Blacklegged Ticks, *Ixodes scapularis* Say, and American Dog Ticks, *Dermacentor variabilis* (Say) Abundance and Distribution on Pastures in Manitoba, Canada.

By

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ABSTRACT

Blacklegged ticks, *Ixodes scapularis* Say, and American dog ticks, *Dermacentor variabilis* (Say) (Acari: Ixodidae), are hard ticks that can transmit pathogens that may pose a threat to livestock, workers, and companion animals. *I. scapularis* can transmit the causative agents of Lyme disease (*Borrelia burgdorferi*), granulocytic anaplasmosis (*Anaplasma phagocytophilum*), and babesiosis (*Babesia microti*), which can affect humans and horses. *D. variabilis* are known to transmit the bacterium *Anaplasma marginale,* which causes bovine anaplasmosis in cattle. As the distribution of these two species spreads further north and west, beef producers in Manitoba want to know if working alongside their animals and in pastures is increasing their risk of acquiring one of these tick-borne pathogens. This study investigated the abundance and distribution of *I. scapularis* and *D. variabilis* in cattle and horse pastures in southern Manitoba, with particular emphasis on habitat associations and the presence of tick-borne pathogens. The abundance and distribution of *I. scapularis* and *D. variabilis* were assessed through environmental drag sampling and tick collections from cattle and horses at seven study sites. Habitat associations were examined using geospatial land-cover data, correspondence analysis, and habitat-distance randomization analyses, while adult ticks were screened for selected tick-borne pathogens using molecular methods A total of 1,946 ticks were collected during the study, including 944 from environmental drag sampling and 1,002 from hosts. *Dermacentor variabilis* was the dominant and most widely distributed species, whereas *Ixodes scapularis* was less abundant and occurred primarily at Dusty Oaks; habitat analyses revealed site-specific associations between *D. variabilis* and several habitat types, including forest, shrubland, grassland, wetland, and water. Molecular screening detected *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in *I. scapularis*, while all *D. variabilis* tested negative for *Anaplasma marginale*.

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CHAPTER 1: GENERAL INTRODUCTION

Ticks are blood-feeding ectoparasites that can transmit pathogens from one host to another. In Canada, ticks such as *Ixodes scapularis* Say, also known as the blacklegged tick or deer tick, and *Dermacentor variabilis* (Say), commonly referred to as the American dog tick or wood tick, have become a growing public health concern. *Ixodes scapularis* was once restricted to the southern United States (Bishopp and Trembley 1945), however, their range has since expanded, encompassing portions of the northeastern United States (Jackson and Defoliart 1970, Spielman et al. 1985), southeastern Ontario (Watson and Anderson 1976), southern Manitoba (Galloway 1989), Nova Scotia (Bell et al. 1992), Québec and, New Brunswick (Ogden et al. 2009, 2006c, Koffi et al. 2012). In contrast, *D. variabilis*, has been documented in North America since the 1900s, and is widespread across a substantial part of the eastern United States (Bishopp and Trembley 1945, Wilkinson 1967, Merten and Durden 2000). *Dermacentor variabilis* extends into Mexico, as well as southeastern Saskatchewan, southern Manitoba, and southern Ontario (Bishopp and Trembley 1945, Wilkinson 1967, Merten and Durden 2000, Dergousoff et al. 2013). *Ixodes scapularis* and *D. variabilis* are currently well established in southern Manitoba and are continuing to expand north and westward (Government of Manitoba 2016, Dergousoff et al. 2025).

The life cycle of both ticks consists of four life stages: egg, larva, nymph, and adult female or male. Ticks feed exclusively on blood obtained from a vertebrate host. Ticks require a bloodmeal to transition from larva to nymph, from nymph to adult, and to produce eggs. After feeding, engorged ticks detach from their host, seek a suitable microhabitat, and either moult or, for mated females, lay eggs. In Northeast America, the entire life cycle of *I. scapularis*, from egg to adult, typically spans two to three years (Yuval and Spielman 1990) and up to three to four years to complete (Lindsay et al. 1998). The life cycle of *D. variabilis* typically takes around two years (Garvie et al. 1978, Burachynsky and Galloway 1985). However, in colder environments or with limited hosts, a few ticks may persist longer, potentially extending the life cycle of *D. variabilis* to three years (Garvie et al. 1978). The likelihood of encountering these ticks depends on life-stage-specific host-seeking behaviour and seasonal patterns (Eisen et al. 2016). While both species are active in the spring and early summer (May-June), *I. scapularis* has a distinct activity peak in October (eTick 2026). The habitat preferences of both ticks also differ: *I.*

scapularis is associated with denser vegetation and high humidity, while *D. variabilis* is more associated with sparse, moderately humid vegetation.

Both ticks are of public concern, as they can carry and transmit tick-borne pathogens, such as bacteria, unicellular eukaryotic parasites, and viruses. *Ixodes scapularis* can transmit *Borrelia burgdorferi* Johnson et al., the bacterium that causes Lyme disease; *Anaplasma phagocytophilum* (Foggie), the bacterium that causes granulocytic anaplasmosis; and *Babesia microti* (Franca), a blood-borne parasite that causes babesiosis, which can affect humans and horses. *D. variabilis* can transmit *Anaplasma marginale* Theiler, a bacterium that causes bovine anaplasmosis in cattle. Active surveillance is the best method for evaluating pathogen prevalence in endemic populations (Koffi et al. 2012). This involves collecting ticks in the field and sampling host mammals found in various environments (Koffi et al. 2012). In Manitoba, extensive sampling to detect the presence of *I. scapularis* occurred in 1991 near Marchand, in southern Manitoba (Galloway et al. 1991), in 2016 near Buffalo Point (Tyler et al. 2018), and in 2017 near Beaudry and Birds Hill Provincial Park (Polk 2017).

This research was done to establish the abundance, distribution, and pathogen prevalence of *I. scapularis* and *D. variabilis* on horses, cattle, and pastures in Manitoba. Chapter 2 reviews the literature, focusing on the biology, distribution, and hosts of *I. scapularis* and *D. variabilis*, and the pathogens associated with them. Chapter 3 presents a research study assessing the abundance and distribution of *I. scapularis* and *D. variabilis* on horse and cattle pastures in Manitoba, as well as the pathogen prevalence in each species and their abundance on horses and cattle. Finally, Chapter 4 discusses this research in the context of current knowledge and future research directions.

CHAPTER 2: LITERATURE REVIEW

Biology and hosts

Ixodidae

Ticks (Arachnida: Acari) are hematophagous ectoparasites that feed on the blood of mammals, birds, and occasionally reptiles. There are three tick families: Nuttalliellidae, Argasidae, and Ixodidae. Nuttalliellidae consists of a single species, *Nuttalliella namaqua* Bedford (Ledwaba and Malatji 2024), which is restricted to southern and eastern Africa, and its life cycle and host associations remain poorly understood . Argasidae, or soft ticks, contains over 190 species (Guglielmone et al. 2010). Argasids are known for their leathery appearance and rapid feeding, often utilizing multiple hosts at each life stage. Soft ticks go through two or more nymphal stages, each requiring a bloodmeal from a host (Nava et al. 2009, Guglielmone et al. 2010). Ixodidae is the largest family, consisting of over 700 species (Nava et al. 2009, Guglielmone et al. 2010), collectively known as hard ticks. The main characteristic that gives hard ticks their name is the presence of a sclerotized scutum, a hard dorsal plate located behind the gnathosoma (the "head"). The gnathosoma houses the tick's specialized mouthparts and palps, which project forward from the body. The mouthparts consist of barbed chelicerae that cut through the skin , and a ventral hypostome, a feeding tube with rear-facing, spine-like denticles that anchor the tick to the host (Sonenshine and Roe 2014). Palps help the tick locate a feeding site, allowing it to attach securely and extract blood from the host. The hard tick life cycle has four stages: egg, six-legged larva, eight-legged nymph, and adult (female or male) (Figure 1).

A bloodmeal is required to transition between life stages and for female egg production. Some hard ticks are opportunistic three-host ticks, meaning that each active life stage takes a bloodmeal from three separate hosts. A bloodmeal refers to a tick piercing the skin of a host animal with its mouthparts to feed on the host's blood. The tick's chelicerae tear the skin and blood vessels, allowing blood to pool around the hypostome where the blood is then drawn through a narrow food canal. During feeding, ticks inject saliva into the host's bloodstream and alternate between salivating and sucking blood until fully engorged. Ticks can feed undetected as saliva contains protease inhibitors that hinder the host's natural blood coagulation and platelet aggregation, acting as an anti-hemostatic agent while also modulating the host's immune system (Chmelař et al. 2017). Certain hard ticks cement their mouthparts to the host's skin during

attachment and detach only after the rapid feeding phase (last 12-36 hours), once most bodily fluids and the bloodmeal have been consumed (Sonenshine and Roe 2014).

Between bloodmeals, unfed hard ticks must locate a suitable host. Many ixodid ticks use an ambush host-seeking strategy known as questing, during which they climb vegetation, secure themselves with their posterior legs, and extend their front pair of legs to contact a passing host (Sonenshine and Roe 2014) (Figure 2). The front legs contain Haller's organ, a sensory structure that allows ticks to detect environmental and host-associated cues, including temperature, humidity, host odours, and carbon dioxide (Sonenshine and Roe 2014). Once contact with a host occurs, the tick grasps the host and subsequently searches for a suitable site for attachment and blood feeding. Questing behaviour is strongly influenced by environmental conditions, particularly temperature and humidity, because ticks must balance opportunities for host contact with the risk of desiccation (Clark 1995, Vail and Smith 1998, 2002, Ogden et al. 2004). When environmental conditions become unfavourable, ticks may retreat from exposed vegetation into the litter or other protected microhabitats to conserve moisture before resuming host-seeking activity when conditions improve (Berger et al. 2014). The likelihood, duration, height, and distance of questing activity are influenced by temperature, humidity (Clark 1995, Vail and Smith 1998, 2002, Ogden et al. 2004), precipitation (Jones and Kitron 2000, Berger et al. 2014), vegetation availability (Jones and Kitron 2000), and host secretions (Carroll et al. 1995, 1996). Consequently, environmental conditions can influence when and where ticks are actively questing and, therefore, their likelihood of encountering a host.

For example, *I. scapularis* are typically seen at the tip of vegetation or within 1 cm of the tip, and the height at which *I. scapularis* climbs up vegetation varies, ranging from 40 to 87.5 cm (Goddard 1992). The questing activity temperature threshold for *I. scapularis* is 4°C (Duffy and Campbell 1994, Clark 1995), although they can quest at lower temperatures (Schulze et al. 2001a). During questing, *I. scapularis* displays both diurnal (Schulze and Jordan 2003, Schulze et al. 2001a) and nocturnal (Durden et al. 1996) activity patterns. *Ixodes scapularis* favour early daytime activity when temperatures are near or at daily minimum and relative humidity is high (Durden et al. 1996, Schulze and Jordan 2003, Dumas et al. 2022, Schulze et al. 2001a). However, when temperatures exceed 30°C, host-seeking activity changes due to increased water loss (Ogden et al. 2004, Nielebeck et al. 2023). This prompts ticks to seek shelter for rehydration

(Berger et al. 2014), and their questing activity shifts to nighttime (Durden et al. 1996). Daily and diurnal changes in temperature and humidity influence the activity and seasonality of *I. scapularis* (Vail and Smith 2002, Berger et al. 2014). *Dermacentor variabilis* uses the same strategy as *I. scapularis*, questing for a suitable host to acquire a bloodmeal (Figure 2).

The period when ticks most actively seek a bloodmeal, often called peak activity, differs by life stage and species. Seasonal adaptations occur through two mechanisms: enhancing individual resistance to adverse conditions and regulating life cycles through population adaptations (Belozerov 2009, Leal et al. 2020). These mechanisms involve both diapause and quiescence, often in combination, where the tick reduces its metabolic activity to survive harsh seasonal conditions, enabling survival and seasonal life cycle adjustments (Belozerov 2009, Leal et al. 2020). Quiescence occurs when environmental conditions are unfavourable; the tick lowers its metabolic rate until conditions improve, conserving energy and enduring temporary hardships. In contrast, diapause is a programmed period of reduced activity and development that allows ticks to synchronize their life cycle with predictable seasonal changes (Belozerov 2009). Changes in day length, potentially coupled with increasing temperatures, play an important role in terminating behavioural diapause and triggering questing behaviour in *D. variabilis* larvae (Diyes et al. 2023). Questing behaviour is subsequently affected by microhabitat variables such as ambient temperature, ground-level temperature, relative humidity, soil humidity, solar radiation, and wind (Harlan and Foster 1990).

Tick-mediated pathogen transmission primarily occurs during blood feeding, involving attachment, feeding, pathogen multiplication, and saliva secretion. Pathogens in the tick's saliva can enter the host's bloodstream, aided by bioactive compounds (Chmelař et al. 2017, Šimo et al. 2017). This can lead to infection and, depending on the tick's ability to harbour multiple pathogens simultaneously, co-infection (Chmelař et al. 2017, Šimo et al. 2017). This interaction underscores the significance of ticks as vectors for various tick-borne pathogens. The family Ixodidae contains various species of medical and veterinary importance, including *Ixodes scapularis* Say and *Dermacentor variabilis* (Say).

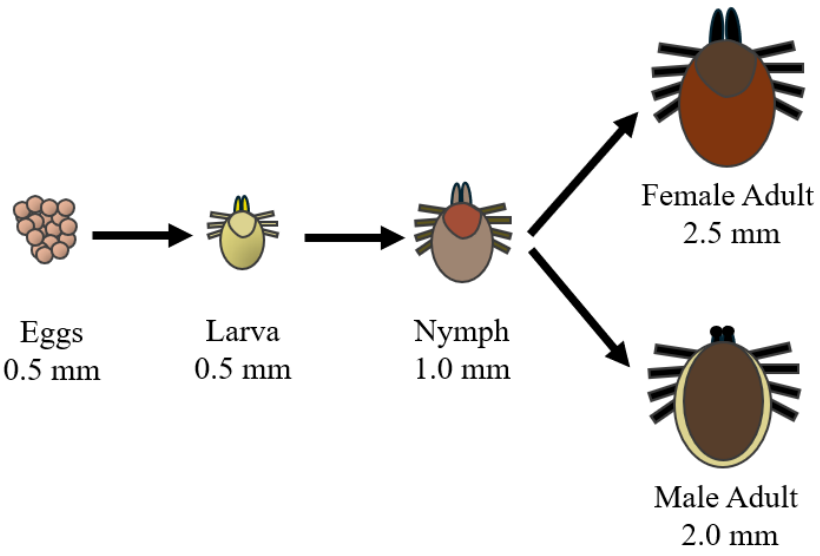

Figure 1: *Ixodes scapularis* life cycle, consisting of four life stages, three of which are active: larva, nymph, and adult (female or male).



Figure 2. Male *Dermacentor variabilis* actively questing on a blade of grass.

Ixodes scapularis Say

Life cycle

Although bloodmeals are essential for transitioning through the life cycle, *I. scapularis* do not necessarily need to feed before mating as the adults can mate directly off host (Kocan et al. 2015). If a female successfully mates, then a bloodmeal is required to complete reproduction. Females take 6-to-11 days to fully engorge (Kocan et al. 2015). The female goes through two feeding phases, slow-feeding and rapid-feeding (Lu et al. 2023). Slow-feeding occurs after the initial attachment to a host; during this phase, the female tick consumes moderate amounts of blood and can take several days (Lu et al. 2023). The bloodmeal consumed during the slow-feeding allows the female to develop new tissue to expand. As females near full engorgement, they take one last "big sip," consuming a large amount of blood over 12–24 hours in what is called the rapid-feeding phase (Lu et al. 2023). Once fully engorged, the female drops off the host and either overwinters or finds a suitable place to lay eggs (Figure 3). The female can take up to 14 days to complete egg laying, during which hundreds to thousands of eggs are deposited (Kocan et al. 2015). Male *I. scapularis* can mate more than once and sometimes stay attached to the female while they feed. After eggs hatch, each active life stage requires a bloodmeal to moult to the next stage. When larvae emerge from eggs, they require a bloodmeal to moult to the nymphal stage, and the newly moulted nymph needs a bloodmeal to moult to the adult stage (Yuval and Spielman 1990). The active life stage may overwinter, moulting the following spring (Yuval and Spielman 1990).

Overwintering survival depends on bloodmeal consumption (Lindsay et al. 1998). Larvae, nymphs and adults can typically only overwinter once without a bloodmeal, although the adults can sometimes survive overwintering without a bloodmeal twice (Lindsay et al. 1998). At northern latitudes, overwintering and feeding success affect survival rates at each stage, the peak host-seeking period, and can prolong the life cycle, which can take up to three or four years to complete (Lindsay et al. 1995, 1998, Hamer et al. 2012). Bloodmeals are essential for *I. scapularis* and are taken from a host, but to find a suitable host, *I. scapularis* must detect it.

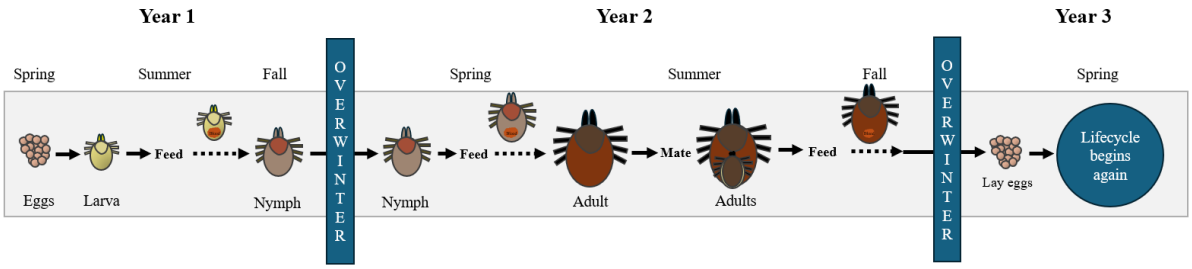

Figure 3. Life cycle of *Ixodes scapularis* in Manitoba, Canada. Dotted lines represent a successful bloodmeal.

Seasonality

All three active life stages of *I. scapularis* start host-seeking during the warm part of the year (Gatewood et al. 2009, Lindsay et al. 1999a, 1999b, Piesman et al. 1987c). In Canada, *I. scapularis* typically start seeking hosts shortly after the snow melts. Because of regional climatic differences, *I. scapularis* seasonality can vary by region. In the northeastern USA, larval peak host-seeking activity occurs in August-September, and nymph peak activity occurs in May-June (Gatewood et al. 2009, Bouchard et al. 2011, Levi et al. 2015). Whereas, in the Midwest, larvae are active from April-November (Hamer et al. 2012, Burtis et al. 2023) and nymphs are active from March-October, with both stages having a unimodal peak host-seeking activity in June (Piesman et al. 1979, 1987c, Wilson and Spielman 1985, Schulze et al. 1986, Gatewood et al. 2009, Bouchard et al. 2011, Hamer et al. 2012, Burtis et al. 2023). This synchronicity in peak host-seeking activity means larvae and nymphs can potentially feed on the same host at the same time. Adults in the Midwest are typically active from mid-March to early December, with peak host-seeking activity in April and October/November (Hamer et al. 2012, Burtis et al. 2023, Lindsay et al. 1999b).

Habitat

Ixodes scapularis are susceptible to desiccation, so they tend to favour habitats that contain dense vegetation, moist leaf litter, forests, woodlands without shrubbery, and areas with dense canopy cover (Talbot et al. 2019). These ticks are particularly fond of areas with oak (*Quercus* spp.) (Neely et al. 2021), cedar (*Thuja* spp.) and maple trees (*Acer* spp.) but tend to avoid areas with ash trees (*Fraxinus*) (Talbot et al. 2019). In open habitats such as pastures, these

ticks tend to stay near wooded edges and avoid areas with sparse vegetation (Schmidtmann et al. 1994). Adult *I. scapularis* tend to disperse more throughout pastures compared to nymphs, especially if the pasture contains large amounts of wooded areas (Carroll and Schmidtmann 1996). This may be because, once attached to a host, they tend to be associated with areas the host prefers, including open habitats (Rynkiewicz and Clay 2014). Nymphs predominantly inhabit leaf litter in forested regions (Ginsberg and Ewing 1989a) and are occasionally found near human habitation (Maupin et al. 1991). In Québec, *I. scapularis* can survive in floodplain forest regions, highlighting its ability to adapt to a variety of woodland habitats (Ogden et al. 2006b). In western regions, although *I. scapularis* can thrive in woodland areas, sparse rainfall and low humidity hinder its establishment in open prairies (Rydzewski et al. 2011, Rynkiewicz and Clay 2014, Eisen et al. 2016).

Hosts

All feeding stages of *I. scapularis* demonstrate a broad host range, parasitizing various mammals, birds and even reptiles (Main et al. 1982, James and Oliver 1990, Galbe and Oliver 1992). However, each life stage prefers hosts of a specific size. Larvae tend to feed on smaller hosts like mice and lizards (Main et al. 1982, James and Oliver 1990), while nymphs prefer small-to-medium hosts such as mice and squirrels (Main et al. 1982). In North America, the primary host that is parasitized by larvae and nymphs is the white-footed mouse (*Peromyscus leucopus* Rafinesque) (Piesman et al. 1979, Anderson and Magnarelli 1980, Main et al. 1982). In Québec, immature *I. scapularis* tend to parasitize deer mice (*Peromyscus maniculatus* Wagner), eastern chipmunks (*Tamias striatus* Linnaeus) and American red squirrels (*Tamiasciurus hudsonicus* Erxleben) (Bouchard et al. 2011). Adult *I. scapularis* prefer to parasitize a large range of medium-large hosts, including deer, humans, dogs, horses and occasionally cattle (Drummond and Medley 1965, Drummond 1967, Main et al. 1981, Chilton et al. 2019, Barnard 1981a). They tend to target mixed-breed medium- to large-sized dogs, especially those in herding and working breeds (DeWinter et al. 2023). In North America, the primary host of adult *I. scapularis* is the white-tailed deer (*Odocoileus virginianus* Zimmermann) (Piesman et al. 1979, Main et al. 1981). As deer density increases, the abundance of *I. scapularis* tends to increase as well (Rand et al. 2003).

Population Establishment

The number of hosts available in an area influences *I.* scapularis abundance, and temperature, precipitation, and relative humidity also influence the establishment of *I. scapularis* populations (Wilkinson 1967, Lindsay et al. 1995, Ogden et al. 2005, Hammond-Collins et al. 2022). Populations of ticks are considered established in an area if all three life stages, larva, nymph, and adult, are found over two consecutive years (Ogden et al. 2006b). Established populations of *I. scapularis* require three conditions for stability: a suitable climate, adequate habitat, and suitable densities of host species (Eisen et al. 2016). *Ixodes scapularis* ticks are particularly vulnerable to desiccation, so their establishment is positively influenced by temperature, precipitation, deer population density (Hammond-Collins et al. 2022), dense vegetation and forests with closed canopies (Mathisson et al. 2021). Microhabitats offering protection, humidity, and warmer temperatures, such as snow cover, leaf litter, and vegetation, contribute significantly to *I. scapularis* survival (Lindsay et al. 1998, Volk et al. 2022). Although this may vary depending on region, for example, *I. scapularis* thrive in deciduous forests in southern Ontario, Canada (Lindsay et al. 1998), while in Québec, Canada, *I. scapularis* establishment is negatively influenced by coniferous and mixed forest habitats (Hammond-Collins et al. 2022). The ability of *I. scapularis* to endure Canada's harsh winter conditions is heavily influenced by snow cover; this tick requires substantial and consistent snow cover to avoid mortality (Lindsay et al. 1995). Another factor contributing to *I. scapularis* survival in winter is acquiring a bloodmeal, with fed ticks having a higher survival rate than unfed ticks (Lindsay et al. 1998). Although *I. scapularis* is a very important vector of pathogens in Canada, *D. variabilis* is another medically important tick capable of transmitting tick-borne pathogens.

***Dermacentor variabilis* (Say)**

Life cycle

Unlike *I. scapularis, D. variabilis* adults require a bloodmeal to mate successfully. Adult *D. variabilis* females will acquire a bloodmeal from a host and will mate on the host (Garvie et al. 1978). Once mated and fully engorged, females drop off the host and find a suitable location to overwinter or lay eggs (Garvie et al. 1978). Males can acquire more than one bloodmeal and will search multiple hosts for a female to mate with. Like *I. scapularis,* male *D. variabilis* can mate more than once. Once the eggs hatch, larvae emerge and require a bloodmeal to moult into nymphs. Fed larvae will overwinter and moult into nymphs in the spring, while unfed larvae overwinter and try to find a bloodmeal the following year (Garvie et al. 1978). If unfed larvae are unsuccessful in their second quest for a bloodmeal, they are unable to overwinter a second time. After nymphs emerge, they must find a bloodmeal to moult into adults. Fed nymphs will overwinter and moult into adults the following year, while unfed nymphs will overwinter and try a second time to find a bloodmeal (Garvie et al. 1978). If unfed nymphs are not successful in their second attempt at feeding, they are able to overwinter a second time, but they cannot overwinter a third time without a bloodmeal. Once adults emerge, they must seek a bloodmeal and the entire life cycle begins again. Adult *D. variabilis* can overwinter twice without a bloodmeal but not a third time. To endure harsh Canadian winters, *D. variabilis* enters diapause and must find a sheltered location, such as leaf litter, to avoid desiccation.

Seasonality

The *D. variabilis* life cycle is influenced by the environmental conditions it encounters. Like *I. scapularis*, all active stages are active during the warm part of the year, with activity beginning in April and continuing until July, although they can be active as late as October (Dergousoff et al. 2013). In Canada, larvae become active in mid-April to mid-May, with a unimodal peak activity occurring between late May and early June (Garvie et al. 1978, Burachynsky and Galloway 1985). However, in Nova Scotia, some larvae show bimodal peak activity, with a second peak in August (Garvie et al. 1978). Larval activity declines in July and early August, with feeding typically ending around mid-September (Garvie et al. 1978). Nymphs are active from early May to early September, with a unimodal peak in June or July (Garvie et al.

1978, Burachynsky and Galloway 1985). Adults are active from late May to September, with a unimodal peak in May or June (Garvie et al. 1978) and activity typically declining in July (Garvie et al. 1978). The length of the *D. variabilis* life cycle varies with environmental conditions, typically taking two to three years to complete in northern latitudes (Garvie et al. 1978, Sonenshine 1979, Burachynsky and Galloway 1985, Lindquist et al. 2016).

Habitat

These resilient ticks tolerate desiccation better than *I. scapularis* and typically thrive in lower-humidity areas, such as open canopy fields with grasses (Mathisson et al. 2021). *Dermacentor variabilis* thrives in regions characterized by warm and humid summers (Wilkinson 1967), often inhabiting areas near rivers, lakes, and in mixed grassland, moist mixed grassland, and aspen parkland ecoregions (Dergousoff et al. 2013). These ticks are adaptable to various environments, ranging from areas with sparse grasses and shrubs to semiarid areas with dense vegetation, including grasses, shrubs, and stands of aspen trees (Dergousoff et al. 2013). Although they occur in diverse habitats, they are especially common in grassy meadows and wooded areas (Campbell and MacKay 1979). In Nova Scotia, adult *D. variabilis* are commonly found more than six inches above the ground (Dodds et al. 1969). The depth and duration of winter snow cover can greatly affect the survival of *D. variabilis* larvae and adults off-host (McEnroe 1982, 1984, 1986, Yunik et al. 2023). Snow cover acts as a refuge, providing increased humidity that benefits *D. variabilis* larvae when exposed to sub-zero temperatures (Diyes et al. 2024). Host secretions influence *D. variabilis activity* in seeking hosts and the height at which they quest (Carroll 1999).

Hosts

At every stage of development, *D. variabilis* feeds on a diverse range of hosts, with size distinguishing the stages. Larvae typically feed on small hosts, while nymphs favour small-to-medium hosts and adults prefer to feed on medium-large hosts. These stages parasitize small rodents like white-footed mice (*P. leucopus*), prairie voles (*Microtus ochrogaster* Wagner), woodland voles (*Microtus pinetorum* Le Conte) (Zimmerman et al. 1987), and shrews (*Blarina*

brevicauda Say). Adult ticks show a preference for medium- to large-sized hosts such as mammals, including dogs, skunks, raccoons, white-tailed deer, and cattle (Dodds et al. 1969, Burachynsky and Galloway 1985, Dergousoff et al. 2013, Dergousoff and Chilton 2016).

Population Establishment

Despite the challenges posed by harsh Canadian winters, both larvae and adults of *D. variabilis* can successfully overwinter and exhibit higher survival rates during winter in southern Canada compared to other regions (Diyes et al. 2021, 2024, Yunik et al. 2015b), where only a select few eggs and nymphs can withstand the harsh conditions (Garvie et al. 1978). *D. variabilis* larvae surviving winter significantly influences nymph and adult abundance in subsequent seasons (Burachynsky and Galloway 1985). Some nymphs manage to survive by feeding and moulting in late summer to early fall, likely contributing to spring adult activity (Garvie et al. 1978).

Distribution in North America

Ixodes scapularis

Ixodes scapularis occurs throughout North America, including the eastern United States and southern Canada (Spielman et al. 1979). This tick species experienced multiple establishment points in eastern North America, forming two clades, southern and northern (Oliver et al. 1993). The earliest recorded instance of *I. scapularis* in Canada dates back to the early 1900s in Ontario (Nuttall and Warburton 1911). Shortly after, *I. scapularis* were recorded in the United States, in the late 1920s in Massachusetts (Larrousse et al. 1928). Consequently, from 1979 to 1992, researchers believed *I. scapularis* (south) and *Ixodes dammini* (north) Spielman, Clifford, Piesman & Corwin were two distinct species. However, hybridization and assortative mating experiments in 1993 revealed that these two species were conspecific (Oliver et al. 1993). This confirmation was further supported by chromosomal studies (Chen et al. 1994), and the scientific name *I. scapularis* took priority (Oliver et al. 1993), and the species was redescribed (Keirans et al. (1996). Despite this nomenclature shift, researchers noted genetic

differences between northern and southern populations of *I. scapularis* (Norris et al. 1996, Xu et al. 2020). The southern population is sparse, stable, and rarely infected with *B. burgdorferi*, whereas the northern population is abundant, expanding, and susceptible to infection (Xu et al. 2020).

Although *I. scapularis* was first recorded in Canada in 1911, the first established population emerged in Long Point, Ontario, in the early 1970s (Nuttall and Warburton 1911, Watson and Anderson 1976). Tick populations are classified into three categories: established, reported (adventitious) or no records (not present) (Canada Health 1991, Dennis et al. 1998). Researchers can determine whether a tick population is established in several ways. Previously, the presence of a minimum of six individual ticks or two of the three active life stages within one sampling year was considered indicative (Dennis et al. 1998). However, since 1991, the criteria shifted to requiring the presence of all three life stages, larva, nymph, and adult, over two consecutive years (Canada Health 1991). Since the 1970s, *I. scapularis* has expanded its range and is now established in southern Manitoba, Ontario, Québec, New Brunswick, and Nova Scotia (Bell et al. 1992, Ogden et al. 2009, 2006c, Koffi et al. 2012). The establishment of *I. scapularis* populations in these southeastern parts of Canada has been attributed to the dispersal of animal hosts to areas with more favourable environmental conditions (Ogden et al. 2008, 2009, Koffi et al. 2012). Unfortunately, this range expansion is expected to persist with the anticipated rise in average annual temperatures (Ogden et al. 2006a). *Ixodes scapularis* has been a concern in southern Canada, but as its distribution extends north and west, Canadians in more northern latitudes now face the risk of encountering this tick and acquiring a tick-borne pathogen.

Dermacentor variabilis

Dermacentor variabilis, commonly known as the American dog tick or wood tick, has been found throughout North America since at least the 1900s. This tick occurs across much of the eastern United States, extending south into Mexico and north into southeastern Saskatchewan, Manitoba, and Ontario (Bishopp and Trembley 1945, Wilkinson 1967, Merten and Durden 2000, Dergousoff et al. 2013). Recent data show that this species is distributed more than 300km further west in Saskatchewan than previously noted (Wilkinson 1967, Dergousoff et

al. 2013, 2025). A geographically isolated population of *D. variabilis* also occurs in Nova Scotia (Dodds et al. 1969), established around the 1900s and likely a result of an infested dog being transported from the northeastern United States (Hall and McKiel 1961). This might also be due to the movement of infested domestic animals, which has been said to be a principal means of spreading ticks (Dodds et al. 1969).

Pathogens

Ixodes scapularis

Ixodes scapularis carries and transmits a variety of tick-borne pathogens, including bacteria, viruses, and parasites. In North America, this tick is an important vector for several pathogens, notably *Borrelia burgdorferi* Johnson et al. (Burgdorfer et al. 1982, Piesman et al. 1987a), the bacterium responsible for Lyme disease; *Anaplasma phagocytophilum* (Foggie) (Telford et al. 1996), the bacterium that causes anaplasmosis; *Babesia* spp., protozoan parasites associated with babesiosis, specifically *Babesia microti* (Spielman et al. 1979, Piesman and Spielman 1980) and *Babesia odocoilei* (Waldrup et al. 1990); and Powassan virus (Costero and Grayson 1996), which leads to Powassan disease.

Borrelia burgdorferi

In North America, the genospecies *B. burgdorferi* sensu stricto is the most frequently documented vector-borne pathogen (Gasmi et al. 2017). By 1989, Lyme disease cases had been reported throughout Canada, except for Prince Edward Island, Nova Scotia, Saskatchewan, and the Territories (Mackenzie 1990). However, as of 2024, Lyme disease has been reported in all the provinces, excluding the territories (Public Health Agency of Canada 2023). Lyme disease became a nationally reportable disease in Canada in 2009, meaning provincial and territorial health personnel must report cases to the Canadian Notifiable Disease Surveillance System (CNDSS) of the Public Health Agency of Canada (PHAC) (Gasmi et al. 2022). In Canada, two tick species transmit Lyme disease: *I. scapularis* (Burgdorfer et al. 1982), which transmits the disease in central and eastern regions, and *Ixodes pacificus* Cooley & Kohls, which transmits the disease in western regions (Ogden et al. 2014b). In central and eastern Canada, Lyme disease is

predominantly transmitted by *I. scapularis* nymphs (Spielman et al. 1985, Falco et al. 1996, 1999, Piesman et al. 1987b). Although nymphs are the primary vector of Lyme disease, *B. burgdorferi* is commonly found in adult *I. scapularis*, moderately present in nymphs, and seldom detected in larvae across Canada (Lindsay et al. 1991). In *I. scapularis*, *B. burgdorferi* is passed from moult to moult, a process called transstadial transmission (Burgdorfer and Gage 1986); it is not transmitted transovarially (Rollend et al. 2013a). Lyme disease progresses through multiple stages and presents as a systemic infection (Hatchette et al. 2014). *Ixodes scapularis* can transmit *B. burgdorferi* to a host within 24-72 h of attachment, and the risk of contracting the bacteria is positively correlated with tick attachment time (Piesman et al. 1987a). During the initial stage, an erythema migrans lesion, an expanding red bullseye rash, can appear as quickly as 8-9 days (Berger 1993), or within 30 days of infection, appearing at the site of the tick bite in around 70% of those affected (Bacon et al. 2008). The rash may occur alongside flu-like symptoms, such as fever, fatigue, headache, myalgia, or arthralgia (Hatchette et al. 2014). Within three months without treatment, *B. burgdorferi* can spread throughout the entire bloodstream (Hatchette et al. 2014). Symptoms of early disseminated Lyme disease encompass various secondary erythema migrans lesions, neurological manifestations, and cardiac symptoms, which, albeit rarely, can lead to fatal outcomes (Centers for Disease Control and Prevention (CDC) 2013). If left untreated, early disseminated Lyme disease can evolve into the late disseminated stage, with arthritis emerging as the predominant manifestation (Bacon et al. 2008).

Borrelia burgorderi can infect horses (Divers et al. 2018) and has been reported in Canadian horses (Burgess 1988). The main reservoirs for *B. burgdorferi* are the white-footed mouse (*P. leucopus*) and white-tailed deer (*Odocoileus virginianus* Zimmermann) (Bosler et al. 1984), although it has been identified in a range of mammalian hosts, including meadow vole, Eastern chipmunks, raccoons, humans, and dogs (Anderson 1988). There is an increased risk of *I. scapularis* attachment to horses in areas with oak trees (Neely et al. 2021), possibly leading to *B. burgdorferi* transmission due to the presence of reservoir hosts attracted to acorns (Watson and Anderson 1976). The peak risk of *I. scapularis* transmitting Lyme disease occurs during late spring-to-early summers months, particularly in May and June (Piesman et al. 1987c). Although recently there has been cases occurring from May to November, with multiple cases reporting symptoms in July (Public Health Agency of Canada 2023). The survival rate of *I. scapularis* in

different temperature-relative humidity situations increases when the individual is infected with *B. burgdorferi* (Nabbout et al. 2023).

Anaplasma phagocytophilum

Anaplasma phagocytophilum, a bacterium responsible for granulocytic anaplasmosis (Chen et al. 1994), is commonly vectored by *I. scapularis* in Canada (Krakowetz et al. 2014). This bacterium was once thought to be three separate species, *Ehrlichia phagocytophila*, *Ehrlichia equi*, and the human granulocytic ehrlichiosis (HGE) agent (Dumler et al. 2001), but is now recognized as one species, *A. phagocytophilum*. In Canada, the first locally acquired case of granulocytic anaplasmosis was reported in Alberta in 2009 (Parkins et al. 2009). In Manitoba, Anaplasmosis became provincially notifiable in 2015, emphasizing the need for heightened awareness about this disease and its potential consequences (Government of Manitoba 2016). *Ixodes scapularis* can transmit two strains of *A. phagocytophilum*, Ap-ha and Ap-variant 1 (Massung et al. 2002, Krakowetz et al. 2014). The Ap-ha variant causes infection in humans, while the Ap-variant 1 does not (Massung et al. 2002, Krakowetz et al. 2014). Geographically, strain prevalence varies, with a higher proportion of ticks carrying Ap-ha in the Prairie and Atlantic provinces than in the Central provinces. Conversely, the Central provinces show a higher prevalence of Ap-variant 1(Krakowetz et al. 2014). *A. phagocytophilum*, can cause disease in people, dogs, and horses (Woldehiwet 2010, Uehlinger et al. 2011, Krakowetz et al. 2014). In horses, *A. phagocytophilum* can cause granulocytic anaplasmosis and has been reported in Canadian horses (Berrington et al. 1996, Uehlinger et al. 2011, Burgess et al. 2012). The white-footed mouse, *P. leucopus*, is the main reservoir of this bacterium, although a wide range of wild and domestic mammals can carry it (James et al. 2019). *Anaplasma phagocytophilum* is passed by transstadial transmission in *I. scapularis* (Kocan et al. 2012). *Ixodes scapularis* can transmit *A. phagocytophilum* to hosts within 24-72 h of attachment, with risk of acquiring the bacteria increasing as tick attachment time increases (Levin et al. 2021). Individuals with *A. phagocytophilum* experience a severe febrile reaction, accompanied by bacteremia and a reduction in white blood cell count, lymphocytes, and platelets within about a week of being bitten by an infected tick (Woldehiwet 2010). This lasts for several days, compromising the

immune system and rendering both humans and animals more susceptible to secondary infections (Woldehiwet 2010).

Babesia spp.

Babesiosis is a tick-borne disease caused by parasites of the genus *Babesia* that infect the host's red blood cells. In North America, *Babesia microti* (França) causes human babesiosis in humans, and *Babesia odocoilei* Emerson and Wright causes cervid babesiosis, which affects bovids and cervids, such as white-tailed deer, elk (*Cervus canadensis*) and caribou (*Rangifer tarandus caribou*) (Holman et al. 2000). The first locally acquired report of *B. microti* in Canada was in 2013 in Winnipeg (Bullard et al. 2014). In Manitoba, babesiosis became a provincially notifiable disease in 2015 (Government of Manitoba 2016), underscoring its importance because it can be acquired through tick bites, blood transfusion (Kain et al. 2001), or organ transplantation (Brennan et al. 2016). *Babesia microti* (Spielman et al. 1985, Rollend et al. 2013b) and *B. odocoilei* (Waldrup et al. 1990, Armstrong et al. 1998) are harboured and transmitted by *I. scapularis*. *B. microti* develops in the salivary gland cells of *I. scapularis* (Karakashian et al. 1983) and can be transmitted through the saliva to a host. *Babesia microti* in *I. scapularis* is transmitted only by transstadial transmission, passed from moult to moult once infected (Spielman et al. 1985). Quantitative PCR can easily distinguish *Babesia microti* and *B. odocoilei* species in *I. scapularis* and humans (Rollend et al. 2013b). White-footed mice are reservoir hosts for *B. microti* and are commonly transmitted by *I. scapularis* (Spielman et al. 1979, 1981, 1985). *Ixodes scapularis* is a competent vector for *B. odocoilei* and this parasite can be transmitted through transstadial transmission (Waldrup et al. 1990, Zembsch et al. 2021b) and transovarial transmission (Milnes et al. 2019). Cervid babesiosis was identified in established populations of *I. scapularis* in southern Ontario in 2019 (Milnes et al. 2019). These researchers also stated that migratory birds harboured these infected *I. scapularis*, suggesting the disease could be moved by avian migration (Milnes et al. 2019). A study in Massachusetts concluded that the highest risk of *I. scapularis* transmitting human babesiosis occurs in late spring and early summer, in May and June (Piesman et al. 1987b). These parasites can co-exist within *I. scapularis*, also known as co-infection, although *B. odocoilei* has been more prevalent than *B. microti* in the USA (Armstrong et al. 1998).

Each of these tick-borne pathogens warrants concern; however, simultaneous infection of *I. scapularis* with multiple pathogens complicates disease identification in an infected host and poses increasing challenges (Schulze et al. 2013). For example, *I. scapularis* can become co-infected with *B. burgdorferi*, *A. phagocytophilum*, *B. microti*, and/or *B. odocoilei* (Armstrong et al. 1998, Holman et al. 2004, Schulze et al. 2013, Hutchinson et al. 2015, Piesman et al. 1987a, Zembsch et al. 2021a). Co-infection can alter disease presentation in hosts and make the disease difficult to diagnose and treat. Raising concerns about co-infections can promote awareness among healthcare professionals and the public, leading to earlier diagnosis and improved treatment of tick-borne pathogens.

Dermacentor variabilis

Dermacentor variabilis can also transmit tick-borne diseases, including Rocky Mountain spotted fever caused by the bacterium *Rickettsia rickettsii* Brumpt (Burgdorfer 1975), tularemia caused by the bacterium *Francisella tularensis* (Dorofe'ev) (Goethert and Telford 2010), and bovine anaplasmosis caused by the bacterium *Anaplasma marginale* Theiler (Lankester et al. 2007, Kocan et al. 2010). In Canada, *D. variabilis* is the primary carrier of medically significant spotted fever group rickettsiae (Wood and Artsob, 2012). This includes *Rickettsia rickettsia*, responsible for Rocky Mountain spotted fever, and *Rickettsia montanensis*, among other *Rickettsia* species (Wood and Artsob, 2012). In North America, *D. variabilis* is responsible for transmitting economically significant pathogens affecting ruminants, such as strains of *A. marginale* (Aubry and Geale 2011), and *Anaplasma ovis*, responsible for bovine and ovine anaplasmosis, respectively (de la Fuente et al. 2008, Kocan et al. 2010). While *R. rickettsii* and *F. tularensis* were previously identified in *D. variabilis* ticks in southern Canada before 1990 (Humphreys and Campbell 1947, Artsob et al. 1984, Lindquist et al. 2016), recent studies have found no evidence of their current presence in *D. variabilis* in Saskatchewan, Manitoba or western Ontario (Dergousoff et al. 2009, Dergousoff and Chilton 2012, 2016, Yunik et al. 2015a). Reports of Rocky Mountain spotted fever and tularemia cases in Canada have been limited (Lindquist et al. 2016). In contrast, southern Canada has experienced several outbreaks of bovine anaplasmosis since 1968 (Howden et al. 2010). Before 2014, cases of bovine anaplasmosis were controlled through culling infected animals (Howden et al. 2010). However,

the discontinuation of the Canadian national surveillance and testing program for this disease in 2014 has heightened the risk of *A. marginale* reintroduction and establishment in Canadian cattle herds, potentially leading to significant economic impacts. Recent outbreaks in Manitoba (Duizer 2021) support this reintroduction risk. Suburbanization and farmlands converting back to woodier habitats have been linked to increased transmission of pathogens by *D. variabilis* (Mount and Haile, 1989).

Tick sampling methods

Currently, tick surveillance is conducted through three primary methods: passive monitoring using specimens or records provided voluntarily by healthcare professionals or the public; active field collection of ticks actively seeking hosts at a local level; or active field collection of host-seeking ticks alongside trapping and examination of vertebrate hosts (Ogden et al. 2014a). Designing experiments demands a meticulous choice of sampling methods, mindful of the target species as well as the timing and location of collections (Mays et al. 2016). When targeting multiple tick species, a combination of methods becomes essential to ensure representative samples that capture all present species (Rynkiewicz and Clay 2014). This is especially crucial in studies examining species diversity and relative abundance (Mays et al. 2016). Failing to account for variation in species and life stages collected with a particular method can bias relative abundance estimates when comparing multiple tick species (Schulze et al. 1997). This emphasizes the need to use the most appropriate sampling method based on the tick species and life stage of interest for a research project. Effective trapping techniques are crucial for accurately estimating and comparing tick presence and abundance, as well as for monitoring pathogen presence and prevalence. Practicality is also important to consider. Typically, ticks are sampled either during questing or while attached to hosts. *I. scapularis* typically inhabits wooded and brushy areas, and field surveillance sites should be chosen accordingly (Lindsay et al. 1998, Schulze and Jordan 2005, Ogden et al. 2006b).

Ticks in environment

Host-seeking ticks can be collected from the environment through many different sampling methods, including dragging, flagging, walking or CO_2-baited traps (Falco and Fish 1992, Schulze et al. 1997, Gherman et al. 2012). Specific sampling techniques vary greatly among research groups based on the research question or surveillance goal (Falco and Fish 1992, Schulze et al. 1997). Each method can be more effective or reliable for a different species and for different life stages, and each comes with its own biases. Two of the most employed techniques for sampling questing ticks include flagging and dragging (Sonenshine 1993). Both drag sampling and flagging use comparable techniques to collect host-seeking ticks (Daniels and Fish 1990, Carroll and Schmidtmann 1992, Falco and Fish 1992). For example, researchers found that drag sampling is the most dependable technique for measuring the density of host-seeking (infected) *I. scapularis* nymphs (Falco and Fish 1992). The Centers for Disease Control and Prevention (CDC) states that dragging is best practice for collecting *Ixodes* species (Centers for Disease Control and Prevention (CDC) 2019). For collecting non-*Ixodes* hard ticks, the CDC recommends either dragging or flagging (Centers for Disease Control and Prevention (CDC) 2020). In Italy, a study found that tick collection efficiency depended on species, life stage, habitat, and seasonal timing, with flagging generally most effective for collecting adult specimens (Dantas-Torres et al. 2013). When using dragging or flagging for assessing host-seeking tick density, it is important to sample a large area ($\geq$750 m^2), check the fabric frequently before ticks dislodge (usually within $\leq$15 m), and sample on two or more occasions during the estimated seasonal peak of the target life stage (Centers for Disease Control and Prevention (CDC) 2019, Salomon et al. 2020). Most surveillance guidelines applicable to *I. scapularis* can be adapted for other medically significant ixodid ticks, and additional guidance is available for species such as *Amblyomma*, *Dermacentor*, and *Rhipicephalus*. In permissive *I. scapularis* habitat, local tick questing activity, measured using flag and drag samples, is primarily influenced by vegetation variables, particularly canopy cover (Ginsberg et al. 2020).

Dragging

The most common passive surveillance technique used to collect host-seeking ticks is dragging. Dragging consists of using a 1 x 1 m white flannel cloth that is attached to two wooden

dowels and is dragged behind the sampler with a rope for a set distance (Figure 4) (Salomon et al. 2020, Barnard 1981b).

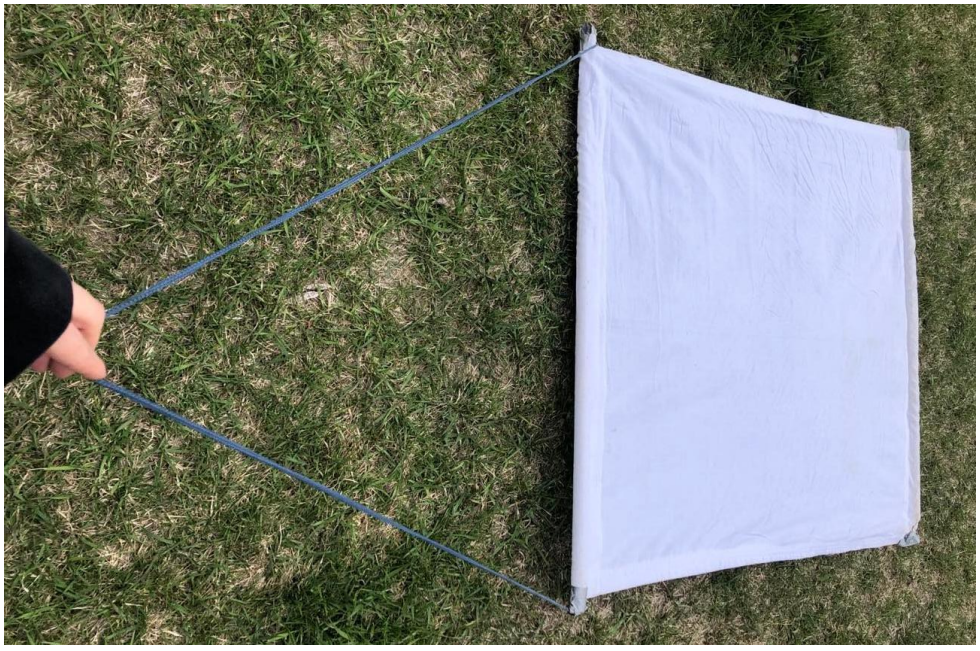

Figure 4. A tick drag, 1 x 1 m white flannel cloth attached to two wooden dowels and dragged by the sampler using a rope attached to one of the dowels.

Other fabrics such as canvas and corduroy have been tested, but flannel cloth is the best for collecting all life stages (Simmons et al. 2021). Flannel makes it easier for ticks to climb onto the drag and stay secured, especially in rough sampling areas (Simmons et al. 2021). Drags are used to sample low vegetation for ticks, and quadrats can be used to determine the abundance of ticks in a sampled area (Barnard 1981b). This technique can easily be conducted in areas with leaf litter and low vegetation (Ginsberg and Ewing 1989b). Dragging is effective for gauging potential contact between ticks and humans because of its high sensitivity (Falco and Fish 1992, Mather et al. 1996). Dragging provides more quantitative results than flagging, as the area dragged can be determined by multiplying the drag width by the distance sampled (number of quadrats) (Rochon et al. 2012). However, dragging may introduce biases, especially differences in sampling techniques among samplers or hesitation to sample areas with thick vegetation to avoid ripping the drag (Ginsberg and Ewing 1989b). Tick presence can vary significantly, so tick dragging should cover a representative area at each study site (Dobson 2013, Schulze et al.

2001b). Researchers have tested varying quadrat sizes to determine the most efficient sample size, including 10-50 m quadrats, and they determined ticks were less likely to drop from the drag if samplers checked every 10 m (Schulze and Jordan 2001, Rochon et al. 2012). Especially in dense vegetation, inspecting the drag every 10 m significantly reduces the number of ticks lost to the vegetation, although in sparse vegetation, checking every 20 m is sufficient (Schulze and Jordan 2001). For dragging, choosing different features at each visit can help represent the entire field rather than only certain areas. Randomizing transects can help mitigate this bias. Although habitat type should be considered when establishing dragging transects, it is also important to note the time at which they are collected. Sampling during 0600 – 0900 hours resulted in the highest number of *I. scapularis* collected, while 1000 – 1300 collected a moderate number of ticks (Schulze et al. 2001a). *D. variabilis* can be collected using more than one method, including dragging, CO_2 and rodent surveys (Rynkiewicz and Clay, 2014). Flagging may work better in dense undergrowth, as it can reach leaf litter, while dragging could be hindered by shrubs (Sonenshine 1993). Conversely, dragging might be more effective in open areas because it has broader surface contact with tick habitat (Sonenshine 1993). In areas with no obstacles, drags might accumulate more ticks than flags (Rulison et al. 2013). This may be because drags, with their larger collecting surface area, contact more leaf litter, even when both methods use materials of the same size (Rulison et al. 2013). While this method is considered the 'gold standard', its implementation demands considerable time and labour, making it unfeasible for large-scale field sampling (Koffi et al. 2012, Ogden et al. 2014a). The dragging method is straightforward and provides results that align with research standards (Mays et al. 2016). It's also more economical than dry ice trapping and requires less maintenance and lower costs than CO_2 dragging (Mays et al. 2016).

Flagging

Flagging typically uses a 90 x 90 cm white cotton cloth attached to a wooden dowel, which is swept side to side (Wilkinson and Gregson 1985), although flag size can vary depending on the research objective or whether the area being sampled is large. Flags are optimal for sampling shrubby vegetation, including areas where drag sampling cannot be accomplished (Lysyk and Moon 1994). Researchers found that stratified random sampling while flagging

yielded more representative quadrats than using simpler fixed quadrats (Wilkinson and Gregson 1985). With flagging, it is also beneficial to sample different areas of the site to randomize and show an overall picture. Flagging is better suited for sampling immature ticks than dragging, which is more effective for adult ticks. Flagging is effective for estimating the relative densities of questing ticks (Ginsberg and Ewing 1989b). However, the proportion of the ticks engaging in questing behaviour can vary significantly between sites with differing abundances of vertebrate tick hosts (Ginsberg and Zhioua 1999).

Walking

Tick sampling by walking involves a sampler wearing light-coloured clothing with pants tucked into socks, then checking the clothing for ticks after a set distance and collecting them (Carey et al. 1980, Schulze et al. 1986, Ginsberg and Ewing 1989b). Light-coloured clothing is essential for this technique, as it makes ticks easier to see, especially nymphs, which can be harder to see with the naked eye. This technique has been noted to be very effective for collecting adult *I. scapularis* (Ginsberg and Ewing 1989b), but it is not efficient for collecting immature *I. scapularis,* as they dwell closer to the ground and are more often found in leaf litter (Ginsberg and Ewing 1989b). Researchers have found that walking surveys collected more *I. scapularis* adults than dragging (Schulze et al. 1997). This method may offer greater precision in evaluating human-tick interactions than drag sampling, flagging, host collection, or traps baited with carbon dioxide (Centers for Disease Control and Prevention (CDC) 2019). However, its effectiveness is particularly notable in areas with growing vegetation facilitating tick movement rather than in leaf litter, where human exposure to ticks often relates to behaviours directly exposing legs or hands/arms to the ground (Centers for Disease Control and Prevention (CDC) 2019). Combining both walking and dragging surveys would be the most efficient and effective way to sample an area, allowing the sampler to potentially retrieve more and be tick-safe at the same time, as they are removing the tick before it has a chance to find a suitable spot to feed.

CO_2 Traps

Although dragging, flagging, and walking surveys can be useful for collecting host-seeking ticks from the environment, these techniques can be time-consuming for the sampler. Another alternative for tick collection includes carbon dioxide (CO_2) traps. Dry ice can attract and trap ticks, since it evaporates into CO_2 gas. These traps are known as dry ice baited traps and can be labour-effective, as the sampler only needs to collect trapped ticks from the trap. CO_2 can also be used with drags and flags by pumping CO_2 through the fabric as the researcher samples (Mays et al. 2016). CO_2-baited traps are useful for collecting ticks attracted to and influenced by CO_2 emissions. They are especially useful for collecting *Amblyomma americanum* Linnaeus, a tick known for using a 'hunting' questing strategy in which it "runs" towards a host that it senses (Falco and Fish 1991, Ginsberg and Ewing 1989b). are useful in collecting ticks such as *Amblyomma americanum* Linnaeus, *I. scapularis* demonstrate a decreased response, suggesting that this method varies in effectiveness depending on the species of interest (Falco and Fish 1991, Ginsberg and Ewing 1989b). This was attributed to the lower mobility and less aggressive host-seeking behaviour of *I. scapularis compared* with *A. americanum* (Falco and Fish 1991). In contrast, *D. variabilis* collection using CO_2 has been successful (Mays et al. 2016). For example, dragging and CO_2-baited traps collect similar amounts of *D. variabilis*, especially in woodland or grassland habitats (Petry et al. 2010). CO_2-based methods (dragging and flagging), while generally comparable to conventional ones, can present challenges (Mays et al. 2016). Carrying a CO_2 tank adds weight and bulk, hindering sampling in dense vegetation or rough terrain; ensuring proper gas flow through tubing demands extra attention, and exposed hoses need protection from vegetation to prevent damage (Mays et al. 2016). Furthermore, CO_2-based methods often cost more than traditional approaches (Mays et al. 2016). Obtaining and transporting the required quantity of dry ice for extensive trapping endeavours can pose significant difficulties and expenses (Mays et al. 2016).

Ticks on hosts

Ticks can also be sampled directly from hosts, such as cattle, horses, humans and many other animals. There are different techniques that can be used to collect ticks from hosts, among them are capturing a host and collecting ticks that are attached, employing a designated nest box

and then collecting the ticks that drop off the host there, or by suspending animal skins over water to collect ticks as they fall (Sonenshine 1993, Wilson 1994). When directly examining a captured host for ticks, the sampler can determine whether ticks are present and can also determine infestation levels by counting the number of ticks on hosts at a given time (Lysyk and Moon 1994). Accurate assessment of tick numbers on hosts not typically handled day to day, such as pasture-grazed cattle, can be done by restraining them in a squeeze chute (Lysyk and Moon 1994). It has been hypothesized that sampling cows through direct tick sampling such as 'scratching' would be less laborious than a sampler sampling the pasture (Wilkinson and Gregson 1985). They noted that although round-up and examination of the cow could be disadvantageous, the ability of the cow to cover lots of land may result in more efficient tick collection (Wilkinson and Gregson 1985). Lindsay et al, (1999b) reported that adult *I. scapularis* collected from white-tailed deer in Ontario, Canada, were mostly found on the neck, front, and head areas of the animal. Another researcher found that female *I. scapularis* on cattle are often attached to the head, ears, neck, dewlap, or shoulders (Barnard 1981a). This researcher also noted that when male *I. scapularis* were found on cattle, they were often found wandering around the head, dewlap, and back-end area (Barnard 1981a). They suggested that these ticks are niche in their attachment sites on cattle (Barnard 1981a). In contrast, in Oklahoma, researchers found that *I. scapularis* were commonly collected from the chest and front axillary region of horses (Sundstrom et al. 2021). This suggests *I. scapularis* may prefer areas of the host that are near the front of the animal; this may be due to the herbivorous habits of the hosts and the inability to groom these areas.

Voluntary submissions

Dragging and scratching are great examples of tick collection methods, but they can be time-consuming and costly. Another collection method is voluntary submissions, also known as passive surveillance. Voluntary submissions can be useful as they are inexpensive, produce large amounts of data and can be more representative. These submissions involve collecting information and specimens from members of the public who collect them themselves. Researchers can use or make a form to collect important data such as date, location, host, and number of ticks collected. Public tick submissions may have limitations, such as submission

fatigue over time, labour-intensive work, under-reporting, and the need for identification education. Passive surveillance offers significant advantages to public health as it requires minimal time and financial resources, in contrast to active surveillance methods (Johnson et al. 2004). Passive surveillance can identify adventitious ticks but may inaccurately pinpoint areas of breeding tick populations (Ogden et al. 2006c). This is especially likely when using ticks obtained from dogs (Ogden et al. 2006c), although data from passive surveillance can forecast the emergence of regions at risk for Lyme disease (Gasmi et al. 2019). The limitations of tick passive surveillance include the risk of inaccurate or insufficient data due to underreporting or misclassification of ticks, which can create gaps in understanding tick distribution and disease risk. Furthermore, relying solely on passive surveillance may overlook specific regions or populations, leading to incomplete or biased assessments of tick-borne disease risk. Passive surveillance is often criticized for its limited sensitivity because it detects only a small portion of tick bites on humans and lacks the precision to pinpoint the exact location of a known tick population (Johnson et al. 2004, Koffi et al. 2012).

Spatial data and ticks

GIS integrates spatial data of three distinct types: points, lines, and polygons, each accompanied by their associated coverage attributes. Spatial data can be recorded for tick abundance, vegetation association and host interaction. These data can be used to map and statistically analyze relationships between two features. Such features can include tick distance to different habitat types, cow distance to different habitat types, etc. Global Positioning Satellites (GPS) can record samplers' locations in sampling areas while they sample for ticks. Typically, a handheld GPS is the most efficient and cheap method of recording positions. For instance, Diuk-Wasser et al. (2006) utilized GIS and park trail maps to designate random points along trails for tick dragging. GIS can overlay numerous geographic factors (coverages) and correlate them with the spread of a disease vector, providing a vital tool for epidemiological research on spatial disease patterns and their temporal fluctuations (Kitron et al. 1991).Tick abundance can be geospatially recorded using handheld GPS units. Using a geographic information system (GIS) allows researchers to compare tick locality and vegetation to determine whether features correlate (Glass et al. 1994).

Vegetation association

Vegetation types can also be geospatially recorded using handheld GPS units or with databases provided by the government. A database that can be used in Canada to show different vegetation types and crops is the Annual Crop Inventory provided by the Government of Canada (Government of Canada 2021, 2022). Temperature and precipitation can also be used, for example Environment Canada provides hourly and daily weather forecasts for areas across Canada (Government of Canada 2026). By combining GIS and statistical analysis, researchers demonstrated that *I. scapularis* abundance correlates with distance to deciduous forests, water bodies, elevation, land cover, and soil type, and that areas with sandy soils, lower elevations, and moderate distances to forests and water are positively correlated with *I. scapularis* encounters (Bunnell et al. 2003). They also noted other environmental factors negatively correlated with these ticks, such as wetlands, increasing distance to water, higher elevations, siliceous soils, and skeletal and fine-loamy soil types (Bunnell et al. 2003). Limitations in accurately determining pathogen prevalence in tick populations include the mobility of people, pets, and wildlife, as well as the potential unreliability of exact tick collection locations (Pasternak and Palli 2022).

Host interaction

Host interaction with ticks can also provide important information. GIS can analyze spatial relationships between hosts and tick habitats, predicting areas of high abundance based on environmental factors. By integrating host distribution data and tick habitat, GIS can help identify hotspots of tick activity, informing targeted interventions and public health strategies for managing tick-borne disease. Some animals such as cattle or deer can also be geospatially recorded using various GPS devices, such as collars. Global navigation satellite system (GNSS) collars are devices equipped with a global positioning system (GPS) and are often used in research and wildlife tracking. These collars can accurately track the movements and locations of animals. Researchers found that GNSS collars on cattle do not affect their behaviour, specifically in Charolais cattle (Manning et al. 2017).

Based on the published literature, I predict that *I. scapularis* will be more abundant in pasture areas with greater tree cover and increased shading, whereas *D. variabilis* will be more

abundant in areas with lower canopy cover and higher sun exposure. These predictions are based on microclimatic differences, such as temperature and humidity, associated with vegetation structure. By quantifying tick abundance across landscape features, this study evaluates how habitat characteristics may structure tick distributions within pasture systems. These landscape-level patterns may, in turn, affect host exposure risk, although this study did not directly measure host movement and behaviour.

CHAPTER 3: ASSESSMENT OF TICK ABUNDANCE, *IXODES SCAPULARIS* AND *DERMACENTOR VARIABILIS*, AND PATHOGEN PRESENCE IN MANITOBA PASTURES: IMPLICATIONS FOR LIVESTOCK AND HUMAN HEALTH.

Abstract

This two-year study conducted in Manitoba assessed the abundance and distribution of *Ixodes scapularis* and *Dermacentor variabilis* in cattle and horse pasture systems, their occurrence on livestock, and the presence of associated pathogens. Seven study sites across the Parkland, Westman, and Pembina Valley regions were sampled using tick dragging and host inspections, supplemented by opportunistic collections from horses, humans, and dogs, while geospatial analyses evaluated tick distributions and habitat associations. A total of 1,946 ticks were collected, with *D. variabilis* as the dominant species (n = 1,872), detected at all seven sites, whereas *I. scapularis* was less abundant (n = 70) and occurred primarily at Dusty Oaks. Habitat associations for *D. variabilis* were site-specific, with tick-positive quadrats showing significant relationships with combinations of grassland, forest, shrubland, wetland, water, and other habitat types; at Dusty Oaks, *I. scapularis* was associated with forest across both habitat-distance comparisons. Molecular screening detected *Borrelia burgdorferi*, *Babesia odocoilei*, and *Anaplasma phagocytophilum* in *I. scapularis*, while all *D. variabilis* tested negative for *Anaplasma marginale*. The high abundance and widespread distribution of *D. variabilis*, combined with pathogen-infected *I. scapularis* and substantial tick burdens on horses at Dusty Oaks, demonstrate that pasture environments can support diverse tick populations and highlight the importance of continued surveillance of ticks and associated pathogens in Manitoba livestock systems.

Introduction

In Manitoba, two tick species are known for transmitting tick-borne pathogens to a variety of hosts: the blacklegged tick, *Ixodes scapularis* Say, a recent addition to the Manitoba tick fauna (Galloway 1989) and the American dog tick, *Dermacentor variabilis* (Say), a tick species collected in Manitoba for over 100 years (Hewitt 1915). Both species belong to the family Ixodidae (Ixodida: Ixodiade), and are three-host ticks, meaning they typically obtain bloodmeals from three different hosts throughout their life cycle. This method of blood feeding can lead to the transmission of tick-borne pathogens. *I. scapularis* is a competent vector for multiple pathogens, including *Borrelia burgdorferi*, the bacterium that causes Lyme disease (Burgdorfer et al. 1982, Piesman et al. 1987a) and *Anaplasma phagocytophilum*, the bacterium that causes granulocytic anaplasmosis (Telford et al. 1996), which can affect horses and humans (Woldehiwet 2010, Uehlinger et al. 2011, Krakowetz et al. 2014). *Dermacentor variabilis* is known for transmitting *Anaplasma marginale,* the bacterium that causes bovine anaplasmosis in cattle and bison. The peak activity of these two species differs in that adult *I. scapularis* are highly active in spring and fall, and *D. variabilis* are very active in mid-summer. The hardy *D. variabilis* are tolerant to desiccation, and because of this they are mostly found in lower humidity and spacious vegetation such as open grassland fields. *I. scapularis,* on the other hand, are very intolerant to desiccation (Lindsay et al. 1995, 1999b), meaning they tend to be found in forested areas with more humidity (Ginsberg et al. 2020, Ogden et al. 2006b). Manitoba pastures often contain both open grasslands and forested areas, and animals grazing those pastures, such as horses and cattle, may be exposed to ticks in both environments. The distribution range of both tick species has expanded north and west into the Canadian Prairies, a region known for crop farming and beef cattle operations. With this expanding distribution, Manitoba beef producers want to know if they and their animals are at risk of acquiring a tick-borne pathogen. In this two-year study conducted in 2021 and 2022 in Manitoba, our objectives were to quantify the abundance of *I. scapularis* and *D. variabilis* on pastures, assess the presence of pathogens in ticks collected on pastures, and examine the relationships between these ticks, hosts (cattle, horses, and humans), and vegetation. We predict that 1) the abundance of *D. variabilis* in open grass areas of the pasture will be greater than *I. scapularis*; the abundance of *I. scapularis* in wooded areas of the pasture will be greater than *D. variabilis*, 2) *Borrelia burgdorferi* will be the most prevalent pathogen in *I. scapularis*; *Anaplasma marginale* will not be prevalent in *D.*

variabilis and 3) cattle will be more exposed to *D. variabilis* than *I. scapularis* while in the pasture.

Materials and Methods

Site Selection

We selected seven sites in southern Manitoba, comprising four cattle pastures and three horse pastures. These sites were selected based on the intensity of cattle production and the potential presence of *I. scapularis* and *D. variabilis* in the area. Where horses were not present at participating beef farms, a nearby horse pasture was selected to ensure representation of both cattle and horses. All study sites were located within Manitoba's Prairie ecozone and were distributed across three distinct regions: Parkland, Westman, and Pembina Valley (Figure 5).

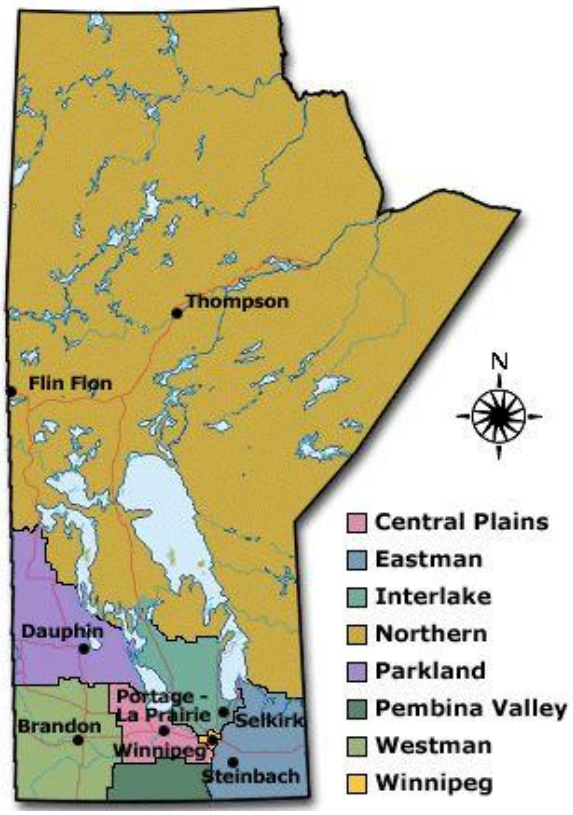

Figure 5. The regions of Manitoba, Canada available from Manitoba's Tourist Regions (macap.ca).

The seven sites varied in livestock composition, working companions, grazing practices, and farm type. Continuous grazing was defined as cattle having unrestricted movement throughout available pasture areas, whereas strip grazing involved the use of moveable fencing to confine animals to smaller grazing areas. Rotational grazing involved dividing larger pastures into smaller paddocks that were grazed systematically in a planned sequence. Small-scale hobby farms were defined as agricultural properties managed by individuals for personal purposes, typically involving smaller-scale livestock production and cultivation.

All seven sites contained forested habitat; however, forest composition varied among sites. Forested areas were dominated by either trembling aspen (*P. tremuloides*) or bur oak (*Q. macrocarpa*). At some sites, livestock had unrestricted access to the forested areas within the pasture, whereas others fenced off the forested habitats, excluding them from the grazing area. Four of the seven sites contained dense vegetation, which was classified as areas where plants grew closely together, creating thick vegetation cover and high biomass per unit area. Forested areas with dense canopy cover and an understory layer were also classified as dense vegetation. Natural grasses within pastures consisted primarily of wheatgrass, needle grass, and North American fescues. Planted grasses included Kentucky bluegrass (*Poa pratensis* L.) and Orchard grass (*Dactylis glomerata* L.). We also included alfalfa (*Medicago sativa* L.) in this category, although it is a legume (Fabaceae), not a grass.

Two sites were in the Parkland region: the Alonsa community pasture (beef cattle site) (AL; 50.6985, -99.0827) and Atkinson Farm (horse site) (AT; 50.4457, -99.5837) (Figure 6). The Alonsa community pasture is managed by the Association of Manitoba Community Pastures (AMCP) and encompasses a total area of 171.5 km^2. The pasture included in this study was leased to a single herd of beef cattle and covered a total area of 6.36 km^2 (AMCP personal communication). During both 2021 and 2022, one bull and 25 cows grazed the pasture continuously from May to September with unrestricted access. Staff at the community pasture use horses when working with cattle within the pastures and may be accompanied by herding dogs. Although working horses were present at Alonsa, they did not graze the same pasture as the cattle and were not readily accessible for sampling. Consequently, a “horse site” was included to represent horses within the Parkland region. Atkinson Farm is a family-operated hobby farm that maintained approximately 130 cattle, 11 horses, three dogs, and numerous sheep

during both study years. The area included in this study covered 1.72 km². Neither site restricted livestock access to forested areas. Both sites contained dense vegetation, trembling aspen (*Populus tremuloides*), and native grasses.

Three sites were in the Westman region: two cattle sites, Brookdale (BR; 50.0510, -99.9245) and Johnson (JO; 49.8760, -99.9063), and a nearby horse site, Dusty Oaks (DO; 49.6214, -99.9772) (Figure 6). Brookdale and Johnson are beef cattle operations managed by Manitoba Beef and Forage Initiatives (MBFI). The Brookdale site covered a total area of 3.28 km² and maintained two bulls and 50 cow-calf pairs in 2021, increasing to 56 cow-calf pairs in 2022. Cattle access to pastures was managed using strip grazing. The site also included five individuals involved in livestock management. The Johnson site covered a total area of 3.72 km² and maintained three bulls and 85 cow-calf pairs in 2021, decreasing to 72 cow-calf pairs in 2022, also managed by strip grazing. Livestock access to forested areas was restricted by fences at both sites. The Brookdale site was characterized by the presence of trembling aspen (*P. tremuloides*) and planted grass species, whereas the Johnson site contained bur oak (*Quercus macrocarpa* Michx.) and planted grasses. Horses were absent from both sites; therefore, we selected an additional site nearby. Dusty Oaks was selected to represent horses within the Westman region. It is a hobby farm with four horses, one dog, and four people, covering a total area of 0.93 km². Horses at DO had unrestricted daily access to natural pasture and forested areas containing dense vegetation, bur oak (*Q. macrocarpa*), and native grasses.

The final two sites were in the Pembina Valley region: one cattle site, Darlingford (DA; 49.2105, -98.3756) and one horse site, Gould Farm (GO; 49.1774, -98.4435) (Figure 6). DA was a beef cattle farm that maintained 17 head in the first year of the study and 27 in the second, and used a rotational grazing system. The study area at DA covered 1.56 km² and fences restricted access to forested areas. This site was similar to the Johnson site, including bur oak (*Q. macrocarpa*) and planted grasses. As horses were not present at Darlingford, a nearby small-scale hobby farm, Gould Farm, consisting of three horses, three dogs, and two people, was selected to represent horses within the Pembina Valley region. Gould Farm covered a total area of 0.09 km². Horses at this site had unrestricted access to pasture areas containing dense vegetation, trembling aspen (*P. tremuloides*) and native grasses.

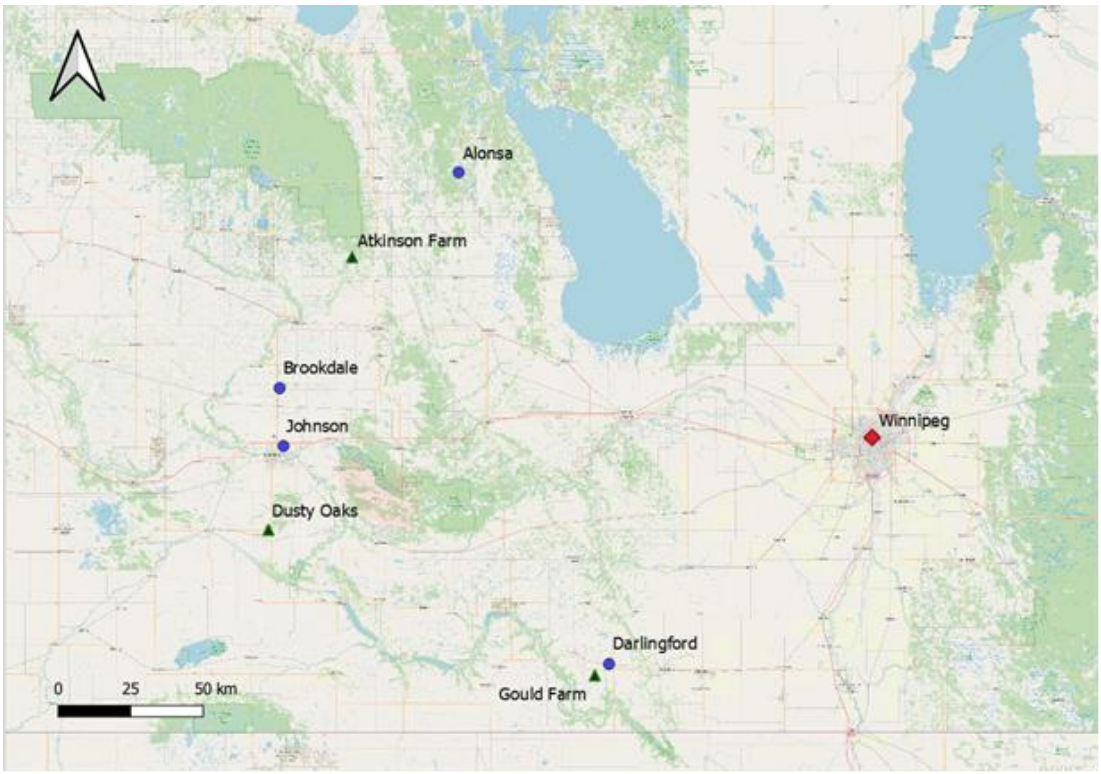

Figure 6. Research sites: cattle pastures in blue (circles) and participating horse owners in dark green (triangles).

Tick Sampling

Environmental Sampling

Drag sampling was conducted at each site one week before animals were put to graze, one week after the onset of grazing, and at the end of the grazing season (September in 2021, October in 2022). At sites with rotational grazing, the sampling area was selected within pastures where cattle had been present for the previous seven days (approximately), which would be where the animals would have encountered and acquired ticks. Tick drags consisted of a white flannel sheet (1 x 1 m) held flat by two PVC (polyvinyl chloride) pipes (1 cm diameter x 1 m length) sewn and taped to the bottom and top of the sheet, and a 2 m rope attached to the top dowel (Rochon et al. 2012). Sampling is performed by pulling the drag along a linear transect using the rope, ensuring the drag maintained contact with the ground and checking the underside every 10 m for ticks (Rochon et al. 2012). Any ticks found were collected into a vial and the

numbers were recorded on a data collection sheet. Because tick drags are 1 x 1 m, each 10 m length represents a sampled quadrat with an area of 10 m^2; sampling transects were 200 m, or 20 quadrats, representing a sampled area of 200 m^2. Before sampling, we established the number of steps each sampler took to walk 10 m to ensure consistency. Each transect was randomly assigned within these recently grazed areas to include a variety of habitats, and transects differed for each visit. We dragged a minimum of 2,000 m at each site (200 quadrats), ensuring that dragging was conducted across different habitat types, such as sloughs, tree lines, and fence lines. Each sampler recorded GPS (Global Positioning System) coordinates at the start and end of each transect. This information, segregated by sampler and site, was integrated into QGIS (Quantum Geographic Information System) for spatial analysis. Collected ticks were transported from the site back to the University of Manitoba, where they were identified to species using dichotomous keys (Keirans and Litwak 1989, Durden and Keirans 1996, Lindquist et al. 2016, Egizi et al. 2019), sexed, and stored in 70% ethanol at 5°C until pathogen testing.

Tick density (D) was calculated as the number of ticks collected per 100 m² of sampled area using the equation $D = (T/A) \times 100$, where T is the total number of ticks collected during the sampling period and A is the total area sampled (m²).

Host Sampling

Cattle Inspection

We aimed to do cattle inspections at each cattle site just before grazing started (usually end of May) to ensure the animals did not already have ticks; one week after grazing started (June) to increase the chances of getting ticks before they detached; and at the end of the grazing season (September/October) to collect any blacklegged ticks that could be active before the end of grazing. Due to weather conditions, cattle were put to pasture one month later than usual in both sampling years. In 2021, conditions were very dry, slowing plant growth; we sampled twice in June and once in September. In 2022, conditions were very wet with a lot of flooding (Figure 7); we sampled twice in June and once in October. None of the cattle were treated specifically against ticks, but animals at the Brookdale and Johnson farms were treated with BOSS® pour-on insecticide (5% permethrin) to control horn flies. This insecticide is labelled to control Rocky Mountain wood ticks in cattle.



Figure 7. Represents the outcome of Manitoba's record flood at one of our cattle sites, Brookdale, in 2022.

Cattle were rounded up into holding pens and each animal was walked through a squeeze chute (Figure 8) to be inspected by hand, a technique known as "scratching," focusing on the head, dewlap, backside, and tail. We focused on these areas because they were safer for both the sampler and the animal, and *I. scapularis* and *D. variabilis* on cattle have mainly been found on the dewlap and hind area (Barnard 1981a). We sampled at least 25 animals per scratching event, selecting them at random. Where fewer than 25 animals were available, they were all sampled. All ticks removed from cattle were collected in vials containing 70% ethanol labelled with the animal's tag number and transported back to the University of Manitoba, where they were counted, sexed, and identified to species using dichotomous keys (described above), and stored in 70% ethanol at 5°C.



Figure 8. A cattle squeeze chute used to safely restrain cattle for scratching, which was done to collect tick specimens.

Horse Sampling

We assessed tick abundance on horses through voluntary submissions from horse owners. Horse owners were provided with tick collecting kits, comprising vials with ethanol, a recording sheet, pencils, and detailed instructions on tick removal. We requested owners to conduct tick collection for one week before our scheduled drag sampling period to supplement our data collection efforts. The tick collecting kits were collected and replenished in September/October, during our last drag sampling event. All ticks collected were identified to species and sexed, counted, and stored for further pathogen testing.

Tick prevalence and intensity were calculated to evaluate tick infestation among inspected hosts. Prevalence represents the proportion of hosts that had at least one tick.

$$Tick\ Prevalence\ (\%) = \frac{number\ of\ infested\ hosts}{total\ hosts\ examined}\ x\ 100$$

Mean intensity represents the average number of ticks collected from infested hosts.

$$Mean\ intensity = \frac{total\ ticks\ collected}{number\ of\ infested\ hosts}$$

Prevalence and mean intensity were calculated separately for each tick species and study site.

Pathogen Detection

All adult *I. scapularis* were sent to the National Microbiology Laboratory (NML) (Public Health Agency of Canada) in Winnipeg for pathogen testing using a series of PCR assays. The testing protocol, described by Dibernardo et al. (2014), included assays targeting *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Borrelia miyamotoi*, *Babesia* spp., and Powassan virus. Initial screening for *B. burgdorferi* and *A. phagocytophilum* was conducted using a multiplex real-time PCR targeting 23S rRNA and *msp2*, respectively. Specimens positive for *B. burgdorferi* were subsequently confirmed using real-time PCR targeting *ospA*. Specimens negative for *B. burgdorferi* were tested for *B. miyamotoi* using real-time PCR targeting the intergenic spacer (IGS) region, and positive specimens were confirmed using real-time PCR targeting *glpQ* (Dibernardo et al. 2014). Ticks negative for *B. miyamotoi* were further tested using nested PCR targeting the 16S-23S region to screen for *Babesia* spp., including *Babesia microti* and *Babesia odocoilei*. Additionally, quantitative real-time PCR was used to screen for Powassan virus (Dibernardo et al. 2014).

All adult *D. variabilis* were sent to the University of Saskatchewan for molecular screening. A broad-range quantitative real-time PCR (qPCR) assay targeting members of the family *Anaplasmataceae* was used for initial screening of *Anaplasma* DNA (Dahmani et al. 2015). Samples that tested positive for *Anaplasmataceae* were further tested using a species-specific real-time PCR assay targeting *Anaplasma marginale* to confirm infection status (Carelli et al. 2007). This assay targeted the *msp5* gene, which is commonly used for the detection of *A. marginale* (Carelli et al. 2007).

Geospatial Data Analysis

We used QGIS (QGIS Development Team 2025) to integrate spatial data from handheld GPS units, elevation models and other baseline geographic datasets.

Garmin GPSMap-series handheld Global Navigation Satellite System (GNSS) units were used by each field sampling team to record waypoints at the start and end of 200 m transects. These GNSS units were set to "auto" track mode, recording the field sampling team member's location in the pasture, adjusting dynamically based on movement, logging more points when changing direction or speed, and fewer points when moving steadily to generate a "GPS Tracklog."

Tick data were collated into a spreadsheet and organized by quadrat ID, grouping all observations from the same quadrat for analysis and comparison. Quadrat IDs were then used to create transect IDs, which incorporated the date, site, and quadrat number. From this, a unique ID was generated by combining the transect ID, quadrat ID, date, and site, ensuring each observation could be accurately tracked and analyzed without duplication.

The waypoints for the start and end of each transect were uploaded into QGIS. A line feature connecting these points was created, and then points representing the sample quadrats were equally placed along the transect lines. These points were spatially joined to the tracklog data using the 'Join attributes by nearest' tool in QGIS. This tool joins each feature (i.e. sample quadrat) to the nearest features in another layer (i.e. GPS tracklog) and copies the attributes of the latter based on proximity (tracklog point with the shortest distance from the quadrat), even if the features do not touch. This provided the exact time of day for each sample quadrat.

Each quadrat in the spatial layer was then linked to the tick analysis dataset using a relational join. Relational joins link tabular data between two or more tables based on a shared attribute or ID, rather than by location. For this join, the quadrat ID attribute in the spatial layer was linked to the equivalent quadrat ID attribute in the tick analysis dataset, allowing us to add collected tick data (*I. scapularis* and *D. variabilis*) to the spatial file (a one-to-one join with no NULLs or duplication). Tick presence and count for each transect and quadrat were then mapped in QGIS. One transect containing 10 *D. variabilis* was removed due to an inconsistent timestamp at the Darlingford site in 2022.

Spatial Error Filtering and Geofencing

To define the spatial extent of each study site, geofences were created around each pasture in QGIS (Figure 9). Fence lines were digitized from aerial imagery and verified using cadastral layers to confirm property and pasture boundary locations. Fence lines were buffered by 40 m (Figure 9). A 40 m buffer was selected as a compromise to account for uncertainty associated with fence placement during digitization and the spatial resolution of the habitat layers (25 m). At sites where fences had been moved or breached, the geofence was locally extended to represent the true pasture boundary. This adjustment was made for Alonsa, where cattle breached the fence in 2022. All points outside the geofence were removed prior to further analysis in QGIS.

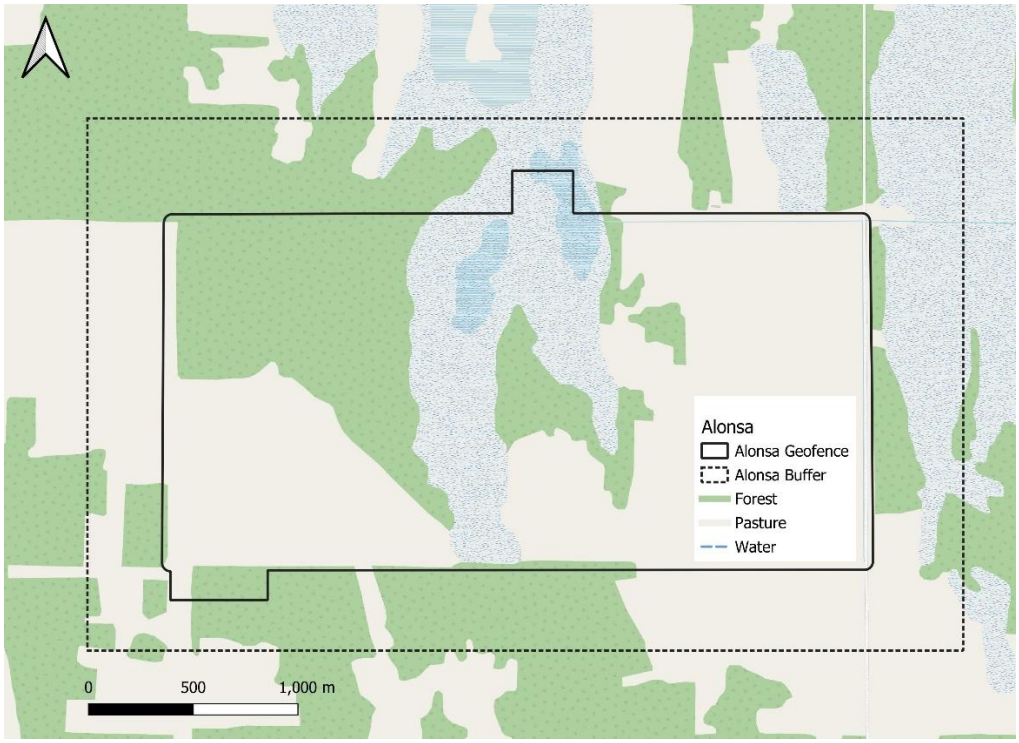

Figure 9. Example of the digitized pasture boundary (geofence) and the 40 m buffer created around the study site Alonsa in QGIS using an OpenStreetMap basemap. The buffered geofence accounted for GPS positional error, habitat layer resolution, and uncertainty in fence digitization.

Baseline and Habitat Data

Baseline data (Elevation, Roads, and Hydrology) and land cover data (Annual Crop Inventory) were compiled in QGIS. These data were obtained from federal and government sites, including geospatial data from Canada Open Government and the Manitoba Land Initiative. Elevation data were initially compiled as a baseline spatial layer but were not included in the final analyses. Cadastral data were also used as a reference to verify digitized study-site boundaries but were not included in subsequent spatial analyses. These layers include:

1. 2021 Census - Road Network File; Vector, no scale; available from: https://open.canada.ca/data/en/dataset/9fd79520-bcbf-4ddf-9ce3-d946a39ec3fa/resource/30daff86-e103-4093-b82a-1b141902446a

2. Annual Crop Inventory (2021); Raster, 30 m resolution; available from: https://open.canada.ca/data/en/dataset/ba2645d5-4458-414d-b196-6303ac06c1c9

3. Manitoba Hydrology (2009); Vector 1:20,000 scale; available from: https://mli.gov.mb.ca/water_resources/index.html

4. Manitoba Orthophotograph Series (various years 2007-2014); Raster, 1 m resolution; available from: https://mli.gov.mb.ca/ortho/index_ortho_refresh_all.html

All baseline layers were clipped to an area approximately 0.5 km outside of the geofence. This buffer was included to provide additional landscape context for mapping and visualization of the study sites, allowing habitat features adjacent to the geofence to be displayed. These layers were added to a QGIS project, grouped by sample site, and organized within a Land Base sub-group.

Habitat Classification

The Annual Crop Inventory (ACI) datasets for the years 2021 and 2022 were used to characterize land cover within each study site. The ACI provides comprehensive maps of land use and agriculture classes across Canada (Government of Canada 2021, 2022) and is derived from classified Landsat data at 30 m resolution. To streamline our analysis, we amalgamated the original ACI vegetation classes into nine broader land cover categories (Table 1). Exposed

land/barren, urban/developed, grassland, pasture/forages, shrubland, wetland, and water were retained as their original ACI classifications. Forest was an amalgamated category that included broadleaf, mixed wood, and coniferous forest classifications. Agriculture was an amalgamated category that included all individual agricultural crop classifications within the ACI, such as barley, spring wheat, canola, corn, and soybeans. These categories represented the standardized land cover classification used in this study for subsequent habitat analyses and aligned with the definitions outlined in the ACI dataset (Government of Canada 2024. Table 1; Figure 10);) The raster ACI layers were converted to vector format and projected to NAD83 UTM zone 14N (EPSG: 26914). To create a finer spatial framework for land cover classification, ACI polygons were divided into 5 x 5 m squares. We used a combination of data sources, including high-resolution Google satellite imagery, the original ACI classification, hydrology layers, and high-resolution orthophotography from each site, to verify that the land cover categories represented in the geospatial layer matched what was observed in these sources (Figure 10). If the land cover category in the geospatial layer did not match the likely land cover category indicated by the high-resolution reference sources, the category was reassigned based on those high-resolution sources.

Table 1. The nine land cover categories and definitions used to amalgamate Annual Crop Inventory (ACI) classifications into a standardized land cover layer for habitat analysis.

Label	Definition
Exposed land/barren	Areas devoid of significant vegetation cover.
Urban/developed	Areas characterized by human development and infrastructure.
Agriculture	Areas where crops are actively managed and cultivated.
Grassland	Areas dominated by native grasses.
Pasture/forages	Areas planted and cultivated with forages.
Shrubland	Low predominately woody vegetation.
Forest	Areas including coniferous, broadleaf, and mixed forests.
Water	Bodies of water, such as reservoirs, lakes, streams, etc.
Wetland	Areas with a nearby/at/above water table, such as sloughs and bogs.

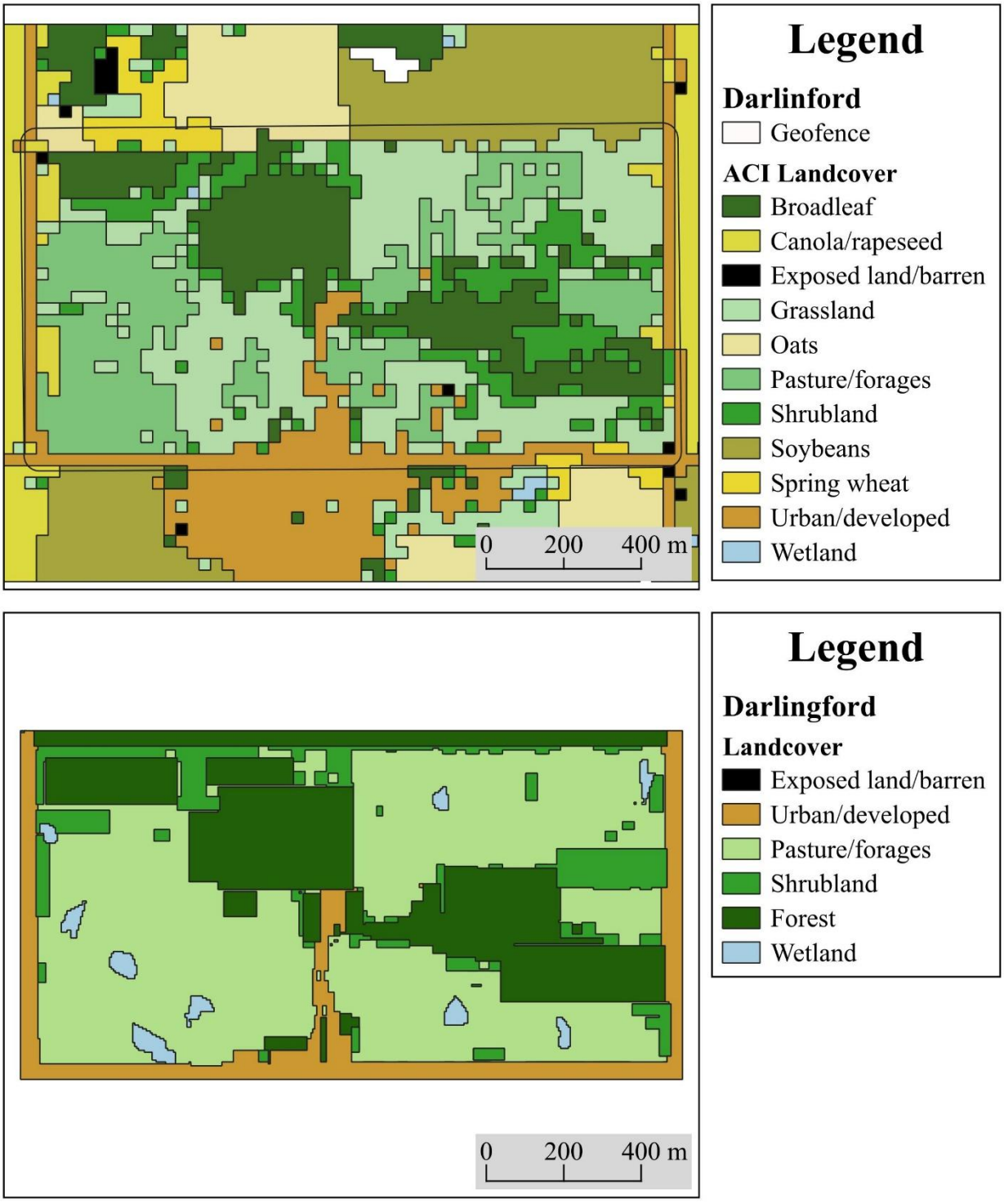

Figure 10. Original 2021 Annual Crop Inventory (ACI) classification (top) and the finalized standardized land cover layer (bottom) for Darlingford. Original ACI land cover classes were amalgamated into nine broader land cover categories and refined using high-resolution satellite imagery, hydrology layers, and orthophotography before being used for habitat analyses.

Land Cover Methods and Analysis

Habitat Composition

To quantify local habitat composition at tick quadrat sampling locations, buffers with a 60 m radius were used to sample the high-resolution 5 by 5 m land cover habitat layer. The 60 m radius was selected to ensure inclusion of multiple habitat cells while capturing the surrounding landscape relevant to tick occurrence.

Buffers were centred around quadrats containing ticks and quadrats where no ticks were detected to allow comparison between tick and no-tick locations. Within each 60 m buffer, the total area of each habitat type was calculated using the vectorized land cover layer. These areas were converted to proportional habitat composition for each quadrat.

This resulted in a habitat composition matrix in which each quadrat was associated with the proportional area of each habitat type within 60 m. This matrix was used for subsequent multivariate analyses.

Habitat data were used to characterize the environmental features associated with both quadrat sampling locations and tick collection sites along those quadrats. The finalized habitat layers were placed within the Landbase subgroup of the QGIS geospatial database and used with the GPS unit tracklog data to accurately locate sampling sites. By overlaying collection locations onto the habitat layers, we were able to identify the habitat types where tick collections occurred and assess habitat associations across the study areas.

I used the geofence-clipped land cover layer in QGIS to calculate and sum the area of each site.

To calculate the proportion of each habitat type contained within each site we used the following equation:

$$Proportion\ Overall=(\frac{Habitat\ \ Type\ Area}{Total\ Site\ Area})\ x\ 100\%$$

We calculated the proportion of each habitat type within the area we sampled as follows:

$$Proportion\ Sampled=(\frac{Sampled\ Habitat\ \ Type\ Area}{Total\ Sampled\ Area})\ x\ 100\%$$

Lastly, to determine the proportion of each sampled habitat that contained ticks we used the following equation:

$$Proportion\ Sampled\ with\ Ticks=(\frac{Sampled\ Habitat\ \ Type\ Area\ with\ Ticks}{Total\ Sampled\ Area\ with\ Ticks})\ x\ 100\%$$

Multivariate Analysis

Correspondence Analysis

To examine similarities and differences in habitat associations among the seven study sites, correspondence analysis was conducted in R (R Core Team 2024) using the following packages; ggplot2 (Wickham 2016), ggrepel (Slowikowski 2024), readr (Wickham et al. 2024), sf (Pebesma 2018, Pebesma and Bivand 2023), tidyverse (Wickham et al. 2019), and vegan (Oksanen et al. 2025). Correspondence analysis is a multivariate ordination technique based on a chi-square distance matrix derived from a contingency table and is commonly used to analyze categorical ecological data (Legendre & Legendre, 1998).

Habitat composition was used as the input for the correspondence analysis. For each quadrat, habitat composition was calculated as the proportion of each of the nine ACI-derived habitat types within a 60-m-diameter buffer centred on the quadrat. In this study, rows of the contingency table represented the total habitat cover of each study site, the pooled mean habitat composition of tick-positive and tick-negative quadrats, the pooled mean habitat composition of all sampled quadrats, and the pooled mean habitat composition of random quadrats. Columns represented the nine ACI-derived habitat types.

The CA produced four axes, with each axis representing a decreasing proportion of the total variation in habitat composition. Habitat types, site, and quadrats were displayed simultaneously in low dimensional biplots, allowing visualization of associations and similarities among sites (Legendre & Legendre, 1998). The first two axes were used for visualization and interpretation because they accounted for the greatest proportion of the total variation. Correspondence analysis was used to compare habitat composition among sites and to visualize associations between tick-positive and tick-negative quadrats and habitat types, as well as differences between sampled and random quadrats.

Habitat Distance Analysis and Randomization

Once the high-resolution habitat layer was completed, mean distances from tick collection locations (tick-positive samples) to nine focal habitat types, exposed land/barren, urban/developed, grassland, agriculture, forest, shrubland, water, and wetland, were calculated. These habitat types were selected due to their known importance for ticks and their hosts. To test whether ticks were located proximate to a given habitat type (i.e. selecting for a habitat) or distally (i.e. avoiding a habitat), two different randomization strategies were used;

1. Unconstrained randomization: Monte Carlo simulation under simple random sampling (SRS) of availability (R randomization script). Tick positive sample distances were determined to each focal habitat, and the mean distances were calculated as the observed values. For the null model, within each Monte Carlo replicate, locations were generated by independent simple random sampling from the study area to match the observed sample size. Distances to each of the focal habitats were calculated for each generated point independently, with sampling conducted with replacement and repeated across replicates to approximate the randomization distribution. While formally a repeated measures within and across replicates, given the spatial extent, coordinate precision and independence of the X and Y coordinates, in practice, replacement did not occur. Statistically this method is in practice an unconditional true randomization test. Within each Monte Carlo replicate, observations were sampled without replacement, whereas sampling across replicates was conducted with replacement.
2. Transect-constrained randomization: Case–control (tick-positive and tick-negative sample) design with resampling from empirical availability. For this approach, availability is not defined over the full spatial domain, but over the sampled frame. Distances to each of the focal habitats were calculated for tick positive and negative samples. The mean focal distances of the tick positive samples were treated as observed. For the null model, within each Monte Carlo replicate, a random subset of tick negative quadrats, equal in size to the observed positive (case) sample, was drawn without replacement from a fixed set of empirically observed tick negative locations; this procedure was repeated with replacement across replicates to obtain a Monte Carlo approximation to the conditional randomization distribution.

For each approach, the observed mean distance from each focal habitat was compared to the corresponding (conditional) randomization distribution generated using Monte Carlo resampling. The p-values were calculated as the proportion of simulated mean values that were as or more extreme than the observed mean, relative to the specified alternative hypothesis. This approach follows common methods for randomization testing and distance-based ecological analysis (Manly, 2006; Legendre & Legendre, 2012).

Results

Tick Sampling

From 2021 to 2022, a total of 1,946 ticks were collected from the environment and hosts; 944 ticks were collected from the environment using tick drags and 1,002 ticks were collected from hosts (cattle, horses, humans, and dogs). The most common tick species collected was *D. variabilis* (n = 1,872 adults), followed by *I. scapularis* (n = 70 adults), *Haemaphysalis chordelis* Packard (n = 4 adults) and one unidentified tick.

Environmental Sampling

During the 2021 and 2022 sampling seasons, a total area of 150,250 m^2 was sampled across all seven sites (Table 2). Tick dragging resulted in the collection of 944 ticks, consisting of *D. variabilis* (n = 929 adults), *I. scapularis* (n = 14 adults) and one *H. chordeilis* adult male recovered from the environment at Alonsa in October of 2022. The number of D. variabilis includes 10 ticks collected in 2022 on the transect removed from analysis because of an inconsistent timestamp.

Ixodes scapularis were less frequently collected during drag sampling and were only detected at Brookdale (n = 1) and Dusty Oaks (n = 13) (Table 2). In 2021, no *I. scapularis* were recovered from cattle pastures, while eight were collected from Dusty Oaks in October. In 2022, six *I. scapularis* were collected from two sites: five were collected from Dusty Oaks in May (n = 4) and October (n = 1), and one was collected from a Brookdale in October.

Dermacentor variabilis was collected at all seven study sites, with the highest abundance observed at Dusty Oaks (n = 284), Darlingford (n = 218), Brookdale (n = 192), and Alonsa (n = 124) (Table 2). *D. variabilis* were collected in June (n = 223) in 2021 and in 2022 they were collected during May-July (n = 706). No *D. variabilis* were collected at the Johnson site in 2021. No fall sampling was conducted at Brookdale or Johnson in 2021 because drought conditions resulted in cattle being removed from the pastures before the planned fall sampling period. The horse sites (Atkinson, Dusty Oaks, and Gould) were sampled for the first time in October 2021, and no *D. variabilis* were found.

Host Sampling

A total of 1,002 ticks were collected from hosts including cattle, horses, humans, and dogs, during 2021 and 2022. In addition to 537 ticks from cattle and 331 from horses, 129 were collected from humans, and five from dogs. As with environmental collections, the majority of ticks collected from hosts were *D. variabilis* (n = 943), with some *I. scapularis* (n = 56) and three *H. chordeilis*. Only one *I. scapularis* was collected from a human, at Dusty Oaks in October 2022; no *I. scapularis* were recovered from cattle or humans in 2021.

Tick collections from humans and dogs were excluded from subsequent analyses because they were inconsistent across sites and sampling years, precluding meaningful comparisons. Host infestation analyses were therefore limited to cattle, where we conducted sampling, and horses at Dusty Oaks, where sampling effort was more consistent.

Both male and female ticks were collected from hosts, with female *D. variabilis* more abundant than males on both cattle and horses, while *I. scapularis* collected from horses were predominantly male (Table 3).

Table 2. Abundance and density (ticks/100m^2) of adult *Ixodes scapularis* and *Dermacentor variabilis* collected by drag sampling at seven study sites in 2021 and 2022.

Site	*Ixodes scapularis*						*Dermacentor variabilis*					
	May-Jul			**Sep-Oct**			**May-Jul**			**Sep-Oct**		
2021	**area sampled (m^2)**	***n***	**density (/100 m^2)**	**area sampled (m^2)**	***n***	**density (/100 m^2)**	**area sampled (m^2)**	***n***	**density (/100 m^2)**	**area sampled (m^2)**	***n***	**density (/100 m^2)**
Alonsa	4,800	0	0	6,400	0	0	4,800	79	1.65	6,400	0	0
Brookdale	11,180	0	0	-	-	-	11,180	50	0.45	-	-	-
Darlingford	10,000	0	0	4,700	0	0	10,000	94	0.94	4,700	0	0
Johnson	5,400	0	0	-	-	-	5,400	0	0	-	-	-
Atkinson Farm	-	-	-	4,000	0	0	-	-	-	4,000	0	0
Dusty Oaks	-	-	-	5,620	8	0.14	-	-	-	5,620	0	0
Gould Farm	-	-	-	1,800	0	0	-	-	-	1,800	0	0
	May-Jul			**Sep-Oct**			**May-Jul**			**Sep-Oct**		
2022	**area sampled (m^2)**	***n***	**density (/100 m^2)**	**area sampled (m^2)**	***n***	**density (/100 m^2)**	**area sampled (m^2)**	***n***	**density (/100 m^2)**	**area sampled (m^2)**	***n***	**density (/100 m^2)**
Alonsa	13,400	0	0	6,400	0	0	13,400	45	0.34	6,400	0	0
Brookdale	8,900	0	0	3,200	1	0.03	8,900	142	1.60	3,200	0	0
Darlingford	17,750	0	0	3,800	0	0	17,750	124	0.69	3,800	0	0
Johnson	9,600	0	0	6,800	0	0	9,600	10	0.10	6,800	0	0
Atkinson Farm	7,700	0	0	3,000	0	0	7,700	16	0.21	3,000	0	0
Dusty Oaks	5,800	4	0.07	3,400	1	0.03	5,800	284	4.90	3,400	0	0
Gould Farm	4,600	0	0	2,000	0	0	4,600	75	1.63	2,000	0	0

Cattle Sampling

Animals were sampled at random multiple times over the two years of the study, resulting in 505 individual inspections across the four cattle sites and 537 ticks collected (Table 3), including 532 *D. variabilis*, two *I. scapularis* and three *H. chordeilis*. All *H. chordeilis* were recovered from cattle at Alonsa during October of both sampling years, with two specimens collected in 2021 and one in 2022, resulting in a prevalence at this site of 1.83% with a mean intensity of 1.5 *H. chordeilis* per infested host.

Ixodes scapularis was collected from one animal at Alonsa (October 2022) and one at Brookdale (June 2022). Prevalence was 0.92% at Alonsa and 0.55% at Brookdale, with a mean intensity of 1.0 *I. scapularis* per infested host at both sites.

Dermacentor variabilis was collected from cattle at all cattle study sites, with the highest prevalence and mean intensity at Alonsa (Table 4). In 2021, *D. variabilis* was collected from cattle only at Alonsa and Brookdale, with Alonsa having the highest abundance (n = 434), prevalence (50.00%), and mean intensity (16.69 ticks per infested animal). In 2022, *D. variabilis* was collected from cattle at all four sites, with Alonsa again having the highest abundance (n = 41) and prevalence (33.33%). All *D. variabilis* removed from cattle were collected in June in both sampling years, except at Darlingford, where none were found in 2021 and four were recovered in July 2022. No ticks were found on cattle at Johnson in 2021.

Horse Sampling

Three sites were designated as "horse sites": Atkinson, Dusty Oaks, and Gould Farm. The staff at the Alonsa pasture used horses for work but did not collect or report ticks on their animals. During both years of the study, we collected 278 *D. variabilis* and 53 *I. scapularis* from horses (Table 3). No other tick species were collected from horses during this study.

Ixodes scapularis was collected only from horses at Dusty Oaks, where 53 individuals (39 males and 14 females) were recovered from four horses in 2021 (Table 3). Males accounted for the majority (73.6%) of *I. scapularis* collected from horses. *Ixodes scapularis* prevalence was 100% with a mean intensity of 13.25 ticks per infested horse. No *I. scapularis* were recovered from horses in 2022.

Table 3. Number of adult (male and female) *Dermacentor variabilis* and *Ixodes scapularis* collected from cattle and horses at each study site during 2021 and 2022.

		Ixodes scapularis				***Dermacentor variabilis***			
		Cattle		**Horses**		**Cattle**		**Horses**	
Site	**Total ticks**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Alonsa	520	0	1	0	0	178	297	17*	27*
Brookdale	53	0	1	-	-	21	31	-	-
Darlingford	4	0	0	-	-	2	2	-	-
Johnson	1	0	0	-	-	1	0	-	-
Atkinson Farm	0	-	-	0	0	-	-	0	0
Dusty Oaks	284	-	-	39	14	-	-	95	136
Gould Farm	3	-	-	0	0	-	-	0	3

* Opportunistic and incomplete inspection of a single horse working at this location, not included in totals or analyses.

Table 4. Abundance, prevalence, and mean intensity of adult *Dermacentor variabilis* collected from cattle at four study sites in 2021 and 2022.

Site	**Individual inspections**	**Ticks (*n*)**	**Prevalence (%)**	**Mean intensity**
2021				
Alonsa	52	434	50.00	16.69
Brookdale	100	32	15.00	2.13
Darlingford	21	0	0	0
Johnson	59	0	0	0
2022				
Alonsa	57	41	33.33	2.16
Brookdale	82	20	14.63	1.67
Darlingford	51	4	3.92	2.00
Johnson	83	1	1.20	1.00

Dermacentor variabilis was collected at two of the three sites. Dusty Oaks had the most (n = 231), recovered from four horses in late May–June 2022, resulting in a prevalence of 100% and a mean intensity of 57.75 ticks per infested horse. Three *D. variabilis* were collected from horses at Gould Farm in June 2022; however, because the number of infested horses was not recorded, prevalence and mean intensity were not calculated. No ticks were reported from horses at the Atkinson site in either year of the study. A single opportunistic, incomplete collection from one horse at Alonsa (a cattle site) in June 2022 yielded 44 *D. variabilis*.

Pathogen Detection

We sent a total of 70 adult *I. scapularis* to the NML for pathogen testing. In 2021, 61 *I. scapularis* were collected at Dusty Oaks: 53 from horses and eight from the environment. Overall, 23 (37.7%) tested positive for *B. burgdorferi*, including three from the environment and 20 from horses. One tick was infected with *B. odocoilei*, and one was coinfected with both *B. odocoilei* and *B. burgdorferi* (Figure 11, top); both were collected from horses.

In 2022, nine *I. scapularis* were collected from three sites: Alonsa (one from cattle), Brookdale (one from cattle and one from the environment), and Dusty Oaks (one from a human and five from the environment). Overall, three (33.3%) tested positive for tick-borne pathogens. Two (22.2%) were positive for *B.burgdorferi* (Figure 11, bottom); one from the environment at Dusty Oaks and one from cattle at Alonsa. One tick collected from a human at Dusty Oaks tested positive for *A. phagocytophilum* (Figure 11). None of the *I. scapularis* collected in this study tested positive for Powassan virus, *B. microti*, or *B. miyamotoi*.

D. variabilis (n = 1,872) were recovered at every site and on every host type in both years of the study. We sent a total of 1,863 *D. variabilis* to the University of Saskatchewan; all were negative for *A. marginale*. The three *H. chordeilis* collected during this study were not tested for pathogens.

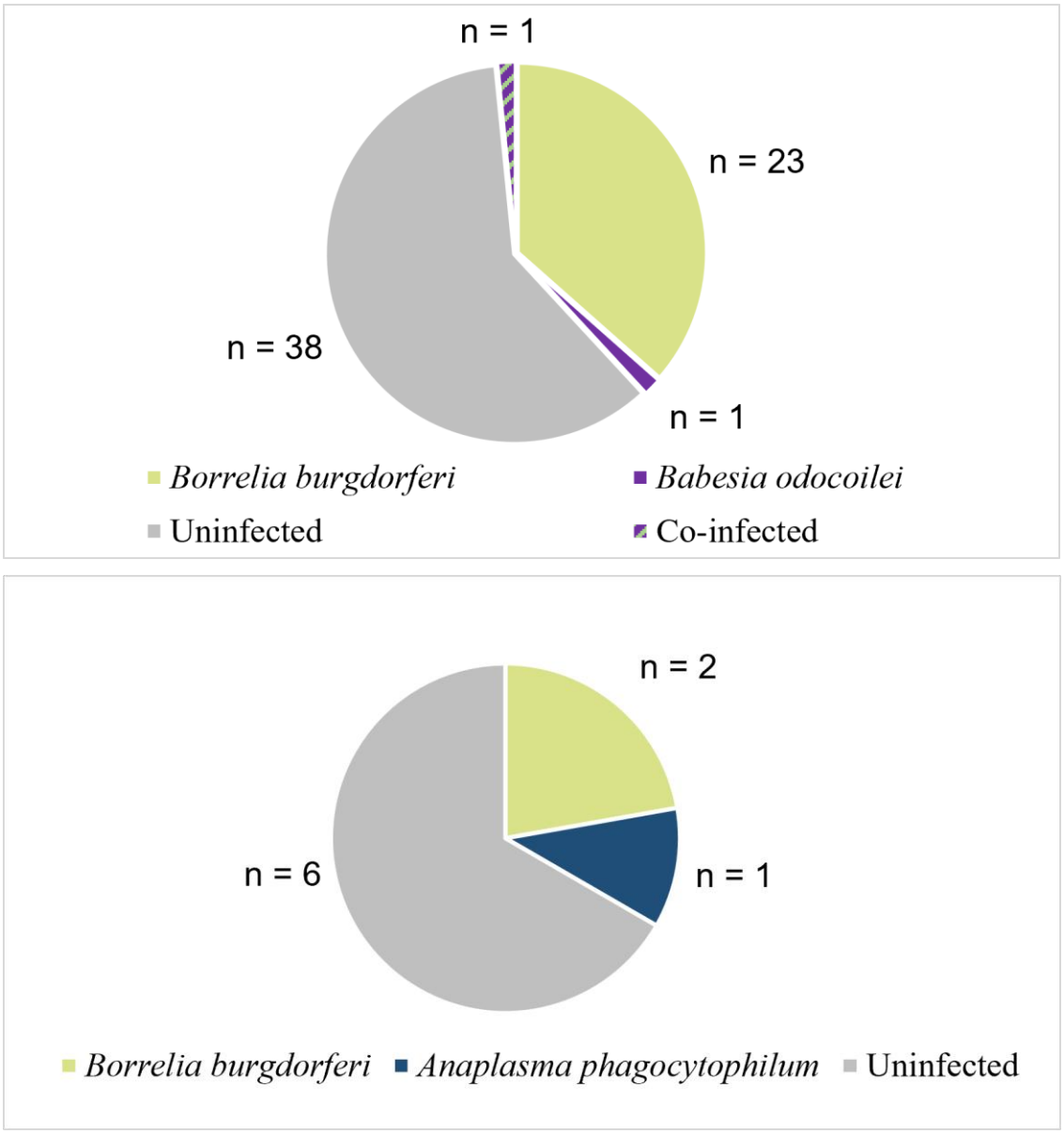

Figure 11. Number of *Ixodes scapularis* collected in 2021 (top) and 2022 (bottom) infected with tick-borne pathogens.

Geospatial Analysis

The following section summarizes the proportional cover of the land cover types described in the Methods. Cover composition is first presented using proportional bar charts and then mapped to illustrate the locations of sampled quadrats and status as either tick positive or negative (Figure 12-Figure 26). As described previously, a total of 929 *D. variabilis*, 14 *I. scapularis*, and one *H. chordeilis* were collected through drag sampling across the seven study sites during 2021 and 2022 (Table 2). These were mapped, with the exception of ten *D. variabilis* with inconsistent timestamps (as described in the Methods) thus, 919 *D. variabilis* and 14 *I. scapularis* are presented below in the geospatial analyses. Note that *I. scapularis*-positive quadrats occurred at two study sites, Brookdale and Dusty Oaks. However, only one *I. scapularis* was collected at Brookdale (Figure 15), limiting interpretation of its spatial distribution at this site.

Alonsa

Alonsa was the largest of the seven study sites, covering a total area of 6.36km^2. Of the nine habitat classes found across all study sites, agriculture and pasture/forages were absent from this site and were therefore excluded from percentage calculations. Grassland was the most dominant habitat type, accounting for approximately 50% of the total land cover (Figure 12). The habitat composition of the sampled quadrats, tick-positive quadrats, tick-negative quadrats, and random points was generally similar to the overall site land cover, with grassland remaining the predominant habitat type across all groups (Figure 12). Wetland was the next most common type followed by forest and shrubland. Other classes represented only minor components of the landscape. However, in tick-positive quadrats, wetlands were absent, while forest and shrubland were proportionally overrepresented relative to the landscape as a whole.

A total of 124 *D. variabilis* were collected through drag sampling at Alonsa during 2021 and 2022 (Figure 13). Tick detections occurred at multiple sampling locations, with counts ranging from one to nine ticks per quadrat. Tick-positive quadrats were distributed primarily through the central portion of the site, extending along the wetland, with most detections occurring toward the eastern side. These quadrats were primarily associated with grassland, followed by shrubland and forest. More ticks were found where grassland bordered shrubland and forest.

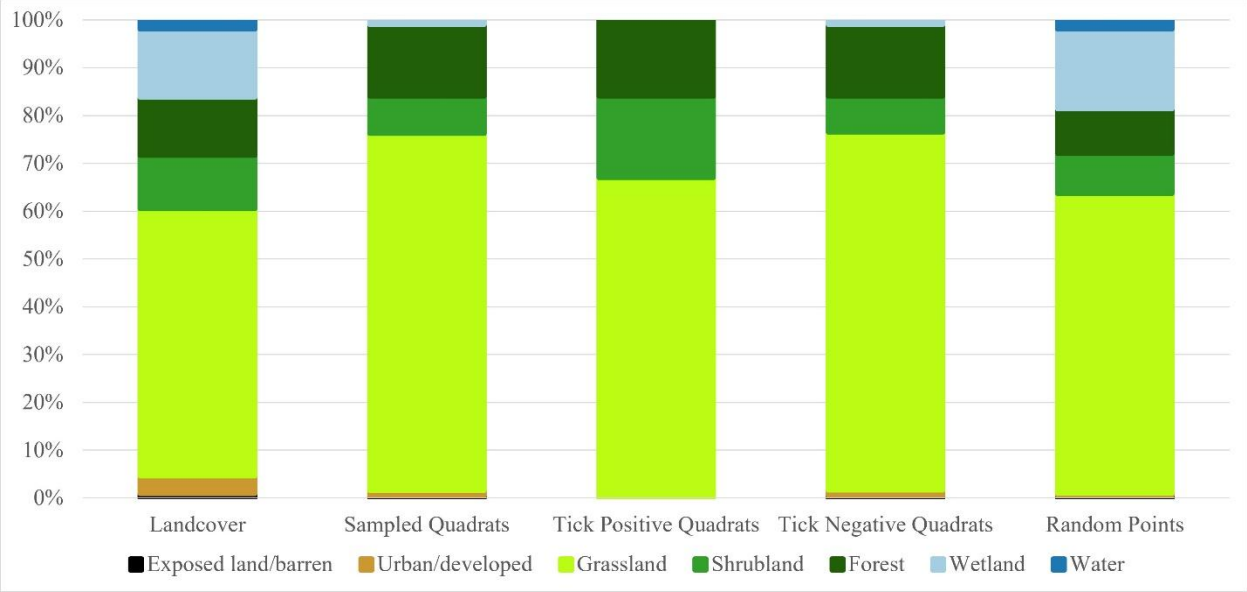

Figure 12. Proportional stacked bars representing the relative cover (%) of the seven habitat types at the Alonsa study site, associated with the total site land cover, sampled quadrats (areas sampled through tick dragging in 2021 and 2022), tick positive-quadrats (sampled quadrats containing *Dermacentor variabilis*), tick-negative quadrats (sampled quadrats where no ticks were detected), and random points (10,000 randomly generated points used for the unconstrained randomization analysis).

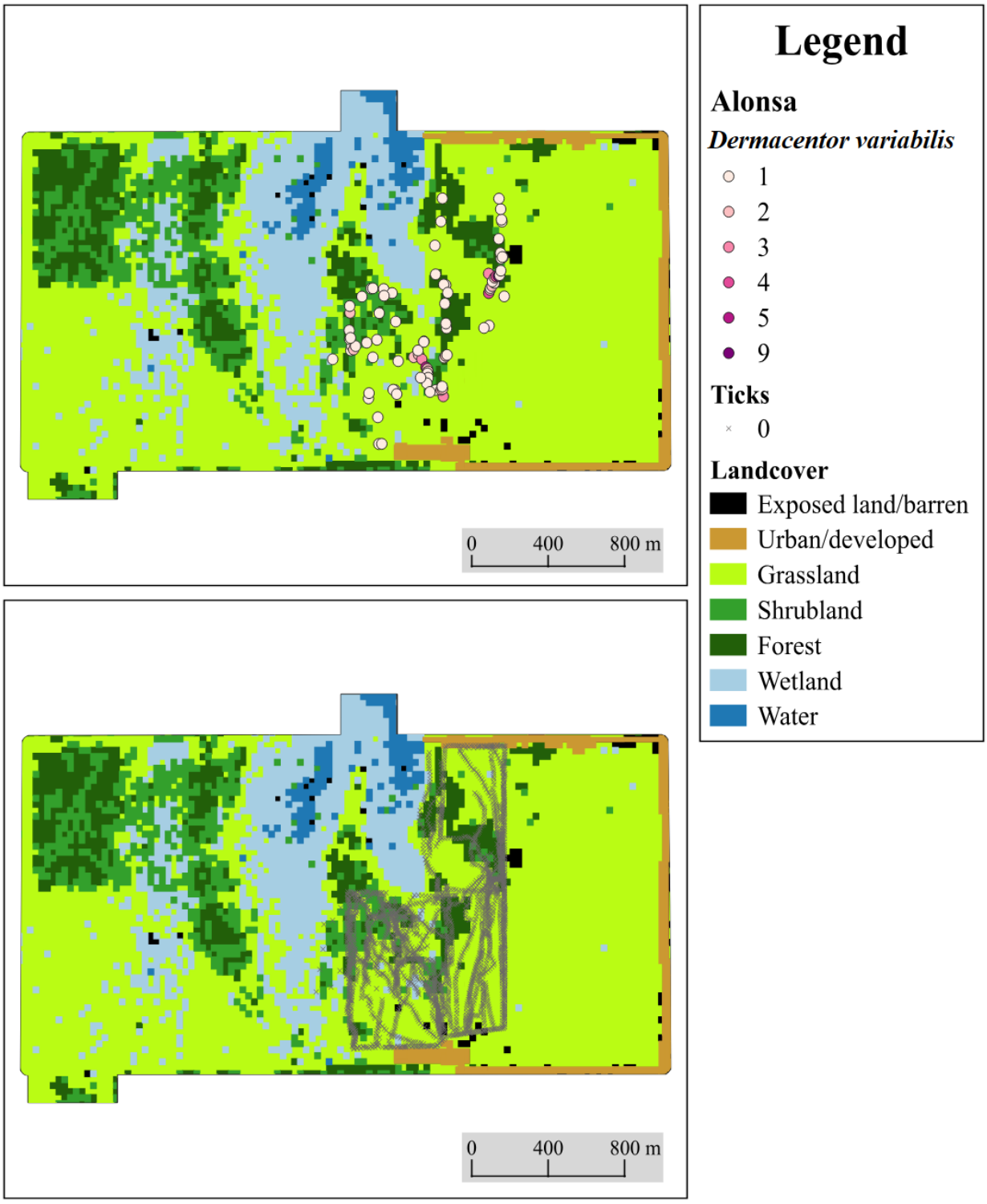

Figure 13. Top: Spatial distribution of *Dermacentor variabilis* (n = 124, circles) collected by drag sampling at Alonsa in 2021 and 2022. Bottom: Quadrats where no ticks were collected.

Brookdale

Brookdale was the third largest site of the seven sites, covering 3.28 km². Of the nine habitat types assessed, grassland was absent from Brookdale and was not included in the percentage calculations. Only one *I. scapularis* was detected at this site; therefore, habitat associations presented here are based on the 137 *D. variabilis* collected. Pasture/forages was the predominant habitat type, making up over 50% of the land cover and comprising most of the habitat composition across sampled, tick-positive, tick-negative, and random points categories (Figure 14). Land cover and random points distributions showed similar habitat composition, while sampled quadrats and tick-negative quadrats were comparable. Forest covered less than 5% of the total land cover but increased in tick-positive quadrats, accounting for approximately 10% of habitat composition.

A total of 192 *D. variabilis* and one *I. scapularis* were collected through drag sampling at Brookdale in 2021 and 2022. Tick abundance was relatively high, with counts ranging from one to six ticks per quadrat. Tick-positive quadrats were primarily located in the northern portion of the site and were associated mainly with pasture/forages, with wetland being the most common adjacent habitat type bordering these quadrats (Figure 15).

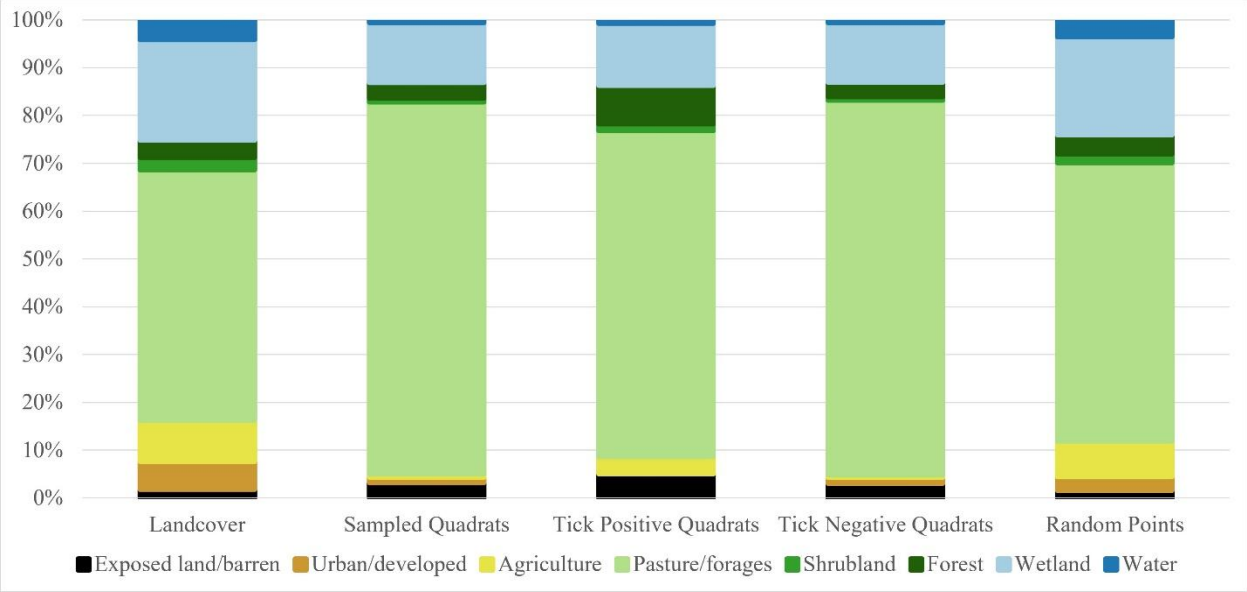

Figure 14. Proportional stacked bars representing the relative cover (%) of the eight habitat types at the Brookdale study site, associated with the total site land cover, sampled quadrats (areas sampled through tick dragging in 2021 and 2022), tick-positive quadrats (sampled quadrats containing *Dermacentor variabilis*), tick-negative quadrats (sampled quadrats where no ticks were detected), and random points (10,000 randomly generated points used for the unconstrained randomization analysis).

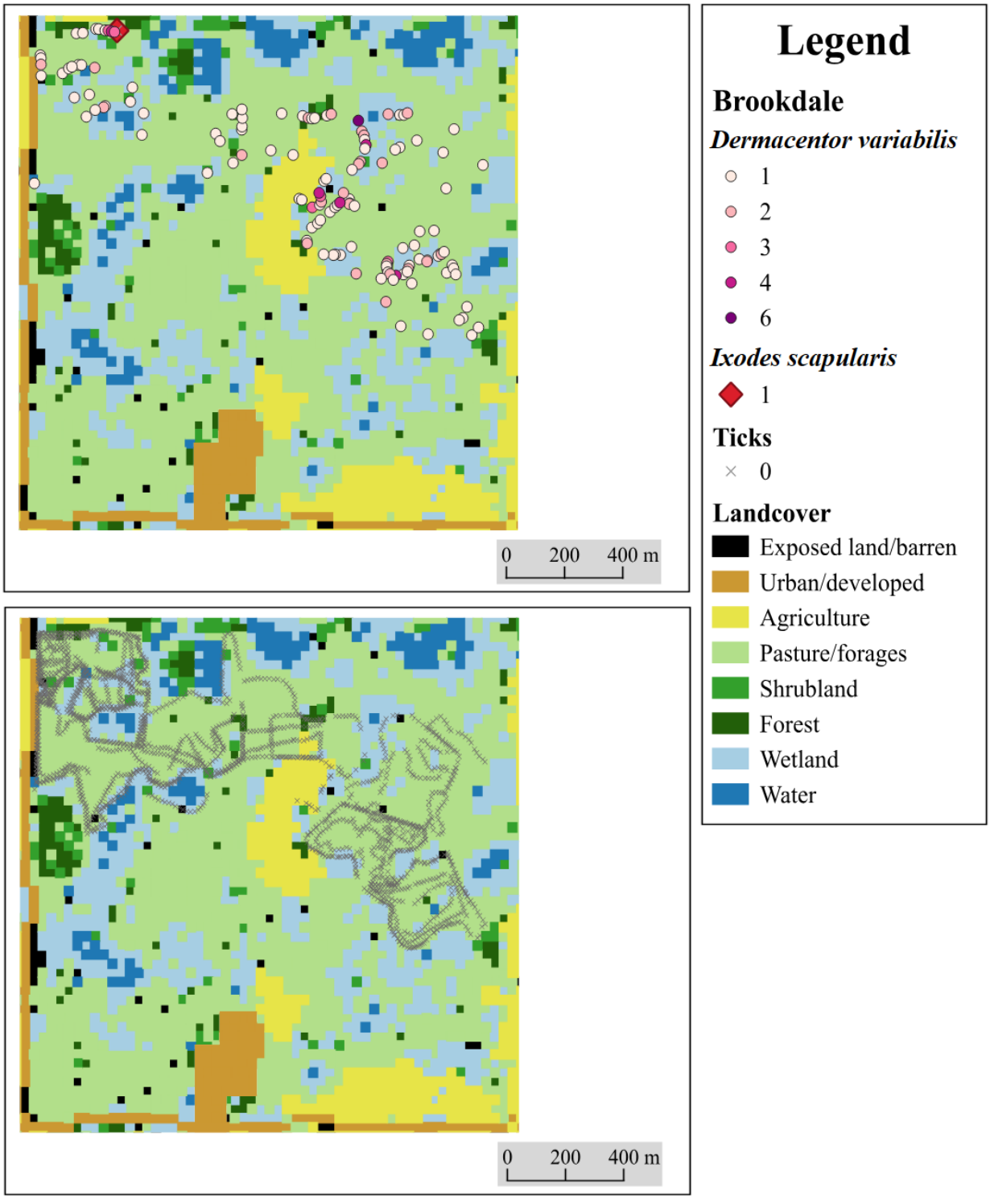

Figure 15. Top: Spatial distribution of *Dermacentor variabilis* (n = 192, circles) and *Ixodes scapularis* (n = 1, triangles) collected by drag sampling at Brookdale in 2021 and 2022. Bottom: Quadrats where no ticks were collected.

Darlingford

Darlingford was the smallest of the four cattle sites, with a total area of 1.56 km^2. Grassland, agriculture, wetland, or water classes were absent from this site and were not included in the calculations. Darlingford held a very small area of exposed land/barren, which may be difficult to discern in figures (Figure 16). The two most common habitat types at Darlingford were pasture/forages (>50%) and forest (~20%). Shrubland was the third most common habitat type, comprising ~5% of the overall site land cover and > 10% for tick-positive quadrats (Figure 10A).

A total of 218 *D. variabilis* were collected through drag sampling at Darlingford in 2021 and 2022. Tick abundance was high, with counts ranging from one to nine ticks per quadrat. Tick-positive quadrats were distributed throughout the site but occurred primarily in the northeastern portion. These quadrats were mainly associated with pasture/forages, with additional tick-positive quadrats occurring in shrubland and forest. Most tick-positive quadrats in pasture/forages were adjacent to forest (Figure 17).

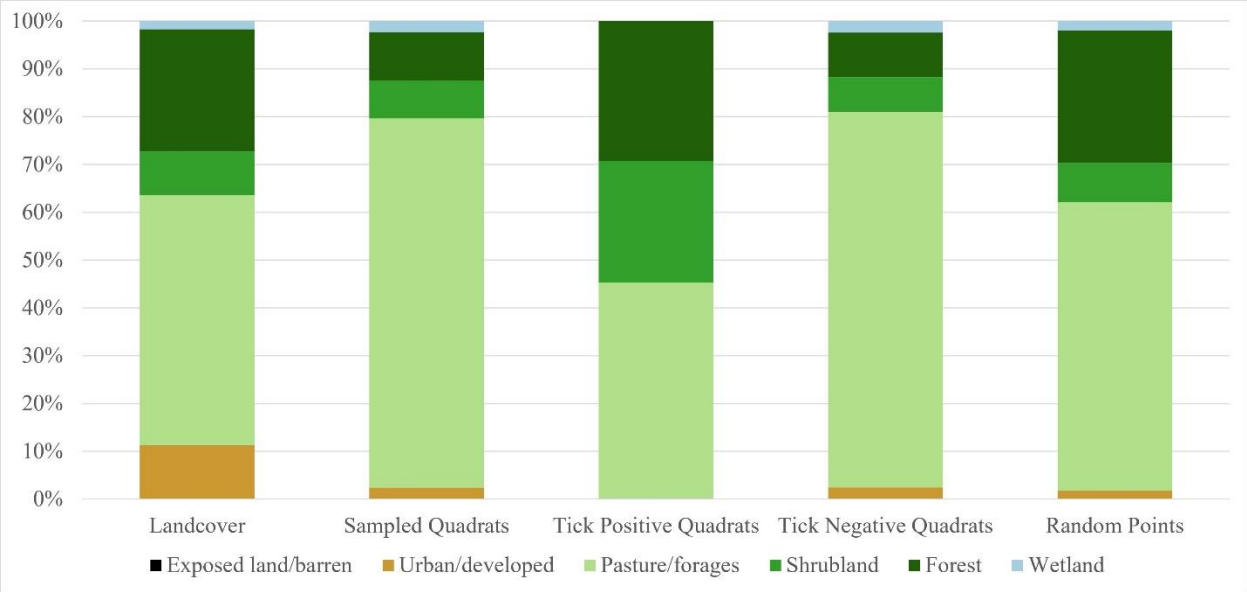

Figure 16. Proportional stacked bars representing the relative cover (%) of the four habitat types at the Darlingford study site, associated with the total site land cover, sampled quadrats (areas sampled through tick dragging in 2021 and 2022), tick-positive quadrats (sampled quadrats containing *Dermacentor variabilis*), tick-negative quadrats (sampled quadrats where no ticks were detected), and random points (10,000 randomly generated points used for the unconstrained randomization analysis).

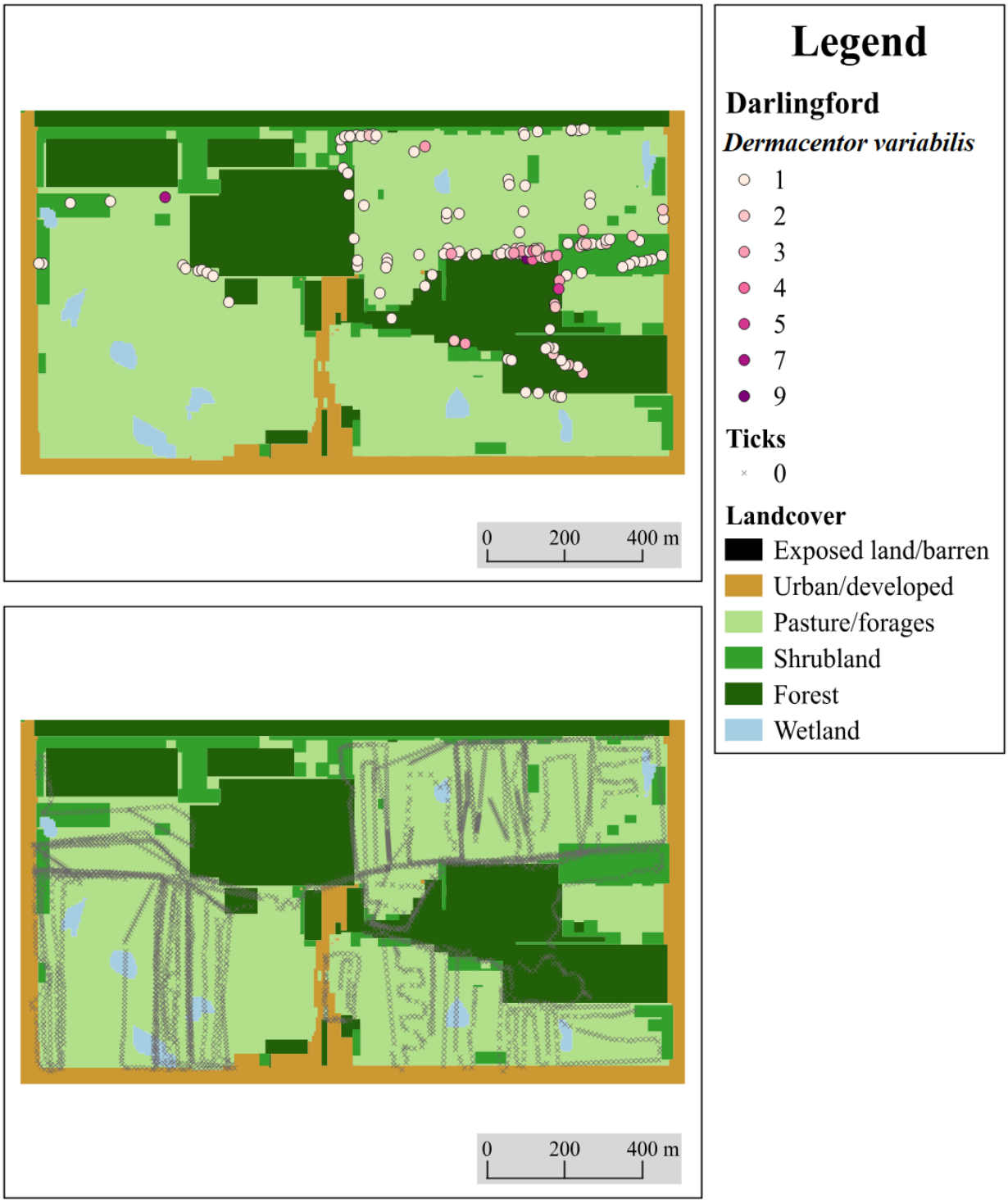

Figure 17. Top: Spatial distribution of *Dermacentor variabilis* (n = 218, circles) collected by drag sampling at Darlingford in 2021 and 2022. Bottom: Quadrats where no ticks were collected.

Johnson

Johnson was the second largest of the seven sites in this study, encompassing an area of 3.72 km². The grassland and forest habitat types were absent from Johnson and were excluded from the percentage calculation. Pasture/forages was the dominant habitat type, accounting for over 70% of the land cover and comprising most of the habitat composition across the other percentage categories (Figure 18). Although water represented a small portion of the total site land cover, it was the second-most common cover type associated with tick-positive quadrats.

A total of 10 *D. variabilis* were collected through drag sampling at Johnson in 2022 only. Tick abundance was very low, with counts ranging from one to two ticks per quadrat. Tick-positive quadrats were primarily clustered in the southeastern portion of the site, with one additional tick-positive quadrat in the northeast. These quadrats were primarily associated with pasture/forages and water and were located adjacent to dugouts, with urban/developed the most common adjacent habitat type (Figure 19).

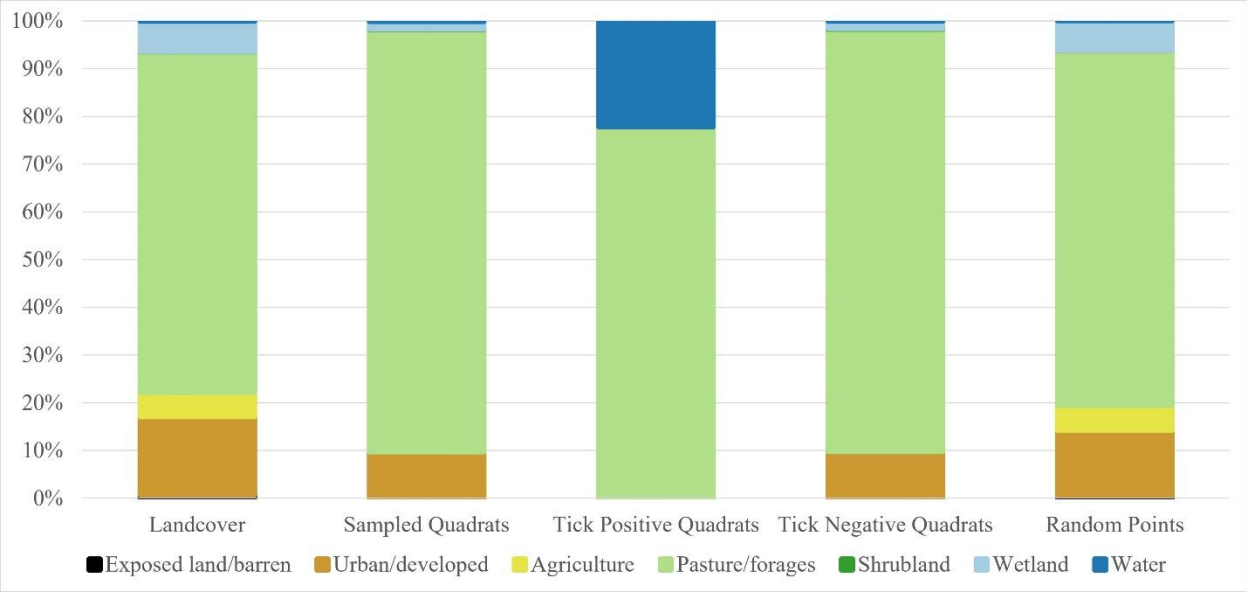

Figure 18. Proportional stacked bars representing the relative cover (%) of the seven habitat types at the Johnson study site, associated with the total site land cover, sampled quadrats (areas sampled through tick dragging in 2021 and 2022), tick-positive quadrats (sampled quadrats containing *Dermacentor variabilis*), tick-negative quadrats (sampled quadrats where no ticks were detected), and random points (10,000 randomly generated points used for the unconstrained randomization analysis).

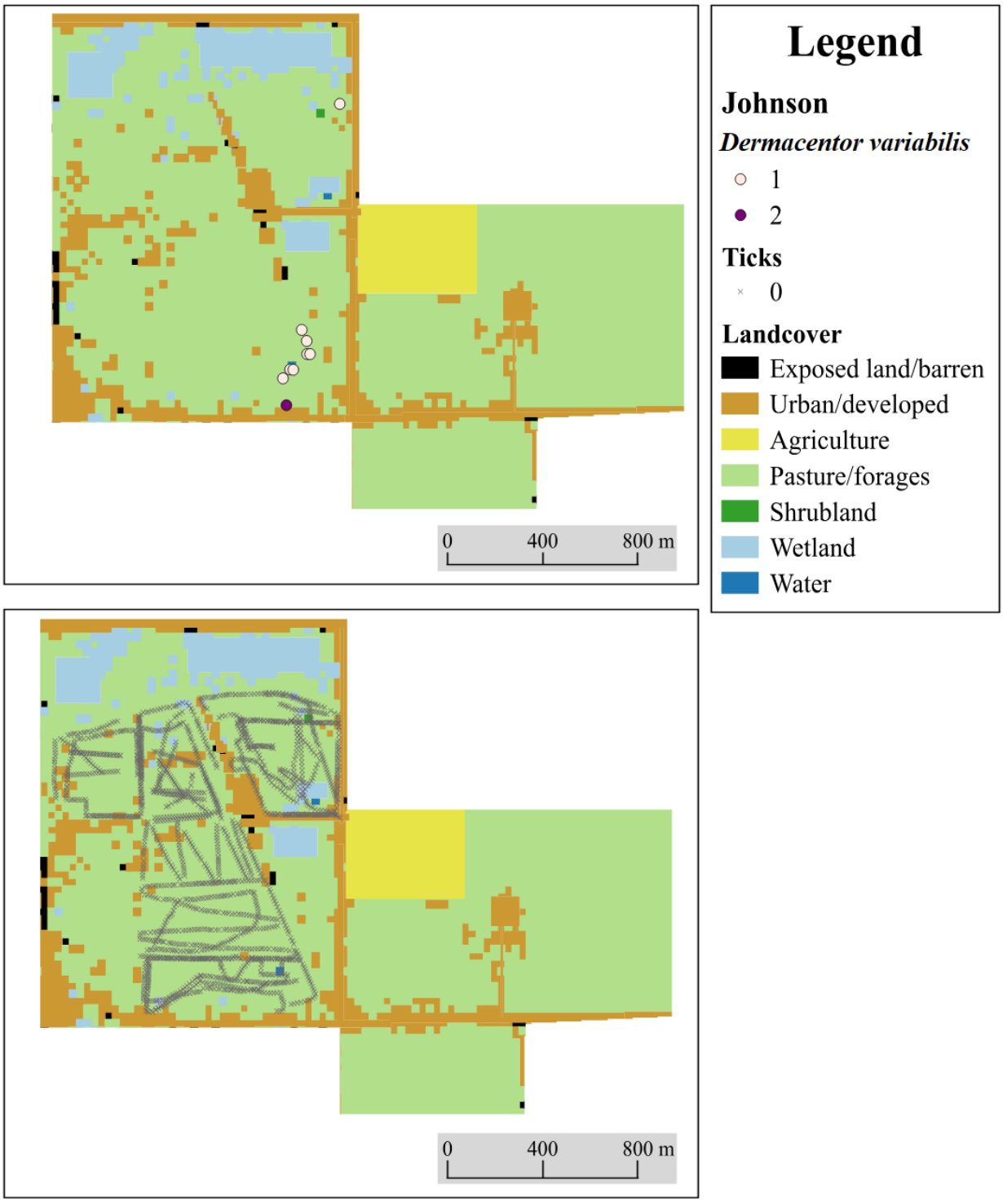

Figure 19. Top: Spatial distribution of Dermacentor variabilis (n = 10, circles) collected by drag sampling at Johnson in 2021 and 2022. Bottom: Quadrats where no ticks were collected.

Atkinson Farm

Atkinson Farm was the largest horse site and the fourth largest site overall, with a total land cover area of 1.72 km². Grassland was absent from this site and was excluded from the percentage calculations. Pasture/forages was the dominant habitat type within the site, accounting for approximately 55% of the total area, and represented a large proportion of habitat across the other sampling categories (Figure 20). Although forest occupied a smaller proportion of the overall site land cover, it represented a larger proportion of the sampled, tick-positive, and tick-negative quadrats.

A total of 16 *D. variabilis* were collected during drag sampling at Atkinson Farm 2022 only. Tick abundance was low, with counts ranging from one to two ticks per quadrat, and only one quadrat contained two ticks. Despite the low number of *D. variabilis*, tick-positive quadrats were primarily located in the central-western portion of the site and were mainly associated with forest and pasture/forages bordering shrubland and forest (Figure 21).

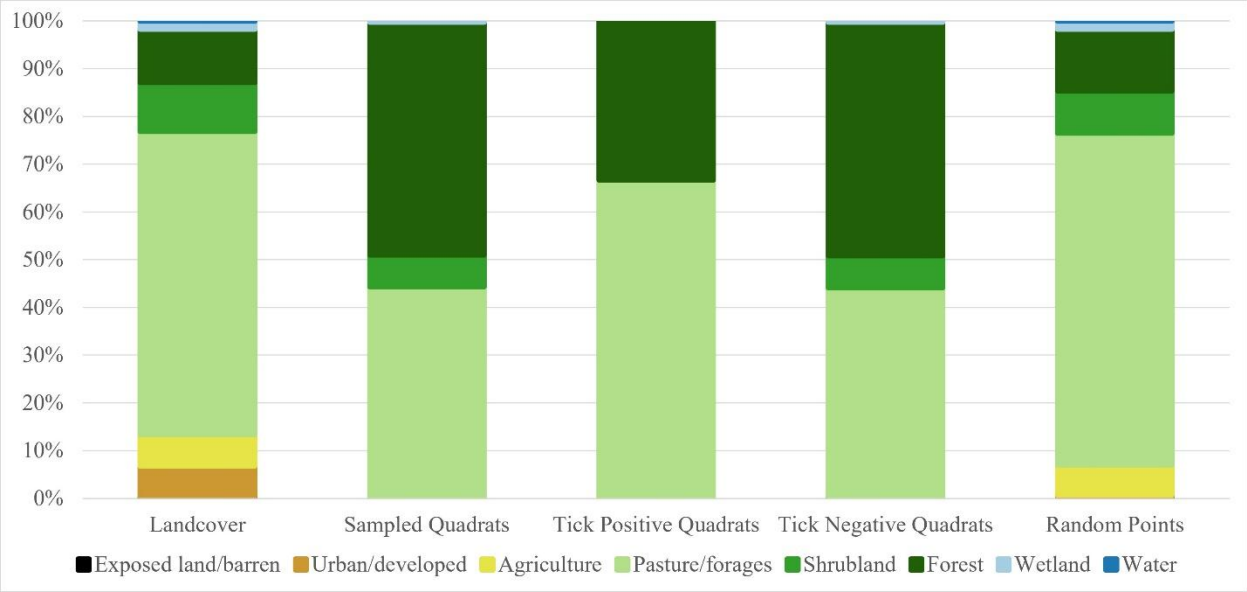

Figure 20. Proportional stacked bars representing the relative cover (%) of the eight habitat types at the Atkinson Farm study site, associated with the total site land cover, sampled quadrats (areas sampled through tick dragging in 2021 and 2022), tick-positive quadrats (sampled quadrats containing *Dermacentor variabilis*), tick-negative quadrats (sampled quadrats where no ticks were detected), and random points (10,000 randomly generated points used for the unconstrained randomization analysis).



Figure 21. Top: Spatial distribution of *Dermacentor variabilis* (n = 16, circles) collected by drag sampling at Atkinson Farm in 2021 and 2022. Bottom: Quadrats where no ticks were collected.

Dusty Oaks

Dusty Oaks was the second largest horse site and the second smallest site overall, covering 0.93 km². The site lacked agriculture and pasture/forages habitat types, so these were excluded from the percentage calculations. The predominant habitat types at the site were grassland and water, with grassland as most common across all categories. Although forest occupied a smaller proportion of the overall site land cover, it represented a larger proportion of the sampled, tick-positive, and tick-negative quadrats (Figure 22).

Dermacentor variabilis (n = 284) were collected at Dusty Oaks by drag sampling exclusively in 2022, and 13 *I. scapularis* were collected in 2021 and 2022. Tick abundance was very high, with counts ranging from one to nine ticks per quadrat. Tick-positive quadrats were mainly found in the central area of the site, especially west of the water body, and were primarily in grassland and forest, with some detections in shrubland. *D. variabilis*-positive quadrats mostly bordered forest, whereas *I. scapularis*-positive quadrats tended to occur near water (Figure 23).

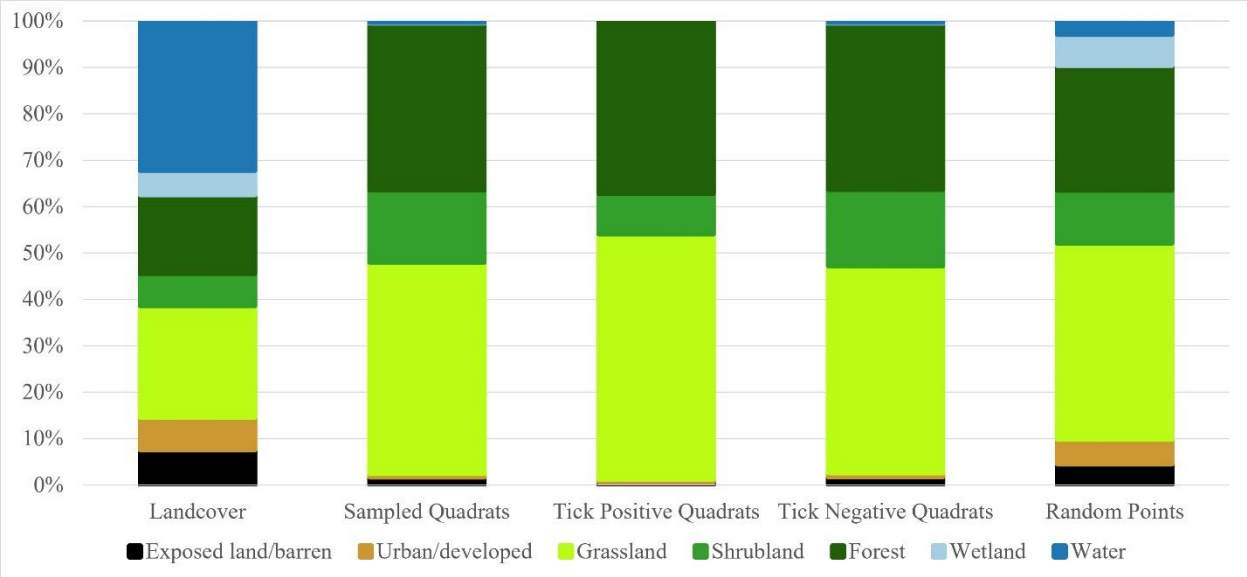

Figure 22. Proportional stacked bars representing the relative cover (%) of the seven habitat types at the Dusty Oaks study site, associated with the total site land cover, sampled quadrats (areas sampled through tick dragging in 2021 and 2022), tick-positive quadrats (sampled quadrats containing *Dermacentor variabilis* and *Ixodes scapularis*), tick-negative quadrats (sampled quadrats where no ticks were detected), and random points (10,000 randomly generated points used for the unconstrained randomization analysis).

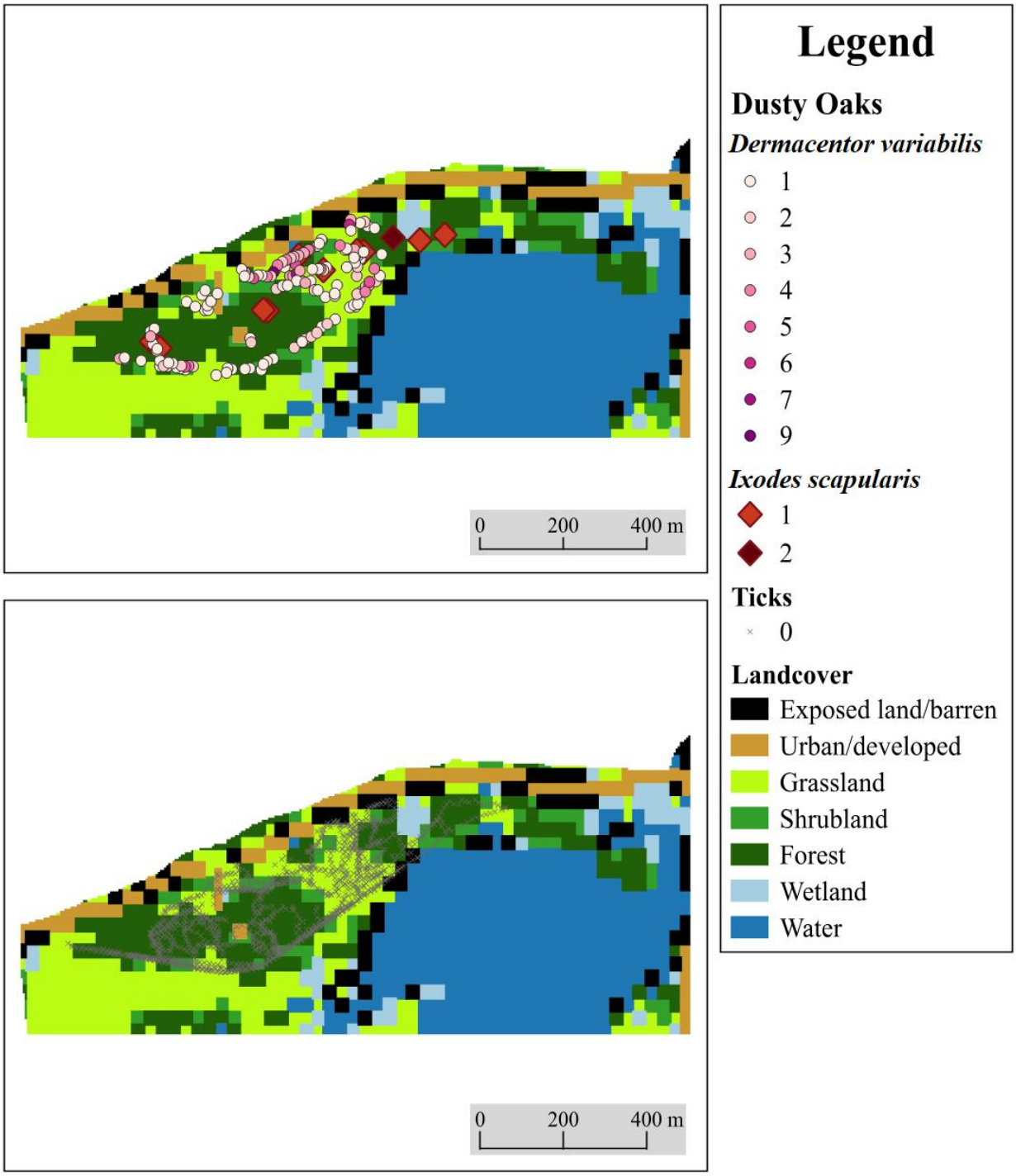

Figure 23. Top: Spatial distribution of *Dermacentor variabilis* (n = 284, circles) and *Ixodes scapularis* (n = 13, triangles) collected by drag sampling at Dusty Oaks in 2021 and 2022. Bottom: Quadrats where no ticks were collected.

Gould Farm

Gould Farm was the smallest site of the seven study sites, with a total area of 0.086 km². Exposed land/barren and pasture/forages were absent from this site and were therefore excluded from the percentage calculations. Forest was the dominant habitat type, accounting for approximately 50% of the site's land cover and representing the largest habitat category across the percentage groups (Figure 24). Agriculture was the second most abundant habitat type, comprising approximately 25% of the site. Urban/developed habitat were more common among tick-positive quadrats, accounting for approximately 15%, compared with only 5% of the overall site land cover and of sampled quadrats.

A total of 75 *D. variabilis* were collected during drag sampling at Gould Farm in 2022 only. Tick abundance was low, with counts ranging from one to three ticks per quadrat. Tick-positive quadrats were distributed throughout the site but occurred primarily in the northern portion, extending through the central and eastern areas. These quadrats were mainly associated with forest, with some detections in agriculture and urban/developed habitats, and several near forest and water (Figure 25).

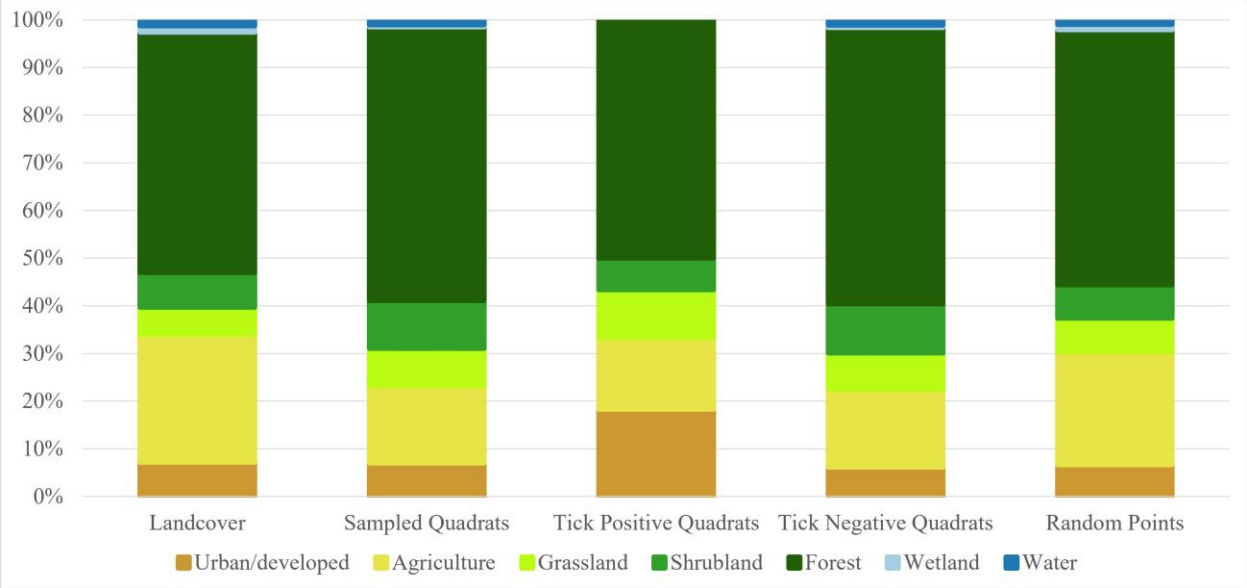

Figure 24. Proportional stacked bars representing the relative cover (%) of the seven habitat types present at the Gould Farm study site, associated with the total site land cover, sampled quadrats (area sampled by tick dragging in 2021 and 2022), tick-positive quadrats (sampled quadrats containing Dermacentor variabilis), tick-negative quadrats (sampled quadrats where no ticks were detected), and random points (10,000 randomly generated points used for the unconstrained randomization analysis).



Figure 25. Top: Spatial distribution of *Dermacentor variabilis* (n = 75, circles) collected by drag sampling at Gould Farm in 2021 and 2022. Bottom: Quadrats where no ticks were collected.

Land Cover Analysis

Habitat Composition

The proportion of each habitat type varied across the seven study sites (Figure 26), but pasture/forages or grassland were predominant at most sites. Cattle sites generally had more pasture/forage and grassland, with Brookdale, Darlingford, and Johnson dominated by pasture/forage, while Alonsa had the largest proportion of grassland. Horse sites were more variable in their land cover composition. Atkinson Farm was similar to the cattle sites, with pasture/forage making up most of the site, while Dusty Oaks had a more mixed composition, with larger areas of water, grassland, forest, and pasture/forage. Gould Farm differed most from the other sites, with large proportions of forest and agriculture and relatively little pasture/forage or grassland. Overall, the cattle sites were more similar to each other and were generally dominated by pasture/forage or grassland, whereas the horse sites were more variable, with some sites containing larger proportions of forest, agriculture, or water.

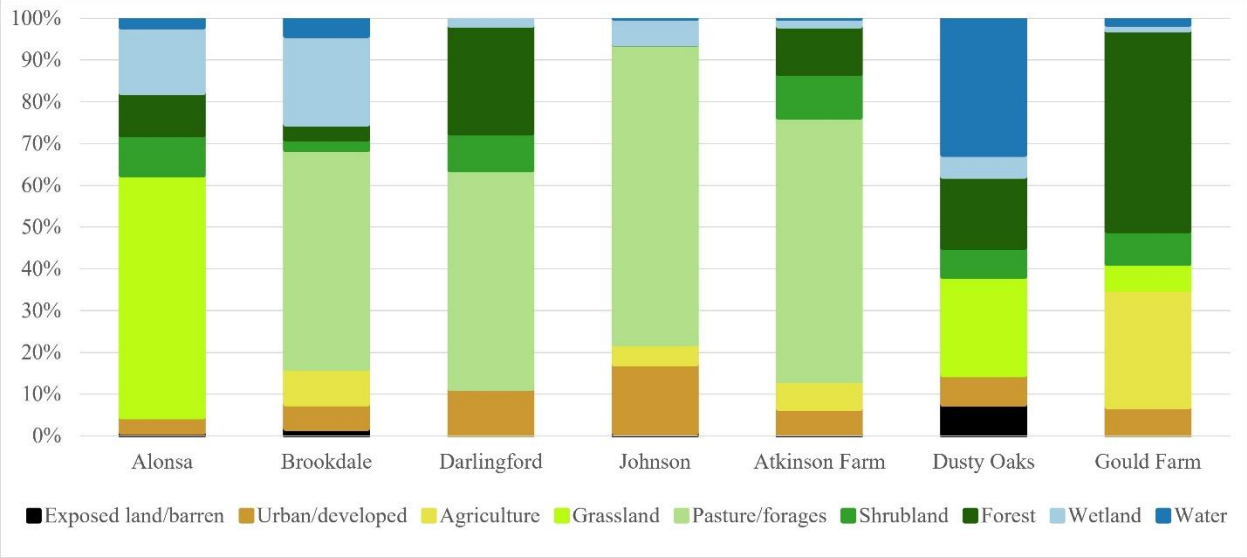

Figure 26. Proportional stacked bars representing the relative cover (%) of the nine habitat types at the seven study sites. Cattle grazed the first four sites, and horses grazed the last three.

Multivariate Analysis

Correspondence Analysis

A Correspondence Analysis (CA) was conducted to examine patterns in habitat composition among habitat types, study sites, sampled quadrats, tick-positive quadrats, tick-negative quadrats, and random quadrats (Figure 27-Figure 29). The first two axes accounted for 68.99% of the total variation, with CA1 explaining 46.69% and CA2 explaining 22.30% of the variation. Because these axes represented the largest proportion of the variation in the dataset, CA1 and CA2 were used to visualize the primary patterns in habitat associations among study sites and quadrats. For clarity of interpretation, this CA is presented with ordination scores for total site cover, tick and no-tick and sampled and random quadrats on separate plots.

Although each CA figure highlights a different sample type, the land cover habitat scores are common to all three (Figure 27-Figure 29). The pattern across the ordination space has pasture/forages, wetland, and urban/ developed associated with negative scores on CA1 (left side of the plot), while grassland, shrubland, exposed land/barren, and water are associated with positive scores (right side of the plot). Forest and agriculture are strongly positive on CA2, while water was strongly negative.

When examining study sites ("Site") ordination scores alone, they were generally positioned near their dominant habitat types ("Habitat") within the ordination space (Figure 27). Sites positioned near a habitat type indicate that habitat was predominant at those sites. Alonsa and Dusty Oaks were associated with grassland habitat. Brookdale, Johnson, Darlingford and Atkinson Farm were all positioned near pasture/forages, with Brookdale also associated with wetland, while Darlingford and Atkinson Farm were associated with urban/developed habitat. Gould Farm was an outlier associated with agriculture and forest. Overall, land cover habitat type patterns in the ordinations were consistent with the differences in habitat composition among sites summarized in Figure 26.



Figure 27. Correspondence analysis plot showing the ordination locations of total cover of habitat types (black ×) by site (orange circles) association showing variation in habitat composition among the seven sites (orange). Proximity between points reflects the strength of the association.

Tick-positive (“Tick”) and tick-negative (“No Tick”) quadrats for a given site are closer together on the ordination plot when their overall habitat composition is similar and further apart when it differs (Figure 28). Tick-positive and tick-negative quadrats generally clustered within the ordination space, with quadrats from the same study site generally plotting near one another. These quadrats also tended to cluster near their respective study sites in Figure 27. Tick-positive and tick-negative quadrats separated at all sites, indicating differences in habitat composition between these quadrat types. Of these, tick and no-tick quadrats at Atkinson, Darlingford and Johnson showed the greatest compositional differences, whereas those at Gould and Dusty Oaks showed the least. However, the direction of separation between tick and no-tick quadrats varies by site, indicating that habitat composition differences are site-specific rather than reflecting a consistent shift toward a particular habitat type.

The pooled sampled quadrats are compared with random quadrats in Figure 29. The random points represented the range of available habitat types across the study areas, whereas the sampled quadrats represented the habitat composition of the areas where sampling occurred. The clustering of sampled and random quadrats suggests that sampled quadrats generally represented the range of habitat types available within the study areas, rather than a distinct subset of habitats. The direction of separation between sampled and random quadrats varied among sites, indicating no consistent compositional shift or sampling-related bias. Sampled and random quadrats showed the greatest compositional differences at Atkinson and Dusty Oaks, while Gould, Darlingford, and Brookdale showed relatively little separation. Overall, the CA results demonstrate differences in habitat composition among study sites, while indicating that the sampled areas generally reflected the available habitat composition.

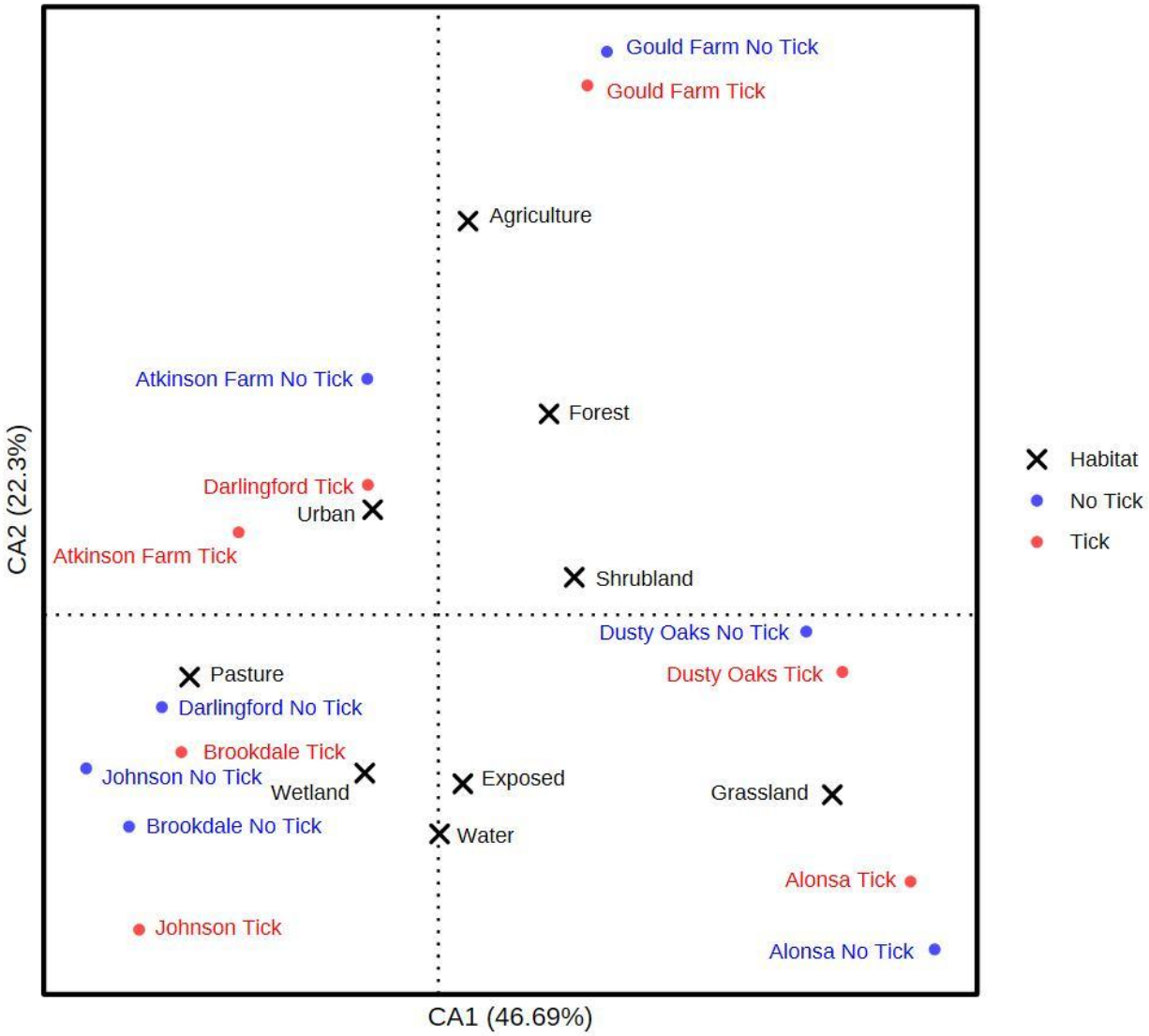

Figure 28. Correspondence analysis of the nine habitat types (black ×) showing variation in habitat composition between the tick-positive quadrats (red circles) and the tick-negative quadrats (blue circles) across all sites.

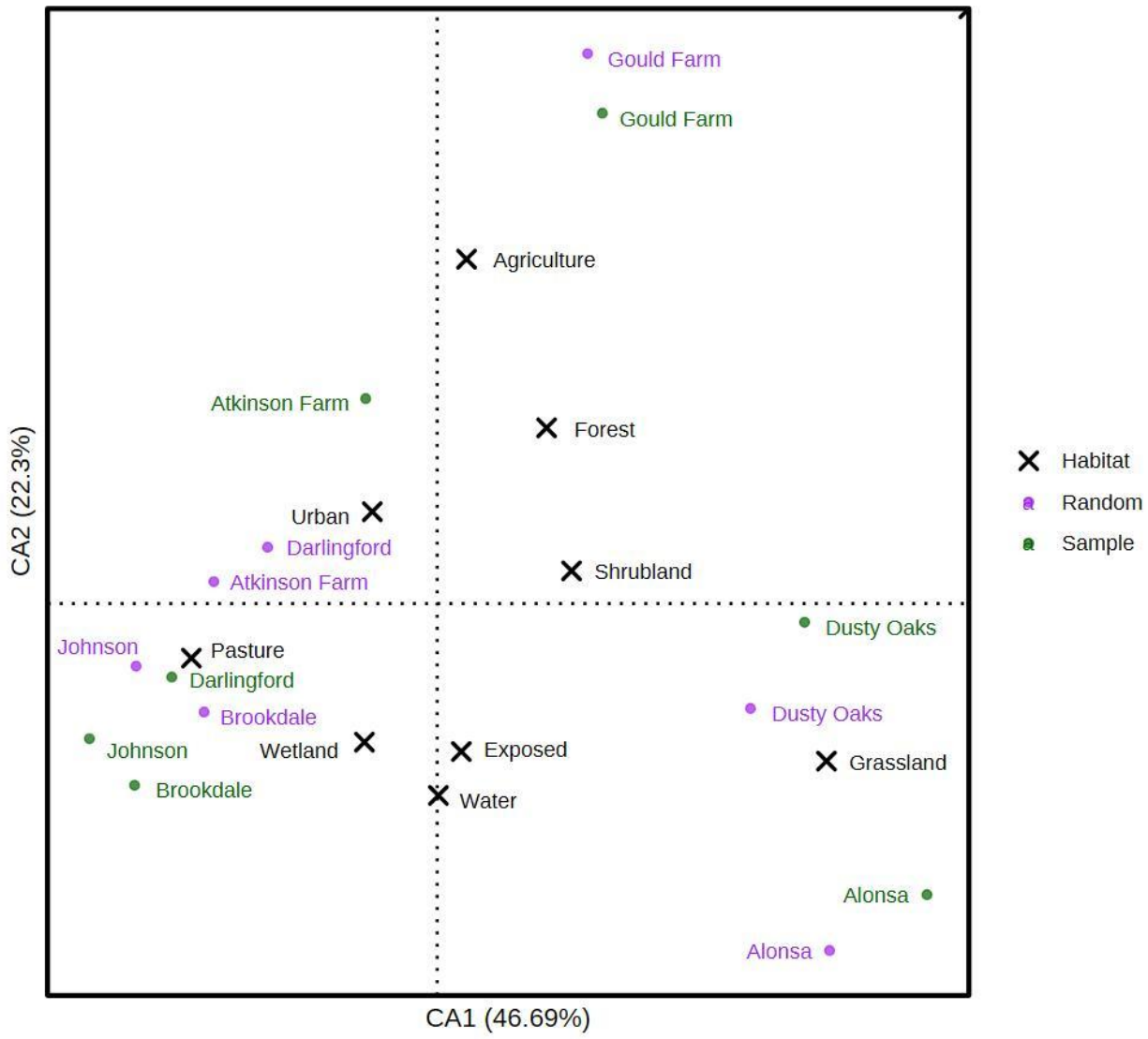

Figure 29. Correspondence analysis of the nine habitat types (black ×) showing variation in habitat composition between random samples (purple circles) and the pooled sampled quadrats (green circles) across all sites.

Habitat Distance Analysis and Randomization

Mean distances from tick-positive quadrats to the nine focal habitat types were compared with distances generated from two randomization approaches to assess whether *D. variabilis* or *I. scapularis* occurred closer to or farther from particular habitat types than expected by chance. The transect-constrained approach compared tick-positive quadrats with randomly selected tick-negative quadrats, whereas the unconstrained approach compared tick-positive quadrats with randomly generated locations across the study area. The results are summarized in Table 13.

At Alonsa, 88 quadrats were positive for *D. variabilis* (June 2021 and 2022). Across both randomization approaches, several consistent patterns were observed (

Table ***5***). Tick-positive quadrats were significantly closer to shrubland ($p < 0.0001$, $p < 0.0001$), forest ($p < 0.0001$, $p < 0.0001$), and water ($p = 0.0137$, $p = 0.0334$) under transect-constrained and unconstrained randomization, respectively. Tick-positive quadrats were significantly farther from grassland ($p = 0.0123$) and urban/developed habitat ($p = 0.0003$) under transect-constrained randomization, but significantly closer to these habitats under unconstrained randomization ($p < 0.0001$, $p < 0.0001$, respectively). Tick-positive quadrats were significantly farther from exposed land/barren under unconstrained randomization ($p = 0.0002$), although this difference was not significant under transect-constrained randomization ($p = 0.4407$). There were no differences in distance to wetland under either randomization approach ($p = 0.4700$, $p = 0.0621$). The consistent association of tick-positive quadrats with shrubland, forest, and water across both randomization approaches may indicate that these habitat types were associated with *D. variabilis* at Alonsa.

At Brookdale, 137 quadrats contained *D. variabilis*. When the tick-positive quadrats were compared using both randomization approaches, several similar patterns were observed (Table 6). Tick-positive quadrats were significantly closer to agriculture ($p < 0.0001$, $p = 0.0031$) and wetland ($p = 0.0001$, $p = 0.0001$), and significantly farther from urban/developed habitat ($p < 0.0001$, $p < 0.0001$) and water ($p < 0.0001$, $p = 0.0072$) under transect-constrained and unconstrained randomization, respectively. Tick-positive quadrats were significantly farther from pasture/forages than random quadrats without ticks ($p < 0.0001$), but significantly closer to that habitat than randomly selected points ($p < 0.0001$). Tick-positive quadrats were significantly closer to forest under transect-constrained randomization ($p = 0.0157$), although this difference

was not significant under unconstrained randomization (p = 0.1361). Similarly, tick-positive quadrats were significantly closer to shrubland under unconstrained randomization (p = 0.0076), although this difference was not significant under transect-constrained randomization (p = 0.2971). There was no difference in distance to exposed land/barren under either randomization approach (p = 0.4826, p = 0.3076). The consistent association of tick-positive quadrats with agriculture and wetland, and their greater distance from urban/developed habitat and water across both randomization approaches, may indicate that these habitat types were associated with *D. variabilis* at Brookdale.

Table 5. Mean distances (in meters) between *Dermacentor variabilis*-positive quadrats (n = 88) and habitat types at Alonsa, compared to randomly selected tick-negative quadrats (n = 88) and 10,000 random points.

	Tick Positive Quadrats	**Transect Constrained Randomization**		**Unconstrained Randomization**	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	223.07	220.96	0.4407	298.30	0.0002
Urban/developed	500.22	419.21	0.0003	778.08	0.0000
Agriculture	-	-	-	-	-
Grassland	4.88	2.93	0.0123	15.82	0.0000
Pasture/forages	-	-	-	-	-
Shrubland	35.30	87.14	0.0000	146.25	0.0000
Forest	30.50	67.33	0.0000	143.07	0.0000
Wetland	147.55	148.67	0.4700	124.35	0.0621
Water	473.05	513.16	0.0137	549.20	0.0334

Table 6. The mean distance (in meters) between *Dermacentor variabilis*-positive quadrats (n = 137) and habitat types at Brookdale, compared to randomly selected tick-negative quadrats (n = 137) and to 10,000 random points.

	Tick Positive Quadrats	Transect Constrained Randomization		Unconstrained Randomization	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	134.40	134.62	0.4826	138.37	0.3076
Urban/developed	645.57	517.06	0.0000	450.25	0.0000
Agriculture	148.48	193.31	0.0000	177.96	0.0031
Grassland	-	-	-	-	-
Pasture/forages	3.41	1.35	0.0000	9.55	0.0000
Shrubland	121.87	118.13	0.2971	107.02	0.0076
Forest	113.41	126.78	0.0157	106.23	0.1361
Wetland	20.47	28.05	0.0001	30.18	0.0001
Water	156.61	125.00	0.0000	134.53	0.0072

There were 136 *D. variabilis*-positive quadrats at Darlingford. Comparing tick-positive quadrats using both randomization approaches revealed several consistent associations with habitat types (Table 7). Under both transect-constrained and unconstrained randomization, tick-positive quadrats were significantly closer to exposed land/barren ($p < 0.0001$, $p < 0.0001$), shrubland ($p < 0.0001$, $p < 0.0001$), and forest ($p < 0.0001$, $p < 0.0001$) than random quadrats or points. In contrast, tick-positive quadrats were significantly farther from urban/developed habitat ($p = 0.0005$, $p < 0.0001$) and wetland ($p < 0.0001$, $p = 0.0065$) under both approaches. Tick-positive quadrats were significantly farther from pasture/forages under transect-constrained randomization ($p < 0.0001$), although this difference was not significant under unconstrained randomization ($p = 0.0946$). These patterns suggest that *D. variabilis* may favour areas near exposed land/barren, shrubland, and forest at Darlingford., and tend to be less associated with urban/developed areas and wetland habitat.

Table 7. Mean distances (in meters) between *Dermacentor variabilis*-positive quadrats (n = 136) and habitat types at Darlingford, compared to randomly selected tick-negative quadrats (n = 136) and to 10,000 random points.

	Tick Positive Quadrats	Transect Constrained Randomization		Unconstrained Randomization	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	747.88	1059.25	0.0000	962.20	0.0000
Urban/developed	209.37	177.22	0.0005	149.00	0.0000
Agriculture	-	-	-	-	-
Grassland	-	-	-	-	-
Pasture/forages	12.86	3.11	0.0000	15.73	0.0946
Shrubland	32.50	73.41	0.0000	65.90	0.0000
Forest	42.18	96.63	0.0000	92.66	0.0000
Wetland	191.42	147.38	0.0000	177.07	0.0065
Water	-	-	-	-	-

For the Johnson site, *D. variabilis* was collected in nine quadrats in June 2022. When the tick-positive quadrats were compared using both randomization approaches, several similar patterns were observed (Table 8). Tick-positive quadrats were significantly closer to agriculture ($p = 0.0015$, $p = 0.0235$), pasture/forages ($p < 0.0001$, $p < 0.0001$), and water ($p < 0.0001$, $p < 0.0001$) under transect-constrained and unconstrained randomization, respectively. Tick-positive quadrats were significantly farther from urban/developed habitat ($p = 0.0053$) and wetland ($p = 0.0186$) under transect-constrained randomization; however, the difference for urban/developed habitat was not significant under unconstrained randomization ($p = 0.2227$), while tick-positive quadrats were significantly closer to wetland ($p = 0.0068$). There were no differences in distance to exposed land/barren ($p = 0.2693$, $p = 0.2500$) or shrubland ($p = 0.1901$, $p = 0.3434$) under either randomization approach. The consistent association of tick-positive quadrats with agriculture, pasture/forages, and water across both randomization approaches may indicate that

these habitat types were associated with *D. variabilis* at Johnson, but very few ticks were collected at this site.

Table 8. Mean distances (in meters) between *Dermacentor variabilis*-positive quadrats (n = 9) and habitat types at Johnson, compared to randomly selected tick-negative quadrats (n = 9) and to 10,000 random points.

	Tick Positive Quadrats	Transect Constrained Randomization		Unconstrained Randomization	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	223.93	207.26	0.2693	270.81	0.2500
Urban/developed	121.16	73.65	0.0053	94.47	0.2227
Agriculture	385.12	599.37	0.0015	617.34	0.0235
Grassland	-	-	-	-	-
Pasture/forages	0.00	0.85	0.0000	9.92	0.0000
Shrubland	888.91	782.36	0.1901	942.08	0.3434
Forest	-	-	-	-	-
Wetland	218.49	136.31	0.0186	408.85	0.0068
Water	98.69	363.80	0.0000	687.09	0.0000

At the Atkinson Farm site, there were 15 *D. variabilis*-positive quadrats (June 2022). Tick-positive quadrats were significantly closer to exposed land/barren ($p < 0.0001$, $p = 0.0003$) and wetland ($p = 0.0003$, $p = 0.0005$), and farther from agriculture ($p = 0.0276$, $p < 0.0001$) and water ($p = 0.0006$, $p = 0.0064$) under transect-constrained and unconstrained randomization, respectively (Table 9). When compared to random points, tick-positive quadrats were significantly closer to forest ($p < 0.0001$) and shrubland ($p = 0.0288$), although these differences when comparing with randomly selected tick-negative transects ($p = 0.1482$, $p = 0.4419$, respectively). There were no differences in distance to urban/developed habitat ($p = 0.3075$, $p = 0.1167$) or pasture/forages ($p = 0.2787$, $p = 0.2772$) under either randomization approach. The

consistent association of tick-positive quadrats with exposed land/barren and wetland, and their greater distance from agriculture and water across both randomization approaches, may indicate that these habitat types were associated with *D. variabilis* at Atkinson Farm.

Table 9. Mean distances (in meters) between *Dermacentor variabilis*-positive quadrats (n = 15) and habitat types at Atkinson Farm, compared to randomly selected tick-negative quadrats (n = 15) and to 10,000 random points.

	Tick Positive Quadrats	**Transect Constrained Randomization**		**Unconstrained Randomization**	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	141.56	248.80	0.0000	262.09	0.0003
Urban/developed	279.23	303.42	0.3075	366.54	0.1167
Agriculture	584.47	493.07	0.0276	315.40	0.0000
Grassland	-	-	-	-	-
Pasture/forages	5.18	7.17	0.2787	8.71	0.2772
Shrubland	54.21	52.85	0.4419	92.90	0.0288
Forest	16.70	30.75	0.1482	154.55	0.0000
Wetland	97.42	182.63	0.0003	231.70	0.0005
Water	926.70	799.97	0.0006	721.06	0.0064

Both *D. variabilis* and *I. scapularis* were collected during drag sampling at Dusty Oaks; therefore, the two tick species were analyzed separately using both transect-constrained and unconstrained randomization approaches. At Dusty Oaks, there were 11 *I. scapularis*-positive quadrats. When *I. scapularis* tick-positive quadrats were compared using both randomization approaches (Table 10) tick-positive quadrats were significantly farther from grassland ($p = 0.0078$, $p = 0.0313$), and significantly closer to forest ($p = 0.0481$, $p = 0.0058$). There were no differences in distance to any other habitat types.

Table 10. Mean distance (in meters) between *Ixodes scapularis*-positive quadrats (n = 11) and habitat types at Dusty Oaks, compared to randomly selected tick-negative quadrats (n = 11) and to 10,000 random points.

	Tick Positive Quadrats	Transect Constrained Randomization		Unconstrained Randomization	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	70.88	90.63	0.1353	65.13	0.3632
Urban/developed	77.95	81.57	0.4143	65.39	0.2432
Agriculture	-	-	-	-	-
Grassland	28.24	11.69	0.0078	15.86	0.0313
Pasture/forages	-	-	-	-	-
Shrubland	25.27	21.37	0.2540	37.43	0.1797
Forest	4.69	13.10	0.0481	18.90	0.0058
Wetland	102.60	108.14	0.3655	102.82	0.5012
Water	133.70	192.80	0.0542	198.68	0.1036

There were also 150 *D. variabilis*-positive quadrats (May and June 2022). When mean distances from those quadrats to various habitat types were compared, distinct patterns emerged (Table 11). In contrast with *I. scapularis*-positive transects, those containing *D. variabilis* were significantly closer to grassland ($p < 0.0001$, $p < 0.0001$) using both randomization approaches. They were also closer to water ($p < 0.0001$, $p = 0.0002$) and significantly farther from wetland ($p < 0.0001$, $p < 0.0001$) than randomly selected negative transects or points. *Dermacentor*-positive quadrats were significantly closer to exposed land/barren under transect-constrained randomization ($p = 0.0001$) but farther from this habitat when random points were selected across the entire landscape ($p = 0.0022$). When compared to random points (unconstrained randomization), transects containing *D. variabilis* were significantly closer to forest ($p = 0.0029$) and shrubland ($p < 0.0001$) and farther from urban/developed habitat ($p = 0.0106$) (Table 11).

The contrast in association between the two tick species with grasslands, and the weaker association of *D. variabilis* with forest habitat compared to *I. scapularis*, suggests the two species were found in different habitats at Dusty Oaks.

Table 11. Mean distance (in meters) between *Dermacentor variabilis*-positive quadrats (n = 150) and habitat types at Dusty Oaks, compared to randomly selected tick-negative quadrats (n = 150) and to 10,000 random points.

	Tick Positive Quadrats	Transect Constrained Randomization		Unconstrained Randomization	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	80.89	93.36	0.0001	65.26	0.0022
Urban/developed	77.57	74.69	0.1147	65.68	0.0106
Agriculture	-	-	-	-	-
Grassland	6.62	13.86	0.0000	15.79	0.0000
Pasture/forages	-	-	-	-	-
Shrubland	20.76	19.29	0.0622	37.39	0.0000
Forest	14.01	15.24	0.1125	18.93	0.0029
Wetland	131.23	109.96	0.0000	103.06	0.0000
Water	151.87	193.21	0.0000	198.38	0.0002

Dermacentor variabilis was collected only in June 2022 at Gould Farm, with 60 tick-positive quadrats. Tick-positive quadrats were significantly closer to grassland ($p = 0.0093$, $p = 0.0017$), wetland ($p = 0.0027$, $p = 0.0002$), and water ($p = 0.0025$, $p = 0.0001$) under both randomization approaches (Table 12). Under transect-constrained randomization, tick-positive quadrats were farther from forest ($p = 0.0494$), but this difference was not significant under unconstrained randomization ($p = 0.2295$). Distance to agriculture, shrubland, and urban/developed habitat did not differ under either randomization approach. The consistent association of tick-positive quadrats with grassland, wetland, and water across both

randomization approaches may indicate that these habitat types were associated with habitat selection by *D. variabilis* at Gould Farm.

Table 12. Mean distance (m) between *Dermacentor variabilis*-positive quadrats (n = 60) and habitat types at Gould Farm, compared to randomly selected tick-negative quadrats (n = 60) and to 10,000 random points.

	Tick Positive Quadrats	Transect Constrained Randomization		Unconstrained Randomization	
Habitat Type	**Mean distance**	**Mean distance**	**P-value**	**Mean distance**	**P-value**
Exposed land/barren	-	-	-	-	-
Urban/developed	35.08	34.63	0.4356	38.73	0.1777
Agriculture	16.25	19.84	0.0746	18.04	0.2791
Grassland	30.59	36.24	0.0093	40.99	0.0017
Pasture/forages	-	-	-	-	-
Shrubland	30.16	26.39	0.0722	32.69	0.2193
Forest	5.82	4.48	0.0494	6.84	0.2295
Wetland	79.30	96.75	0.0027	106.76	0.0002
Water	74.92	90.09	0.0025	97.94	0.0001

Across sites, *D. variabilis*-positive quadrats showed variable associations with habitat types (Table 13). Forest, shrubland, and exposed/barren habitats were frequently associated with tick-positive quadrats, although these patterns were not always consistent between randomization approaches or sites. Ticks tended to be associated with grasslands when that habitat type was present at a site. Tick-positive quadrats were generally located farther from urban/developed habitats than random. The association of tick-positive quadrats with agriculture, pastures, wetland or water varied by site.

Table 13. Summary of comparisons of the mean distance (m) between tick-positive quadrats and habitat types compared to randomly selected tick-negative quadrats (left) or 10,000 random points (right).

Site	Exposed/ Barren	Urban/ Developed	Agricul- ture	Grassland	Pasture/ Forages	Shrubland	Forest	Wetland	Water
Alonsa	n.s. / ++	– – / ++		– / ++		++ / ++	++ / ++	n.s. / n.s.	+ / +
Brookdale	n.s. / n.s.	– – / – –	++ / +		– – / ++	n.s. / –	+ / n.s	++ / ++	– – / –
Darlingford	++ / ++	– – / – –			– – / n.s.	++ / ++	++ / ++	– – / –	
Johnson	n.s. / n.s.	– / n.s.	+ / +		++ / ++	n.s. / n.s.		– / +	++ / ++
Atkinson	++ / ++	n.s. / n.s.	– / – –		n.s. / n.s.	n.s. / +	n.s. / ++	++ / ++	– – / –
Dusty Oaks	++ / –	n.s. / –		++ / ++		n.s. / ++	n.s. / +	– – / – –	++ / ++
Gould		n.s. / n.s.	n.s. / n.s	+ / +		n.s. / n.s.	– / n.s.	+ / ++	+ / ++

n.s., not significant ; +, closer than random $0.05 > p > 0.001$; ++, closer than random $p < 0.001$; –, farther than random $0.05 > p > 0.001$; – –, farther than random $p < 0.001$.

Discussion

This study examined the presence and distribution of *Ixodes scapularis* and *Dermacentor variabilis* within cattle and horse pasture environments at seven sites in southern Manitoba. A total of 1,946 ticks were collected during 2021 and 2022, including 944 ticks collected from the environment using tick drags and 1,002 collected from hosts. *Dermacentor variabilis* was the dominant species, with 1,872 adults collected compared with only 70 adult *I. scapularis*. Four adult *Haemaphysalis chordeilis* and one unidentified tick were also collected. The dominance of *D. variabilis* was expected, as this species is widely distributed throughout Manitoba and is commonly associated with open habitats, agricultural landscapes, and areas with suitable hosts (Bishopp and Trembley 1945, Wilkinson 1967, Burachynsky and Galloway 1985, Dergousoff et al. 2013). Tick abundance varied by year, site, species, and host type. The number of ticks collected from the environment differed substantially between years; in 2021, 231 ticks were collected (8 *I. scapularis* and 223 *D. variabilis*), while in 2022, 702 ticks were collected (6 *I. scapularis* and 696 *D. variabilis*). Although a greater area was sampled in 2022 (96,350 m²) than in 2021 (53,900 m²), the increase in *D. variabilis* abundance remains after accounting for sampling effort.

Overall, *D. variabilis* was much more common on cattle than *I. scapularis*, with 532 *D. variabilis* and only two *I. scapularis* collected from cattle during 505 individual inspections. *Dermacentor variabilis* was collected from cattle at all four cattle sites, with the highest abundance and prevalence at Alonsa. Cattle at Alonsa continuously grazed the same large pasture and were not restricted in their access to particular habitat types, which may have increased their opportunity to encounter ticks throughout the pasture compared with cattle that could not access some habitats due to static or movable fences. Animal movement can influence the spatial distribution of host-seeking ticks by changing the areas where hosts and ticks interact (Schulze et al. 2001b). In contrast, *I. scapularis* was uncommon on cattle, with only one individual collected at Alonsa and one at Brookdale. Although *I. scapularis* can attach to cattle (Barnard 1981a), the low number collected from cattle in this study suggests that cattle were not a common host for this species at the study sites.

The number of ticks collected on hosts also varied substantially between years. In 2021, 466 *D. variabilis* were collected from cattle, compared with 66 in 2022, representing an

approximately 86% decrease. This decline was largely driven by data from Alonsa, where *D. variabilis* collections decreased from 434 ticks in 2021 to 41 in 2022. *Dermacentor variabilis* was also collected from cattle at Brookdale in both years, with a 35% decrease in 2022. This is surprising considering we collected more ticks from pastures in 2022. In contrast, *D. variabilis* was found on cattle at Darlingford and Johnson only in 2022. *Ixodes scapularis* was not detected on cattle in 2021, but two individuals were collected in 2022, one each from Alonsa and Brookdale. The pattern on horses differed somewhat from that observed on cattle. At Dusty Oaks, 53 *I. scapularis* were collected from horses in October 2021 while only eight were collected from the environment. Interestingly, most *I. scapularis* collected from horses were males (39 of 53; 73.6%). Adult males do not require a bloodmeal and are considered to feed rarely, making their high occurrence on horses noteworthy (Kocan et al. 2015). Whether these males were actively feeding or were present primarily for mating is unknown. However, those same horses were hosts to 231 *D. variabilis* in 2022 when tick density was very high. At Brookdale, the decrease in *D. variabilis* collected from cattle in 2022 coincided with a substantial increase in environmental *D. variabilis* during the same year. These differences highlight the importance of considering host type when interpreting tick abundance and suggest that exposure to different tick species can vary considerably among livestock species and between sites. The contrasting patterns between host and environmental collections suggest that differences in tick abundance between years were not simply due to changes in sampling effort, but may have reflected environmental conditions, host activity, and the timing of sampling.

The distinct environmental conditions between 2021 and 2022 may help explain these differences. Manitoba experienced severe drought and unusually hot conditions in 2021, whereas 2022 was substantially wetter and included historical flooding at several study sites (Figure 30). These contrasts were particularly pronounced at Alonsa and Dusty Oaks, although other sites were particularly affected by drought (Johnson) and flooding (Darlingford). The hot and dry conditions in 2021 may have influenced tick and host behaviours. During periods of extreme heat, livestock may seek shade and refuge in forested or otherwise sheltered areas. These areas may provide more favourable microclimatic conditions for ticks by retaining higher humidity and reducing exposure to extreme temperatures. Under hot conditions, adult *I. scapularis* shifts activity toward cooler periods, including nocturnal questing (Durden et al. 1996). For *I. scapularis*, moisture is an important factor influencing activity and survival (Berger et al. 2014,

Ginsberg et al. 2020), while temperature and other meteorological conditions can influence the timing and intensity of questing activity (Schulze and Jordan 2003, Schulze et al. 2001a). Thus, the relatively low number of *I. scapularis* collected through environmental sampling in 2021 may partly reflect a shift to questing during cooler parts of the day or at night, and therefore not be effectively captured by daytime dragging. The same may apply to *D. variabilis* questing activity. Ticks exposed to high temperatures and low humidity lose water rapidly and die more quickly; they also increase questing activity before death (Nielebeck et al. 2023). Cattle and horses spend considerably more time in their pastures than researchers do, "sampling" a greater area and increasing their exposure to ticks. Potentially higher questing rates from stressed ticks, combined with a shift to questing at cooler times of the day, may explain the discrepancy between tick densities on pasture and the prevalence and intensity on grazing hosts. In June 2022, many pastures were flooded, which likely reduced tick questing activity and may have restricted host movement.

In southern Manitoba, cattle usually start grazing in late May. However, in both study years, cattle were moved to their summer grazing grounds near the end of June. In 2021, this delay was due to drought conditions and insufficient plant growth to sustain earlier grazing; in 2022, grazing was delayed because pastures were too wet and any plants that had grown were underwater. Cattle sampling was scheduled one week after the onset of grazing, and *D. variabilis* were collected from hosts in late June, consistent with seasonal adult activity in the Prairie region (Schvartz et al. 2015). At the horse sites, however, sampling began only in October 2021, after the *D. variabilis* questing activity period. Consequently, the lower number of *D. variabilis* collected from horses in 2021 reflects the missed peak seasonal activity rather than a true difference in abundance between years. This sampling-timing effect is important when comparing host collections between 2021 and 2022. The seasonal pattern observed for *I. scapularis* was consistent with its known activity in Manitoba. Adult *I. scapularis* in Manitoba exhibit two periods of activity, with a smaller spring peak from approximately April to June and a larger fall peak from September to November, particularly October (Hamer et al. 2012, Burtis et al. 2023, Lindsay et al. 1999b). In the present study, adult *I. scapularis* were collected during June and September/October, corresponding with these periods of increased seasonal activity. This suggests that detecting *I. scapularis* during both the early summer and fall sampling periods was consistent with expected seasonal adult activity in Manitoba.

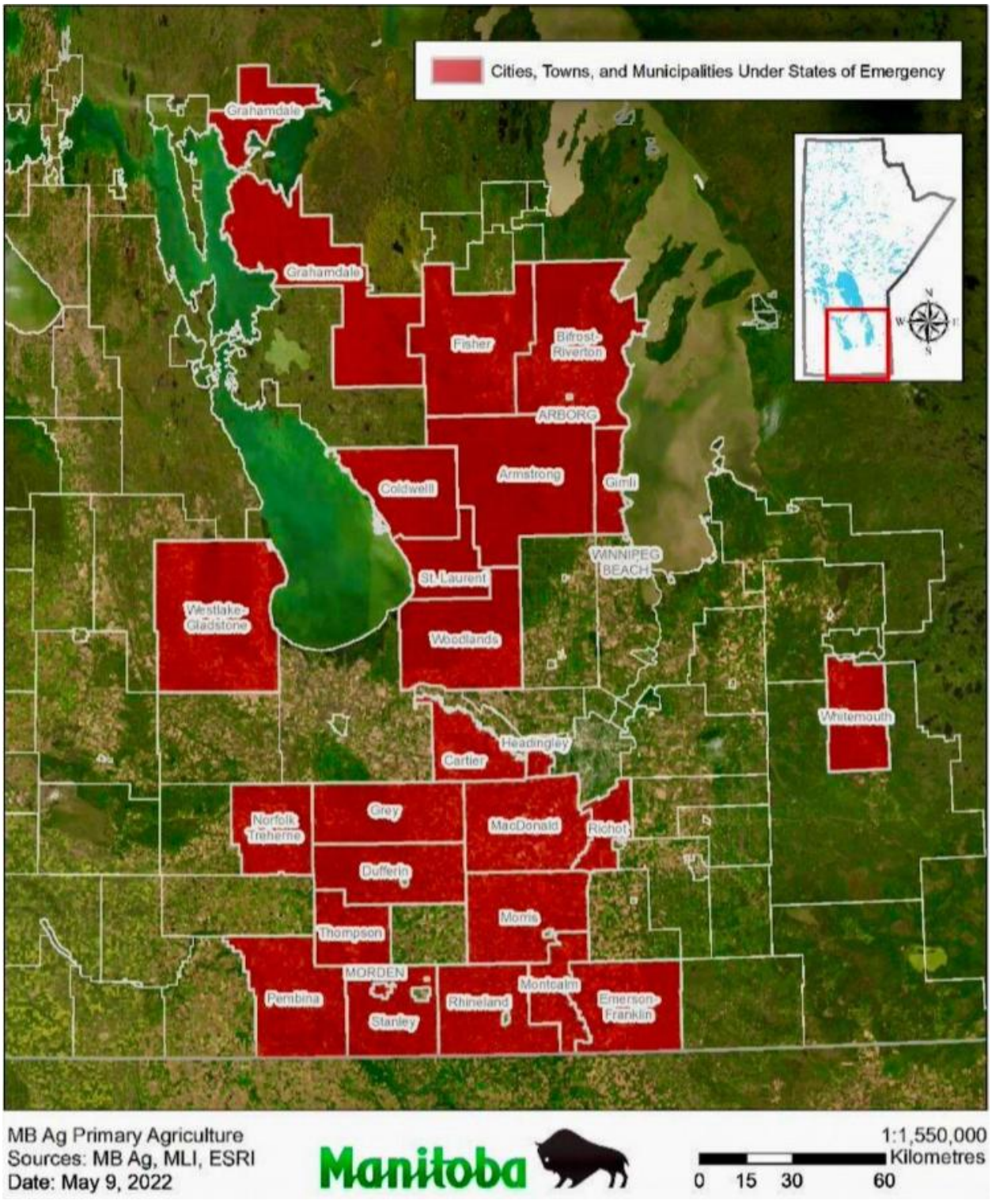

Figure 30. Map from the Government of Manitoba, representing the areas within southern Manitoba that were in a state of emergency due to flooding in 2022.

Together, the host and environmental collections demonstrate that year-to-year differences in tick abundance were complex and likely reflected the combined effects of weather, habitat conditions, host behaviour, and sampling timing. The decrease in *D. variabilis* collected from cattle in 2022 contrasted with the increase in environmental collections, especially at Brookdale. Similarly, *I. scapularis* was uncommon in environmental collections in both years but was locally abundant on horses at Dusty Oaks in 2021. These patterns emphasize that tick abundance measured from livestock or environmental sampling represents only part of the tick population and can be strongly influenced by when, where, and from which hosts ticks are sampled.

The habitat-distance analyses indicated that *D. variabilis* occurrence was associated with several habitat types, but these relationships varied among sites. Forest, shrubland, and grassland were often associated with tick-positive quadrats, as were wetland and water, although the direction and consistency of these associations differed among sites and between randomization approaches. For example, tick-positive quadrats were consistently closer to forest at several sites, while wetland and water showed both closer and farther associations depending on the site.

The repeated association with forest is particularly interesting given that *D. variabilis* is generally considered an open-habitat tick. Rather than indicating that *D. variabilis* requires forest habitat, the proximity of positive quadrats to forest may reflect the importance of habitat edges, shaded conditions, and host activity. Forested areas may provide cooler, more humid refuges during periods of high temperature. Ticks may therefore be concentrated near forest–pasture interfaces because these areas offer opportunities for host contact and microclimatic conditions favourable to survival.

The concentration of ticks near shaded habitat edges may also reflect host activity patterns, as livestock and wildlife, especially small mammals, may use these areas more frequently for shelter. Increased host activity in these areas could increase the likelihood that ticks detach from hosts locally. Mammalian hosts influence the distribution and population dynamics of *D. variabilis*, as host movement can contribute to tick dispersal throughout heterogeneous landscapes (Zimmerman et al. 1987). Similar relationships have been observed for *I. scapularis*, where vertebrate hosts influence tick establishment and distribution by providing opportunities for feeding and movement between suitable habitats (Anderson and

Magnarelli 1980, Spielman et al. 1985). Habitat transitions, such as woods–pasture interfaces, may be particularly important because they combine suitable vegetation structure for tick survival with increased host movement between habitat types (Schmidtmann et al. 1994, Carroll and Schmidtmann 1996). Therefore, the clustering of ticks near shaded habitat edges observed in this study may reflect the combined influence of host activity, vegetation structure, and localized environmental conditions rather than a direct preference for forest habitat.

Differences among sites may also reflect vegetation and grazing management. *Dermacentor variabilis* was more abundant at sites with less-maintained vegetation (grassland), particularly Alonsa, and Dusty Oaks, where a large proportion of the *D. variabilis* were collected during the study. Less-maintained areas may provide greater vegetation structure and shade, potentially creating more favourable microhabitats for tick survival and increasing the likelihood of host activity. They may also harbour greater biodiversity and support small hosts more than seeded pastures. However, because vegetation management was not experimentally manipulated and differed among study sites, these patterns should be interpreted as associations rather than evidence that reduced pasture maintenance directly increases *D. variabilis* abundance.

The relationship between tick-positive quadrats and water in the environment is noteworthy, as environmental conditions over the two years of the study may also have shaped it. We used the 2022 ACI layer to classify habitat types in the landscape, and some areas classified as water in 2022 may have represented lower-lying portions of the pasture that lacked standing water in the drought conditions of 2021 but nonetheless retained more moisture. These areas may therefore have provided localized moisture refuges during the dry conditions of 2021. Thus, ticks may have been concentrated in areas where moisture persisted despite dry conditions, highlighting the potential importance of localized moisture availability for tick occurrence.

Tick-positive quadrats were closer to water at Johnson, Dusty Oaks, Alonsa, and Gould Farm, but farther from water at Brookdale and Atkinson. This variability suggests that the association with water should not be interpreted as a direct habitat preference. Instead, these results may reflect differences in elevation, drainage, moisture retention, vegetation, or changes in water availability between years. Future analyses could incorporate a digital elevation model (DEM) to examine whether fine-scale differences in elevation and topography influence drainage, moisture accumulation, and the spatial distribution of ticks within pasture

environments. This interpretation is consistent with previous research showing that moisture availability and humidity influence tick survival and distribution (Lindsay et al. 1995, 1998, Ogden et al. 2004, 2006b). There may also be some interactions between water and wetland habitats that could be explored: when tick-positive quadrats were closer to water, they were farther from wetland, and vice versa, yet those habitat types tend to be adjacent at many sites. Together, the habitat-distance results suggest that *D. variabilis* was not associated with a single habitat type across the study area. Instead, habitat associations likely reflect site-specific combinations of vegetation structure, moisture conditions, host activity, and landscape context.

The correspondence analysis provided additional context for the relationship between habitat and tick occurrence. Study sites differed in overall habitat composition, with sites clustering near their dominant habitat types, reflecting the heterogeneous landscapes in which sampling occurred. However, tick-positive and tick-negative quadrats showed substantial overlap in ordination space, indicating that broad habitat composition did not clearly distinguish quadrats where ticks were detected from those where they were not. The sampled quadrats also overlapped substantially with the 10,000 random points, suggesting that the sampled areas generally represented the range of habitat types available within the study areas rather than a distinct subset of habitats.

These findings, together with the habitat-distance analyses, suggest that although habitat characteristics may influence localized tick occurrence, broad-scale habitat composition alone may not explain *D. variabilis* presence or absence within pasture environments. Instead, differences among sites and tick-positive quadrats may reflect finer-scale environmental conditions or localized habitat features not captured by the broad land-cover categories used in this analysis. Finer-scale characteristics such as vegetation height and density, canopy cover, temperature, humidity, soil moisture, and proximity to habitat edges may therefore be more important in determining where ticks are encountered. Previous studies have demonstrated that tick occurrence can be influenced by finer-scale environmental conditions, including temperature, humidity, vegetation structure, and moisture availability (Lindsay et al. 1995, 1998, Ogden et al. 2004, 2006b, Eisen et al. 2016).

Although *I. scapularis* habitat requirements are well documented in forested environments, less is known about its occurrence within agricultural pasture systems. In this

study, *I. scapularis* were detected at Dusty Oaks, Brookdale, and Alonsa, suggesting that agricultural landscapes may support low-density populations when suitable environmental conditions are present. The strongest evidence for a habitat association was observed at Dusty Oaks, where positive quadrats were consistently closer to forest and farther from grassland under both randomization approaches. This pattern is consistent with the known association of *I. scapularis* with forested and more humid environments, although the small number of positive quadrats limits the strength of this inference. The association with forest also corresponds with the high number of *I. scapularis* collected from horses at Dusty Oaks in 2021, suggesting that the forest–pasture interface may have been an important area of exposure.

Previous research has shown that *I. scapularis* survival and establishment are strongly influenced by temperature, humidity, and habitat characteristics (Lindsay et al. 1995, 1998, Ogden et al. 2006b). The occurrence of *I. scapularis* within these pasture systems therefore likely reflects localized areas that provide suitable microclimatic conditions rather than the pasture's overall habitat composition.

Although *D. variabilis* was abundant across several of our study sites, we did not find any evidence of *Anaplasma marginale* in the ticks we collected. In contrast, the detection of multiple tick-borne pathogens in *I. scapularis* underlines the potential hazard blacklegged ticks may pose to livestock and workers. *B. burgdorferi* was the most frequently detected pathogen and was found in ticks collected from both horses and the environment. The detection of *A. phagocytophilum* in a tick collected from a human at Dusty further the close association between horses, cattle, humans, and pasture environments, where infected ticks may come into contact with multiple hosts.

The detection of *B. odocoilei*, including one coinfection with *B. burgdorferi*, was unexpected but is consistent with recent evidence that this pathogen occurs in *I. scapularis* populations across Canada. Guillot et al. (2024) found *B. odocoilei*-positive *I. scapularis* throughout south-central and eastern Canada, although prevalence was lower in south-central Canada, including Manitoba, than in eastern provinces. The detection of an infected tick in the present study therefore provides additional evidence that *B. odocoilei* occurs in *I. scapularis* in Manitoba, although the small number of ticks tested limits any conclusions about its prevalence on a larger scale.

Together, the pathogen results demonstrate that even though *I. scapularis* was relatively uncommon compared with *D. variabilis*, the presence of several pathogens highlights the potential for exposure to tick-borne pathogens. No ticks tested positive for Powassan virus, *B. microti*, or *B. miyamotoi*, although the relatively small number of *I. scapularis* available for testing limits conclusions about these pathogens in the study area.

Conclusion

In conclusion, there was a low occurrence of *I. scapularis* on cattle and in cattle pastures. There was a high occurrence of *I. scapularis* at one of our horse sites (Dusty Oaks), which contained both bur oak and trembling aspen forested areas. This pasture also contained areas where horses had full access to heavily treed areas. In 2021, we collected many *I. scapularis* from horses at Dusty Oaks, which may have been influenced by the drought conditions experienced that year. Under dry conditions, *I. scapularis* seeks areas or hosts that offer greater humidity and moisture. Horses may also seek areas with more humidity and shade. Then, in 2022, we experienced severe flooding, which may have contributed to the low number of *I. scapularis* we collected from the pasture and cattle. Flooding may also have altered horse movement and habitat use, causing horses to avoid flooded areas. At Dusty Oaks, the flooded area was very close to the forested area, meaning that much of the habitat that may have been suitable for *I. scapularis* was temporarily inundated.

Although *I. scapularis* were relatively sparse in this study, 26 of the 70 tested ticks (37.1%) were infected with at least one pathogen, including *Borrelia burgdorferi*, *Borrelia odocoilei*, and *Anaplasma phagocytophilum*. This demonstrates that even where *I. scapularis* occur at low abundance, they may still pose a risk for exposure to tick-borne pathogens. Beef producers and horse owners should be aware of the potential for tick exposure and do regular tick checks to reduce the chance of tick attachment and exposure to tick-borne pathogens. Tick management practices can help reduce interactions between ticks and hosts. Although some tick management techniques can be impractical for producers and horse owners, as they can be time-consuming and expensive, placing a woodchip barrier around the borders of frequently used paths may reduce exposure (McKay et al. 2020).

CHAPTER 4: GENERAL DISCUSSION

Future direction

Ixodes scapularis and *Dermacentor variabilis* are two tick species of particular interest in Manitoba, and more research is needed to understand their distribution, seasonal activity, and interactions with livestock and wildlife. *I. scapularis* is a relatively recent addition to the tick fauna of Manitoba, expanding its distribution in northern and western regions of the province, including areas of open prairie that were once thought to be less suitable for this tick. More research is needed to determine if *I. scapularis* is becoming established in these areas or if they are only occurring in small areas that provide suitable habitat. In contrast, *D. variabilis* has been documented in Manitoba for over a century and was considerably more abundant than *I. scapularis* in our study. Although *D. variabilis* was abundant across several of our study sites, we did not find any evidence of *Anaplasma marginale* in the ticks tested. Continued monitoring of both species would help determine if their abundance, distribution, and pathogen presence are changing over time.

Future studies should sample more frequently throughout the tick-active season and during different weather conditions. *I. scapularis* can quest during both the day and night depending on conditions, and temperature and relative humidity strongly influence questing activity (Durden et al. 1996, Schulze and Jordan 2003, Burtis et al. 2023). In our study, the grazing season was delayed by approximately one month due to weather conditions, which led us to conduct host sampling later than originally planned. Adult *I. scapularis* can be active from early spring through early summer (Lindsay et al. 1999b), so sampling earlier in the spring and continuing throughout the grazing season may provide a better understanding of when this species is active in Manitoba pastures. This may be especially important for *I. scapularis*, as we collected considerably fewer of this species compared to *D. variabilis*.

The effects of drought and other extreme weather conditions should also be investigated further. The 2021 sampling season occurred during a severe drought, which may have increased desiccation and reduced tick activity or survival, especially in exposed pasture areas (Berger et al. 2014, Ogden et al. 2021). Previous research has shown that periods of low humidity can reduce *I. scapularis* activity and survival, while greater moisture and precipitation can support higher tick activity (Berger et al. 2014). In contrast, the historical flooding that occurred in 2022

may have changed the amount of suitable habitat available to ticks and may have also changed where horses and cattle used the pasture. At Dusty Oaks, the flooded area was close to the forested area, which may have reduced the amount of suitable habitat available to *I. scapularis*. Future studies should include measurements of temperature, humidity, soil moisture, and vegetation cover to better understand how these conditions affect tick activity and habitat use. Sampling over several years with different weather conditions would also help determine how much tick abundance changes between years.

More research is also needed on where *I. scapularis* are found within livestock pastures. Previous studies have found differences in *I. scapularis* abundance between wooded areas, pasture edges, and open pasture, and that adults and nymphs may use these habitats differently (Schmidtmann et al. 1994, Carroll and Schmidtmann 1996). Our study also found that tick-positive quadrats were associated with certain land-cover types. Future studies could sample from forested areas, forest edges, and open pasture while also measuring temperature, humidity, and vegetation. This could help determine if *I. scapularis* are becoming more established in open prairie habitats or if they are mainly using small areas within the pasture that provide more shade and moisture.

The role of white-tailed deer in the distribution of *I. scapularis* should also be investigated. White-tailed deer movement has been associated with the distribution of *I. scapularis* and may help move ticks between habitats (Main et al. 1981). We observed deer at several of our cattle sites, and some of our study sites contained habitat where deer could reside. This may have contributed to the presence of *I. scapularis* at some of our sites. Future studies could monitor deer abundance and movement to determine if areas with more deer also have higher numbers of *I. scapularis*.

Future research should also look at practical tick-management methods that could be used by Manitoba producers. Methods such as physical barriers, vegetation management, and acaricides may help reduce interactions between ticks and hosts, but more research is needed to determine which methods are effective and practical for livestock pastures. For example, woodchip barriers have been reported to reduce *I. scapularis* occurrence within the barrier by approximately 75% (McKay et al. 2020). However, the study was conducted along recreational trails rather than livestock pastures, so additional research would be needed to determine if the

same approach is effective in large grazing areas. Future studies could compare different tick-management methods to determine which are most practical for producers.

The geospatial information from this study could also be used to help identify areas within pastures where livestock and people may be more likely to encounter ticks. Future work could combine land cover, vegetation, weather conditions, livestock movement, and wildlife activity to identify areas and times when tick encounters may be more likely. This information could help producers make decisions about livestock movement and tick-management practices. Producer education could also be used to increase awareness of ticks, including tick identification and removal, signs of tick-borne disease, and personal protection such as using repellents and wearing appropriate clothing. Continued monitoring of ticks, pathogens, environmental conditions, and wildlife will help improve our understanding of how *I. scapularis* and *D. variabilis* are distributed in Manitoba and how their interactions with livestock may change over time.

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Appendix

Table A1: Tick abundance, density, and sampled area across sites for 2021.

Site	**Area Sampled (m^2)**	***Ixodes scapularis***		***Dermacentor variabilis***	
		n	density (ticks/100m^2)	*n*	density (ticks/100m^2)
Alonsa	11,200	0	0.00	79	0.71
Brookdale	11,180	0	0.00	50	0.45
Darlingford	14,700	0	0.00	94	0.64
Johnson	5,400	0	0.00	0	0.00
Atkinson Farm*	4,000	0	0.00	0	0.00
Dusty Oaks*	5,620	8	0.14	0	0.00
Gould Farm*$	1,800	0	0.00	0	0.00

* These sites were only sampled in October ; $ Site visited only once

Table A2: Tick abundance, density, and sampled area across sites for 2022.

Site	**Area Sampled (m^2)**	***Ixodes scapularis***		***Dermacentor variabilis***	
		n	density (ticks/100m^2)	*n*	density (ticks/100m^2)
Alonsa	19,800	0	0.00	45	0.23
Brookdale	12,100	1	0.01	142	1.17
Darlingford	21,550	0	0.00	124	0.58
Johnson	16,400	0	0.00	10	0.06
Atkinson Farm	10,700	0	0.00	16	0.15
Dusty Oaks	9,200	5	0.04	284	3.09
Gould Farm	6,600	0	0.00	75	1.14



Figure A1: Alonsa.



Figure A2: Brookdale.



Figure A3: Darlingford.



Figure A4: Johnson.



Figure A5: Atkinson Farm.



Figure A6: Dusty Oaks.



Figure A7: Gould Farm.