 2012; Van Duijvendijk *et al.*, 2017), heat (Oorebeek *et al.*, 2008), Type A blood (Žákovská *et al.*, 2018) and vibration (Vassallo & Pérez-eid, 2002). These and other studies, however, examined tick host-seeking/questing behaviour in artificially constructed set-ups, such as Y-tube olfactometers (Van Duijvendijk *et al.*, 2017), arenas containing artificial vegetation (Arsnoe *et al.*, 2015), Petri dishes (Oorebeek *et al.*, 2008; Žákovská *et al.*, 2018) and/or with artificially dispersed CO_2 (Falco & Fish, 1991; Gherman *et al.*, 2012). Less is known about how ticks might respond to a potential host under natural conditions.

The goal of this study is to examine the extent to which adult *I. scapularis* ticks will actively seek out and move towards a nearby stationary host in terrains of varying complexity and under natural conditions.

Methods

Field observations

An experiment was conducted in the late spring and early summer of 2019 (29 May–24 June) to determine (a) whether adult *I. scapularis* ticks will utilize a 'sit-and-wait' or an 'active-search' host-seeking strategy in the presence of a stationary host (the human observer), (b) if ticks are able to detect and navigate to a host within 50 cm in a 30-min period of time and (c) if tick behaviour is altered by the complexity of the terrain separating it from the potential host. Observations were conducted directly on public trails at two locations in Broome County, New York, U.S.A. (latitude 42°N, longitude 75°W), including Chenango Valley State Park, Port Crane, NY and SUNY Broome Community College nature preserve, Binghamton, NY. Ticks were collected from public trails by dragging a 1-m^2 6-wale white corduroy cloth. Each tick was marked with a yellow dot of Testor's enamel paint (Testor Corporation, Rockford, IL, U.S.A.) on the scutum/abdomen for ease of tracking, a technique used in previous studies (Goddard, 1993; Portugal & Goddard, 2016). Only one tick was observed at a time, and all ticks were observed within 5 minutes of collection.

The observation area consisted of an area of ground with four quadrants (numbered 1–4). The observer (potential host) was always positioned in quadrant 1 approximately 50 cm from the start. The same observer performed each trial. After the tick was marked with paint, a sterile swab was used to pick up the tick from the drag, and the swab was inserted into the ground, at the centre of the observation area, with approximately 3 cm of swab exposed. The tick was not oriented in any particular direction. Sterile gloves were worn to prevent the transfer of human cues to the swab or environment.

Ticks were observed directly on public trails with ground cover generally consisting of deciduous leaf litter and grasses, habitats that generally show greater tick abundance (Lubelczyk *et al.*, 2004). The complexity of the terrain was defined by the obstacles separating the tick from the potential host (observer) and climbing opportunity: *Level 1* (easy) – no/minimal opportunity for vertical climb (rocks, bare ground, deciduous leaf litter, coniferous needles, no grass or vegetation); *Level 2* (moderately difficult) – moderate opportunity for vertical climb (bare ground, deciduous leaf litter, patches of loose grass or vegetation occurring over less than half of the study area and rarely exceeding 20 cm in height); and *Level 3* (difficult) - ample opportunity for vertical climb (no bare ground, dense grass and vegetation rarely exceeding 20 cm in height). The final sample size was 15 ticks for Level 1 terrain (8 males and 7 females), 16 ticks for Level 2 terrain (3 males and 13 females) and 14 ticks for Level 3 terrain (5 males and 9 females). Observations were performed in a range of environmental conditions (temperature range: 57 F–74°F, humidity: 59.3%–85.6%, time of day: 8:00 AM–3:00 PM). Ticks were tested on the same terrain and in the same conditions from which they were collected.

Tick behaviour was recorded once per minute for 30 min for a total of 30 observations. At each observation, ticks were recorded as either *moving* (making forward progress of at least one full body length within 3 seconds) or *stationary* (remaining in the same relative position); *questing* (fore legs outstretched and waving, whether tick was on the ground or on vegetation) or *not questing* (fore legs relaxed, not waving); and *hidden* (not visible to the observer, likely under vegetation) or *exposed* (clearly visible). Determination of questing or not required the observer to lean forward for a closer look. If ticks were observed to be moving, the orientation of movement was also recorded: *up* (moving upwards at ≥45° angle relative to the ground), *down* (moving downwards at ≥45° angle relative to the ground) or *horizontal* (moving on or relatively parallel to the ground). In addition, each tick's *horizontal distance from centre* (the swab) and *vertical distance from the ground* (if greater than 5 cm) were recorded at every fifth observation (i.e. every 5 min for a total of six times in 30 min). Each time a tick came within proximity of the observer, the observer would back away from the tick (maintaining at least a 10 cm gap) until the end of the observation period.

At the end of the observation period, each tick was placed in a microcentrifuge tube of 100% ethanol and stored at −20° Celsius.

Statistical analysis

Statistical analyses were performed using R Statistical Software.

Host-seeking behaviour.

Sit-and-wait or active-search. A Mann–Whitney test was used to determine if ticks were more often observed *moving* or *stationary* for 30 total observations per tick. To determine if terrain complexity had an effect on tick host-seeking behaviour, Kruskal-Wallis tests were conducted on the number of observations in which ticks were *moving* (as opposed to *stationary*) per level of terrain (Level 1, 2 or 3) and the number of observations in which ticks were *moving up*, *down* and *horizontally* per level of terrain (Level 1, 2 or 3).

Ability to navigate towards host. To compare the appearance of ticks in the four quadrants, the number of ticks that remained at the start point and that stayed in the four quadrants at t = 30 min were counted. A chi-square test was used to determine whether the probabilities of ticks in the four quadrants were the same or not. A chi-square test was also used to determine if the probability of remaining in quadrant 1 (containing host) was higher than the other quadrants (quadrants 2, 3 and 4 combined).

The time that each tick spent in each of the four quadrants was normalized by dividing by the total time spent in each quadrant, thus obtaining the proportion of time spent in each of the quadrants. Each tick is represented by a vector (thus, there are 45 such vectors, one for each tick) of four proportions that sum up to 1, which mimics a probability distribution over the four quadrants. To check whether the mean of these proportion vectors is equal (0.25, 0.25, 0.25, 0.25), three confidence intervals for the first three proportions are constructed (as the four coordinates sum up to 1, the fourth coordinate is not tested). As this is a multiple comparison problem, the Bonferroni multiple comparison correction method was used.

A Mann–Whitney test was used to compare the maximum horizontal distances travelled by ticks in quadrant 1 (containing host) vs. the maximum horizontal distances in any quadrant that is not quadrant 1. Specifically, for each tick, the maximal value of the maximum distances in quadrants 2, 3 and 4 are compared with the maximum distance in quadrant 1.

Effects of terrain complexity. A general linear model was used to examine the effect of terrain level and time on the horizontal distance and vertical distance ticks travelled. Because the distance in the data is accumulative distance since time 0, the incremental distance was first computed by considering the difference of the horizontal (or vertical) distance between 0 and 5 min, 5 and 10 min, 10 and 15 min, 15 and 20 min, 20 and 25 min and 25 and 30 min. This was done to alleviate the dependency between any two data points from the same tick. The incremental distance was then used as the response (dependent) variable in the general linear model. Potential independent variables are the terrain level (as a factor), the time (as a numerical variable) and/or the interaction between them.

Results

Field observations

Host-seeking behaviour.

Sit-and-wait or active-search. Ticks were *exposed* (as opposed to *hidden*) for 99.98% of observations. Overall, ticks were more often observed *moving* (72% of observations) than *stationary* (28% of observations). In addition, there was a significant effect of terrain on the number of observations where ticks were observed moving, in that ticks in Levels 1 and 2 (easy and moderate) terrain were more often observed moving than ticks in Level 3 (difficult) terrain (df = 2, $H = 6.6779$, $P = 0.03547$). Specifically, ticks were observed moving (whether up, down or horizontal) in 72.6% of observations in Level 1 terrain, 76.5% of observations in Level 2 terrain and 58.6% of observations in Level 3 terrain. Whether moving or stationary, ticks were observed *questing* in 98.3% of observations.

There was a significant effect of terrain on the number of observations where ticks were moving *up* (df = 2, $H = 27.662$, $P < 0.001$) and moving *down* (df = 2, $H = 12.237$, $P = 0.002$). Levels 1 and 2 (easy and moderate) ticks were more often observed moving *horizontally* than Level 3 (difficult) ticks (df = 2, $H = 23.567$, $P = 0.000$). Specifically, the percentage of observations where ticks were observed moving up, down and horizontal are as follows: Level 1: 2.9%, 5.8% and 69.8%; Level 2: 17.7%, 11.9% and 46.9%; and Level 3: 26.4%, 17.9% and 14.3%, respectively.

Ability to navigate towards host. No ticks remained at the start after 30 min. There was a significant difference in the number of ticks that visited each quadrant after 30 min ($X^2 = 47.356$, df = 3, $P < 0.001$). Specifically, the number of ticks (out of 45) that visited quadrants 1, 2, 3 and 4 after 30 min are 31 (68.9%), 7 (15.6%), 2 (4.4%) and 5 (11.1%), respectively. The probability of ticks remaining in quadrant 1 (containing host) was significantly higher than the probability of ticks remaining in the combined region of quadrants 2, 3 and 4 ($X^2 = 6.42$, df = 1, $P < 0.001$) (Fig. 1).

Over the period of 0–30 min, a tick only spent 3.42 min at the start point, on average. Three 99.9% confidence intervals were constructed for the proportion of time spent in each of the first three quadrants. The lower limits of the confidence intervals are (0.361, 0.072, and 0.002), and the upper limits are (0.756, 0.385, and 0.154), respectively. As (0.25, 0.25, 0.25) clearly falls out of the cube characterized by these upper and lower limits, the null hypothesis at the 0.1% level for each of the three coordinates and the null hypothesis that the mean of the proportion vectors is (0.25, 0.25, 0.25, 0.25) at the 0.3% level can be rejected, and

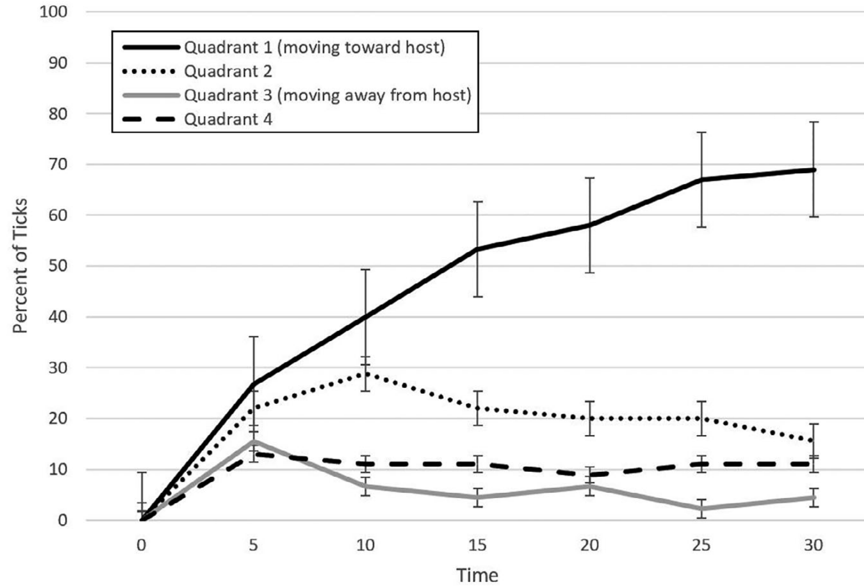

Fig. 1. Percentage of ticks located in each quadrant (regardless of terrain complexity) every 5 min over the 30-min observation period. Means ± SE are shown.

Table 1. Effect of terrain on the horizontal and vertical distances (cm) travelled by ticks (regardless of quadrant) over time (incremental difference in the distance).

	Estimate	SE	t-value	*P*-value
Horizontal distance from start				
Terrain Level 1 (intercept)	6.64	0.72	9.27	$<2 \times 10^{-16}$*
Terrain Level 1 vs. 2	−1.20	0.99	−1.20	0.229
Terrain Level 1 vs. 3	−5.41	1.03	−5.25	3.11×10^{-7}*
Vertical distance from ground				
Terrain Level 1 (intercept)	0.144	0.672	0.21	0.830
Terrain Level 1 vs. 2	1.356	0.936	1.45	0.148
Terrain Level 1 vs. 3	4.249	0.968	4.39	1.63×10^{-5}*
Time	−0.023	0.173	−0.13	0.894
Terrain Level 2 × time	−0.190	0.240	−0.79	0.429
Terrain Level 3 × time	−0.876	0.248	−3.53	4.96×10^{-4}*

*Statistical significance at $\alpha = 0.05$.

it can thus be concluded that ticks spent a greater proportion of time in quadrant 1 (containing the host).

There was a significant difference in the horizontal distances travelled by ticks in quadrant 1 vs. the maximum distances in any quadrant that is not quadrant 1 (W = 1310.5, $P = 0.01507$).

Effects of terrain complexity. When the terrain level is the only independent variable, the results suggest that there is no significant difference in the horizontal movement of ticks between terrain Level 1 (easy) and Level 2 (moderate) ($P = 0.229$), but there is a strong statistically significant difference between terrain Level 1 and Level 3 (difficult) ($P < 0.001$) (Table 1) (Fig. 2). Overall, ticks constantly move horizontally in terrain Level 1 and Level 2, and at a pace much faster than how they move in terrain Level 3. Time is not significant (meaning pace was constant) and was not included in the report.

When the terrain level is the only independent variable, the results suggest that the vertical movement of ticks is significantly different when they are in Level 3 (difficult) terrain compared to terrain Level 1 or 2 (easy or moderate). Time and the interaction between time and terrain were then also included in the general linear model. For each of the three terrain levels, the incremental vertical distance is modelled as intercept + time × slope, where the intercept is the mean (predicted) value of distance if the time = 0, and the slope is the increased prediction value as the time increases by 1 min. Here, the intercept for terrain Level 1 (easy) is 6.64 cm and that for terrain Level 3 (difficult) is 4.25 cm more than in terrain Level 1 ($P < 0.001$) (Table 1). Overall, results suggest that, although ticks do not move much horizontally when they are in terrain Level 3, they do move vertically significantly more compared to terrain Levels 1 and 2 initially. In addition, time has an effect on movement. For ticks in Level 3 (difficult) terrain, for every additional time unit, the vertical movement is reduced by 0.88 cm more than in Level 1 (easy) terrain ($P < 0.001$) (Table 1) (Fig. 3). This may be explained by the limited vertical space for movement, and once ticks have reached the top surface of the terrain, they must sit-and-wait or descend.

Discussion

Although ticks may indeed utilize a sit-and-wait strategy when no host is detected, this study suggests that adult *I. scapularis* ticks utilize an active-search strategy when a potential host is detected nearby. Regardless of the terrain, 31 ticks (69%) were able to successfully move in the direction of the host in natural conditions. Seven ticks (15.5%; three in Level 1 (easy) terrain and four in Level 2 (moderate) terrain) not only reached the host (at 50 cm) within 30 min but ultimately

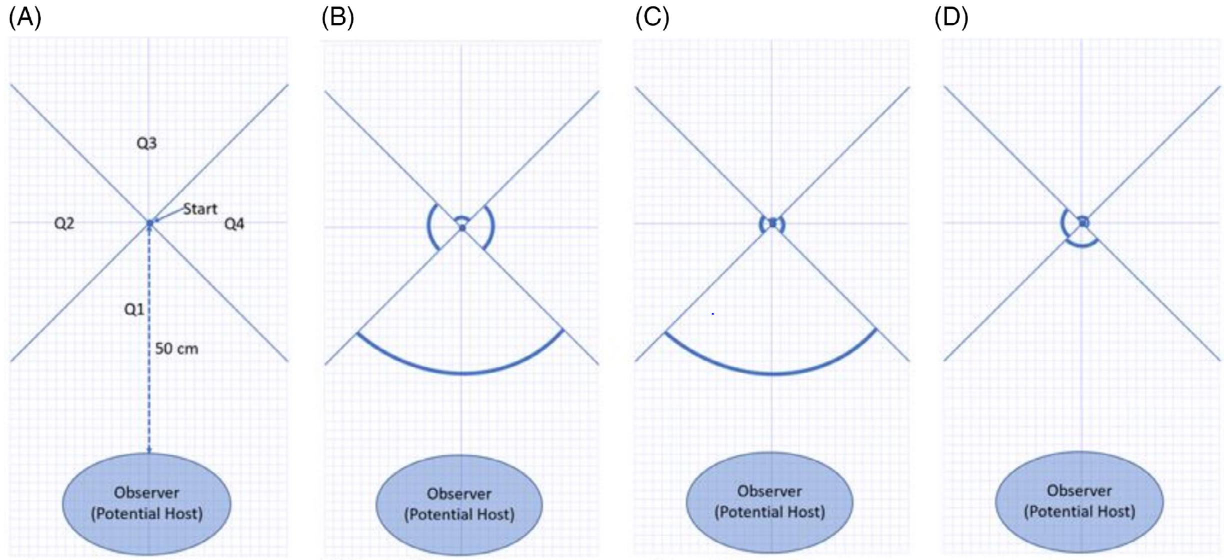

Fig. 2. (A) Experimental design. Q = Quadrant (numbered 1–4). Each square represents 2 cm^2. (B–D) Maximum horizontal distance (cm) travelled by the average tick in each quadrant in (B) Level 1 terrain (easy), (C) Level 2 terrain (moderate) and (D) Level 3 (difficult) terrain. Each square represents 2 cm^2. [Colour figure can be viewed at wileyonlinelibrary.com].

travelled distances ranging from 50 to 110.5 cm towards the retreating observer – a rate of 1.7–3.68 cm/min. Thus, patience is not always a virtue for host-seeking ticks – the transition to more of an active-search approach would likely increase the foraging success of ticks near host animals that have stopped to rest/sleep or feed. Goddard (1993) found the horizontal movement of adult *I. scapularis* ticks to be minimal (the majority was recaptured within 0.5 m of their release point weeks after mark and release); however, no host was present. Thus, the results of this study indicate that the presence of a stationary host within close proximity can have a marked impact on tick movement.

The complexity of the terrain did indeed influence tick host-seeking behaviour, in that ticks in Level 3 (difficult) terrain were more often observed stationary compared to ticks in less complex terrain (although they were still more active than not). In addition, these ticks more often moved vertically (up or down) than horizontally and maintained a higher vertical position throughout the observation period compared to ticks in Level 1 or 2 (easy or moderate) terrain (Fig. 3). The benefit of active vertical climb (whether up or down) was that it allowed for simultaneous horizontal movement as ticks seemed to favour a horizontal shift from one blade to another when intersections were encountered. It is also possible, however, that ticks were switching from one blade to another in search of a vegetation that would allow them to climb higher. Mejlon & Jaenson (1997) found the vertical distribution of *Ixodes ricinus* ticks to be dependent on life stage – adults favoured a questing height comparable to the height of their ‘preferred’ host (60–79 cm). That 8 of the 14 Level 3 ticks in this experiment concluded the observation period in quadrant 1 (moving towards the host) implies that their horizontal movements were directed. However, further examination of the vertical and horizontal movements of adult ticks in complex terrain using *continuous* observation (rather than the incremental data collected here) is needed.

The question remains whether nymphal ticks would behave in the same manner as the adults in this study. In general, the immatures of many species are more likely to sit-and-wait for passing hosts, whereas adults are more active and disperse greater distances (Smittle *et al.*, 1967; Rechav, 1979; ElGhali & Hassan, 2010; Portugal & Goddard, 2016). Presumably, the small size of nymphs (body length <1 mm) compared that of an adult male (~2 mm) or an adult female (~3 mm) prevent them from covering equal distances. Another issue is that nymphal ticks are susceptible to desiccation (Stafford III, 1994) and may not remain exposed for as long as adult ticks, returning to below the leaf litter surface to rehydrate. Indeed, *I. scapularis* nymphs have been found to quest at lower heights when relative humidity is low (Vail & Smith, 2002). Regardless, nymphal size would make it virtually impossible to mark and observe ticks in the same manner as adults in the present study. Although nymphs are considered to be responsible for much of the transmission of *B. burgdorferi* to humans in North America (Fish, 1993), adult *I. scapularis* should not be dismissed as a significant contributor – they are nearly twice as infected (45.5% of adults compared to 27.5% of nymphs) (Roome *et al.*, 2018); are host-seeking a greater proportion of the year (spring and fall for adults compared to summer for nymphs) (Roome *et al.*, 2018); and, as demonstrated by this experiment, can actively and successfully locate stationary hosts.

Another factor to consider is that weather conditions may influence the likelihood of ticks engaging in active-search. Although all observations in this study were performed in dry conditions, ticks may be less likely to actively search when it is raining or vegetation has standing water as ticks avoid liquid water (Kröber & Guerin, 2000). As stated previously, relative humidity can also influence tick host-seeking behaviour (Vail &

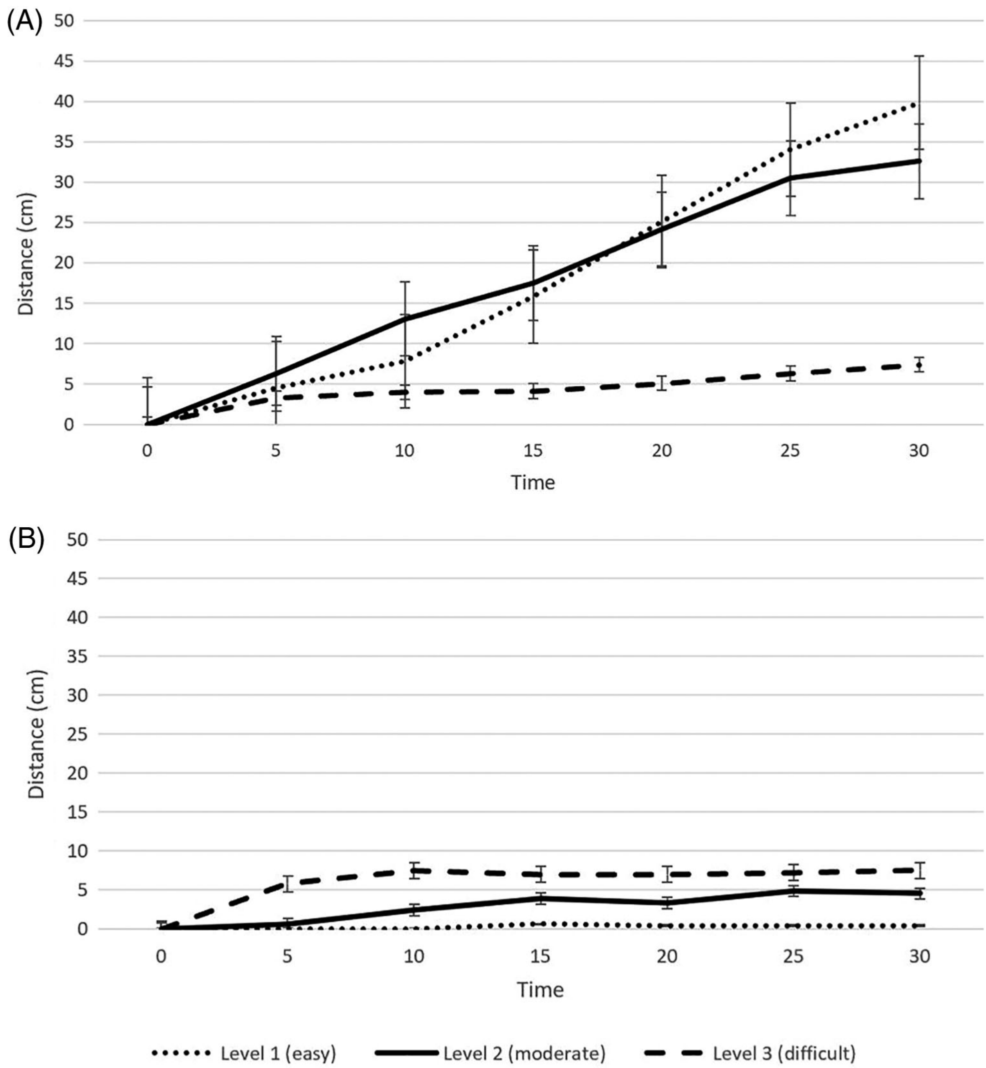

Fig. 3. Effect of terrain on (A) the horizontal distance from start and (B) vertical distance from the ground travelled by ticks (regardless of quadrant) every 5 min over the 30-min observation period. Means ± SE are shown.

Smith, 2002), potentially discouraging ticks from active-search when relative humidity is low.

This study contributes to our understanding of tick host-seeking behaviour and the factors that play a role in human exposure to ticks and the diseases they transmit. The transition of adult *I. scapularis* ticks to an active-search approach would likely increase the foraging success of ticks near host animals that have stopped to rest/sleep or feed. As ticks are increasingly found in built environments often constructed for the purpose of human congregation (Roome *et al.*, 2018), awareness is required for outdoor activities that involve remaining stationary (napping, picnicking, reading, etc.) in areas where ticks may be present. In addition, humans effectively reduce the complexity of terrain (by walking on, manicuring, trimming, landscaping, etc.), thereby promoting the horizontal movement of ticks and the likelihood of tick exposure in a short period of time. Along walkways with high human use, DNA analyses revealed an overall *B. burgdorferi* infection rate of 45.5% in adult *I. scapularis* ticks over a 2-year span in Broome County (NY, U.S.A.) (Roome *et al.*, 2018). This is particularly alarming considering human perception of risk is poor. There is significant need for an increase in human acceptance of personal protective measures (such as wearing protective clothing, using repellent and showering/bathing after being outside) (Eisen *et al.*, 2012). The results of this and other recent studies echo the necessity of taking proper precautions.

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