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Ecology of Ticks (Acari: Ixodidae) in Shelterbelts of Southeastern Nebraska

By

Matthew W. Yans

A THESIS

Presented to the Faculty of  
The Graduate College at the University of Nebraska  
In Partial Fulfillment of Requirements  
For the Degree of Master of Science

Major: Entomology

Under the Supervision of Professor Manuel Roberto Cortinas

Lincoln, Nebraska

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# Ecology of Ticks (Acari: Ixodidae) in Shelterbelts of Southeastern Nebraska

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University of Nebraska, 2011

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Shelterbelts in the agricultural landscape provide valuable habitat to a variety of animals that in turn may be hosts to ixodid ticks. The purpose of this study is to determine if shelterbelt associated factors influence tick ecology. In 2010 and 2011, ticks were collected from several shelterbelts in southeast Nebraska in Lancaster and Saunders counties. Four tick species were identified.

Adjacent land use, shelterbelt composition, and time of day were all factors which did not influence the number of questing ticks collected. Differences in relation to aspect and distance from the shelterbelt were significant. The east side had continually higher numbers of questing ticks than the north, south and west. The duration of tick activity was reduced on the north side. Solar radiation, soil moisture and soil temperature could influence tick emergence from diapause resulting in the first cohort of the year.

Based on my findings, the entomological risk for land management can be augmented, tick control strategies can become more effective and less wasteful, and knowledge of the presence of the various species of ticks can impact policies on public health and education.

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## DEDICATION

The suitcase, misunderstood,  
has come to bear many fruit  
which serve as my inspiration  
through life's endeavors.

## ACKNOWLEDGMENTS

I would like to thank my committee members, Dr. Roberto Cortinas, Dr. Jim Brandle, and Dr. Scott Gardner; your insight, experience and patience have been invaluable to my career at the university. I would also like to thank Steve Spomer for moral support and thesis revisions, Dr. Kathy Hanford for her help with the statistical analysis, and Timothy Hotaling and Allister Bryson for assistance with field collecting. Lastly I would like to thank the land managers and land owners of the various study sites for their hospitality.

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## **Chapter I**

### **Shelterbelts as a Potential Habitat for Ticks (Acari: Ixodidae)**

#### **Introduction**

Farming results in the alteration of large tracts of land. Historical data indicate that removing natural flora to create monoculture fields and grazing land results in increased erosion potential if land management practices fail to account for wind. To account for this problem, shelterbelts are created around agricultural plots to protect the adjacent land from wind erosion.

Additionally, shelterbelts function as sinks by collecting debris (either eroded soil or chemical drift), as habitat for mammals, aves and arthropods, as a source for collected debris and recruited wildlife, and as a corridor for wildlife traversing the landscape (Yunuasa et al., 2002; Brandle et al., 2009).

Shelterbelts are intentionally constructed to alter the microclimate in the immediate area. The change in microclimate and the establishment of diverse assemblage of organisms creates a distinct community compared to the adjacent open land. Shelterbelts provide a wide range of benefits, however they use land resources which then cannot be used for farmland. As a result the farmland, whether it is monoculture fields or grazing land, is pushed to the edges of the shelterbelts. Farmland and shelterbelts become connected and the fauna which use them as refuge will now interact with the adjacent land as well.

Ticks are common ectoparasites that feed on a variety of mammals and aves. With the presence of shelterbelts in the agricultural matrix and subsequently the wildlife habitat, ticks can be present as well (Stein et al., 2008). Ticks can transmit a wide variety of disease-causing pathogens, including viruses and bacteria, while feeding on a host (Table 1), and are among the most important vectors of disease-causing pathogens in the arthropod community. The entomological risk from interacting with ticks and their habitats is therefore of great concern.

The proximity of farmland to shelterbelts creates a potential threat of parasitism by ticks to farm animals and/or humans managing the farmland. How the establishment of shelterbelts affects the microclimate is well known. Wildlife using them for refuge and food is also well known. What is not known is how the microclimate affects tick ecology and seasonality. The purpose of this study is to determine if ticks are present in shelterbelts, which species are present, and to determine if shelterbelt associated factors influence tick ecology.

## Literature Review

The body of research pertaining to shelterbelts and their effect on microclimate is vast. Studies often focus on the effect they have in the agricultural landscape. The body of research pertaining to tick ecology is vast as well. However, how the patch of forest (shelterbelt) influences the microclimate and the subsequent effects on tick ecology have not been studied.

### **The Need for Shelterbelts-**

Nebraska ranks first among the fifty states in land used for farmland practices. United States Department of Agriculture (USDA) census data from 2007 shows total land used for farmland practices in Nebraska at 93% (Vilsack and Clark, 2009). Farmland is inclusive of croplands (47%), woodland (1%), pastureland (50%), other land uses (2%); the percent breakdown is of the 93% land use per the 2007 USDA census. Census data is consistent for the past several decades.

Alteration of the landscape to sustain agricultural practices removes natural barriers to erosion. The most effective way to mitigate the deleterious effects of erosion is to use shelterbelts in the landscape (Caborn, 1971).

Shelterbelts are linear rows of trees and shrubs purposely planted due to the wide range of benefits that they can provide (Cleugh, 2002; Brandle et al., 2009). They reduce air speed which allows for the distribution of percipient, either rainfall or snow which reduces irrigation needs and increases crop yield (Scholten, 1998; Brandle et al., 2004). They enhance livestock production by reducing stress on animals from harsh

climatic conditions (Atchison, 1982), and function as a barrier to protect crops (Kort, 1988). Reduced air speed decreases erosion potential (Huszar and Piper, 1986; Ribaudó, 1986; Pimental et al., 1995; Lyles, 1998), as soil particles can become airborne at six miles per hour (Midwest Plan Service, 2000).

### **Shelterbelt Design and Influence on Microclimate-**

The major function of shelterbelts is to alter microclimatic conditions as part of farmland management practices (McNaughton, 1988; Cable and Cook, 1990; Flora, 2001). They are usually planted with a predetermined composition (Capel, 1988; Brandle et al., 2004, 2009). Elements such as tree type, density, height, and number of rows are used to meet specific site objectives (Fig. 1). Variation in the design and structure creates a diverse set of microclimatic conditions in the sheltered zone (Plate, 1971; Wang et al., 2001; Cleugh, 2002; Mize et al., 2008; Zhou et al., 2008). The bulk of the literature concerning microclimatology defines the microclimate as the sheltered or protected areas (zones) in multiples of the height of the shelterbelt.

The alteration of air flow creates a sheltered area on both the windward and leeward sides (McNaughton, 1988, 1989; Mize et al., 2008). On the windward side the sheltered zone extends from two to five times the height of shelterbelt outward; on the leeward side it is far greater, extending ten to thirty times the height (Heisler and DeWalle, 1988; Brandle, 1990; Wang and Tackle, 1995; Brandle et al., 2009). The sheltered area produces zones where there is limited turbulent mixing. The air in the sheltered area becomes sequestered from the surrounding air in the environment resulting

from differences in air pressure (Wang and Tackle, 1995). Limited interaction between the air in the sheltered area and the air in the macro-environment generates the microclimate associated with shelterbelts (Fig. 2).

### **Humidity**

Humidity is increased in the sheltered zone. Reduced turbulent air mixing allows for greater atmospheric water content in the stagnated air via decreases in the rate of evaporation of soil moisture and transportation of plant transpiration as the air becomes saturated (Lomas and Schlesinger, 1971; McNaughton, 1988; Brandle et al., 2009).

Precipitation captured is increased due to the surface area of the plants and reduction in wind (Scholten, 1988; Shaw, 1988). As the precipitant reaches the soil, capillary action will increase soil moisture content on both sides, regardless of which side is trapping the precipitant (Brady and Weil, 2008). Snowfall accumulation is heavily influenced by design (Fig. 3). Height and density affect the length and depth of the snowdrifts (Brandle et al., 2009).

Elevated water content increases the absorption of solar radiation which decreases radiative heat loss; this decreases the nighttime fluctuation in temperature in the sheltered zone (Prueger et al., 1998; Brandle et al., 2009).



## **Solar radiation**

Solar radiation is radiant energy emitted from the sun and consists of visible light (VL), infrared light (IR) and Ultra Violet light (UV). Radiant flux is the measure of intensity or power of the solar radiation, defined as the amount of energy per unit surface area per unit time (Woodward, 1987). The amount of radiant flux within and adjacent to a shelterbelt varies based on composition, geographic location, season and time of day (Brandle et al., 2009). Shelterbelts with a north-south orientation receive radiant energy on the east side during the earlier hours of the day and on the west side during the afternoon hours. East west oriented lines will receive radiant energy on the south throughout the day and the north side will remain shaded. Duration of shading depends on time of the year –at 40° latitude, Lincoln Nebraska, during 2011, the summer solstice occurs on June 21st, the altitude of the sun is 72.6°, winter solstice occurs on December 22nd with a solar altitude of 25.8°, and the spring and fall equinox occurs on March 22 and September 23rd respectively with a solar altitude of 49.1°.

The vertical surface of a shelterbelt reflects a portion the solar radiation outward which influences the adjacent microclimate (Smith et al., 1997). The soil surface and adjacent vegetation can absorb the reflected radiation increasing the temperature in the microclimate (Fig. 2).

## **Air temperature**

Air temperature on aspects (north, south, east, west) which are actively reflecting solar radiation can be several degrees warmer; conversely, those aspects not receiving

solar radiation may be cooler depending on soil temperatures (Brandle et al., 2009). Soil temperatures are influenced by the amount of radiant energy received and the composition of the soil. Shaded areas on the east side will have higher soil temperatures than shaded areas on the north side due to the early daytime radiation received on the east side but absent on the north side. Air temperatures during the nighttime are warmer in the first meter from the soil surface in sheltered areas than temperatures in the unsheltered area (Zhang et al., 1999; Hodges et al., 2004).

### **Shelterbelts and Wildlife-**

The ecotone created by the shelterbelts serves as a confluence of two distinct habitats, the forested area and the monoculture field or open meadow (Giles, 1978; Forman and Gordan, 1986; Stein et al., 2008). The ecotone is dominated by edge species because of the linear arrangement of the shelterbelt, as it is a continuous edge (Kent and Coker, 1992). Edge species are those which thrive between the forested area and the open field/meadow performing their daily activities within the ecotone (Johnston, 1947; Forman and Gordan, 1986). Animal diversity varies with respect to those that are endemic or migratory in a given region (Mineau and McLaughlin, 1996). The presence of shelterbelts in the agricultural matrix increases species diversity (Yoakum and Dasmann, 1971; Jobin and Belanger, 2001).

Shelterbelts provide habitat for wildlife (Fig. 4) (Capel, 1988; Johnson and Beck, 1988; Mineau and McLaughlin, 1996; Jobin and Belanger, 2001; Alkorta et al., 2003). In landscapes dominated by agriculture, they provide refuge for a variety of aves, mammals

and arthropods (Mineau and McLaughlin, 1996), and are the only available habitat that is suitable for wildlife (Johnson et al. 1994). Wildlife will use them as travel corridors when traversing the agricultural landscape (Johnson and Beck, 1988; Yahner, 1988; Dix et al., 1995; Fluery and Brown, 1997; Lindenmayer and Fischer, 2006).

Shelterbelts effectively concentrate animal inhabitants within a given area to these linear arrangements of fragmented forest (Yunuasa et al., 2002). Range of the animal inhabitants depends on species. Some may stay within a given shelterbelt or set of shelterbelts while others may travel great distances or migrate and use them for refuge (Yahner, 1988).

### **Shelterbelts and Arthropods**

Shelterbelts not only alter the microclimate but also the ecological dynamics of the community. They recruit a variety of flora and fauna, increasing species diversity in the agricultural landscape. Small mammals and aves present in the shelterbelt community are subject to parasitism from ectoparasites including ticks. Ticks also parasitize humans and are capable of transmitting a variety of disease causing pathogens. Establishing communities where ticks can survive and reproduce has potential consequences on public health. Knowing how ticks survive, reproduce, find hosts and transmit disease is paramount in assessing risk to the human population using the adjacent lands.

## **Ixodid Ticks**

Ixodid ticks, family Ixodidae, are known as “hard ticks” because the presence of scutum, a hard plate covering the dorsal side of the tick. The family is comprised of 13 genera and approximately 650 species (Sonenshine, 1991). Ixodid ticks are obligatory blood feeding ectoparasites of many mammals, reptiles, amphibians and aves. The intimate relationship between hard ticks and their hosts results in the distribution of ticks correlating with host distribution (Giles, 1978; Bunnell et al., 2003). Increased numbers of ticks will therefore be found along edges and ecotones in areas dominated by farmland (Semtner et al., 1971; Sonenshine, 1993).

### **Ixodidae Three Host Life Cycle-**

The hard tick life cycle begins as an egg; once hatched, the six-legged, sexually indistinct larvae will begin host seeking (Fig. 5) (Oliver, 1989; Sonenshine, 1991; Goodman et al., 2005). When attached to a host the larvae will feed slowly for the next 3-8 days (Oliver, 1989). After a full blood meal has been obtained, the larval tick will drop off the host, seek shelter, diapause and molt into the nymphal stage. Nymphal ticks repeat the questing behavior, feed 4-9 days, drops off and molts (Oliver, 1989). Following the nymphal molt the ticks are now sexually dimorphic adults. Reference to a tick being either female or male implies that it has reached the adult stage. As adults the questing and feeding behaviors are repeated.

Mating takes place on the host while the female is feeding (except for the genus *Ixodes*, which can mate either on the host or in the environmental substrate) (Kiszewski

et al., 2001). Males may mate with several females; females mate once (Oliver, 1989; Sonenshine, 1991; Goodman et al., 2005). Females will increase to over 100 times their initial body weight during feeding (Oliver, 1989; Sonenshine, 1991; Goodman et al., 2005). When the female has finished feeding and has mated she will drop off the host and seek a sheltered environment in which to lay her eggs (Oliver, 1989; Sonenshine, 1991; Kiszewski et al., 2001; Goodman et al., 2005). Female ticks will lay thousands of eggs over 10-20 days (Kiszewski et al., 2001). Upon completion of egg laying the female dies (Oliver, 1989; Sonenshine, 1991; Goodman et al., 2005).

Photoperiodic entrainment induces a cycle of feeding and dropping off in correlation to photoperiod and host behavior (Smith and Cole, 1941; Amin, 1970; Pound and George, 1988). Photoperiodic entrainment allows for an increased chance of survival as the tick will drop off in optimal habitats, such as a shelterbelt, while the host is at rest (Camin and Drenner, 1978). The presence of hosts in the shelterbelt not only provides an optimal habitat for fed ticks to drop off, it also provides an opportunity for questing ticks to attach and feed. Conversely, dropping off in a hostile location, such as an open meadow with limited shelter and high exposure to the environment has limited survivability (Camin, 1963; Semtner et al., 1971). Survival depends on the ticks' ability to avoid desiccation (the most common cause of mortality), excessive moisture, parasites and predators (Knulle, 1966; Hair et al., 1975; Needham and Teel, 1991).

Ticks are cold-blooded (poikilothermic) and their development is influenced by ambient temperature (Campbell and Harris, 1979; Sonenshine, 1993). Warmer air temperatures increase the rate of development. The developmental timeline of ticks is measured in accumulated degree days (ADD). In warmer climates the life cycle can be

completed in a single year. In cooler climates the life cycle may take up to 3 years to complete (Sonenshine, 1991; Goodman et al., 2005). Cooler climates delay development and/or ticks may enter diapause to overwinter (Sonenshine, 1991; Goodman et al., 2005).

Southeast Nebraska's climate is classified as "warm/humid, continental" according to the Koppen-Geiger method (Map 3) (Peel et al., 2007). Ticks may have a two or three year life cycle in Nebraska depending on their ability to find suitable hosts. Many species of hard tick can survive for a year or more without feeding, extending their life spans (Sonenshine, 1991; 1993).

#### **Ixodidae Host Seeking Behavior-**

Two types of host seeking strategies are used by ticks: active and passive. Active host seeking ticks will leave their resting location in search of a host when the appropriate stimuli are present. Active host seeking is limited among tick species and is often coupled with passive host seeking. Passive host seeking ticks remain static in the questing position after ascending the vegetation in hopes of contacting potential hosts as they pass (Sonenshine, 1991; 1993). Questing ticks are those which are currently seeking hosts (Fig. 6). Questing will only occur when the tick is the proper state of hydration and maturation (Semtner and Hair, 1973; Waladde and Rice, 1982; Randolph and Storey, 1999). The tick will ascend the vegetation and wait for the appropriate stimuli given off by a potential host. Ticks have a variety of sensory organs; the most notable is located on the forelegs, known as Haller's organ. The Haller's organ is complex chemosensory organ used to sense potential hosts in the environment and can detect a variety of

chemicals in the air such as CO<sub>2</sub> and ammonia (Sonenshine, 1993; Goodman et al., 2005). Once potential hosts have entered the tick's area of detection the tick will wave their forelegs in the air in an attempt to make contact with a host (Sonenshine, 1991; 1993).

The tick will quest until a host has been found. If no host is found and the moisture content of the tick has been significantly reduced, the tick will descend the vegetation and rehydrate at the soil level (Semtner and Hair, 1973; Waladde and Rice, 1982; Needham and Teel, 1991; Randolph and Storey, 1999). Once rehydrated, the tick will ascend the vegetation to repeat the process. Desiccation rates increase as the tick moves from the litter layer into the open environment (Lees, 1948; Oliver, 1989). The greater the height a tick quests, the more exposure it gets to sunlight, radiation, and wind, all factors which increase the rate of desiccation (Lees, 1948). The moisture content in the microhabitat is critical to the survival of the tick (Lees and Milne, 1951; Knulle, 1966; Lees, 1969; Hair et al., 1975; Needham and Teel, 1991; Randolph and Story, 1999). A tick will ascend and descend the same vegetation multiple times; questing will continue until a tick has found a host, has died from depleted energy reserves, or is preparing for diapause (Harlan and Foster, 1990).

The actual determinant of questing activity appears to be ambient temperature (Hall and McKiel 1961; Balashov, 1972; Wilson et al., 1972; George and Cook, 1979; Harlan and Foster, 1986; 1990; Short et al., 1989). A study done by Atwood and Sonenshine (1967) concluded that ambient temperature was not as strongly correlated with questing activity as solar radiation for *D. variabilis*. Harlan and Foster reported that ambient temperature at one meter above the soil level was the most important factor

relating to host seeking activity by *D. variabilis* (Harlan and Foster, 1990). The study also suggested that an upper temperature threshold may exist for host seeking activity (Harlan and Foster, 1990). The Harlan and Foster (1990) study recorded several microclimatic factors at various heights in the ticks' microenvironment. The Atwood and Sonenshine study (1967) stated that solar radiation is the best predictor of tick host seeking. Conversely, the Harlan and Foster (1990) study indicated the opposite- that temperature was the main predictor and of all the microclimatic measurements taken, solar radiation was the least important predictor.

Hosts may vary depending on tick species and life stage (Hoogstraal and Aeschlimann, 1982; Oliver, 1989). In general, larvae tend to feed on smaller mammals such as mice and voles while nymphs will feed on raccoons, rabbits, etc. and adults will feed on larger mammals such as deer (Oliver, 1989). Host size may be related to questing behavior as the larger the tick, (i.e. larvae, nymph, adult) the greater distance it can ascend the vegetation and withstand the increasing exposure to the environment (Oliver, 1989). An adult tick may ascend to a distance of a meter or more and therefore be too high to come in contact with smaller mammals (Oliver, 1989; Sonenshine, 1991; 1993).

### **Importance to Public Health-**

Several tick-borne diseases are increasing in the United States (CDC). Ticks can transmit a variety of pathogens including bacteria, viruses and parasites (Sauer et al., 1995). In livestock production, ticks and tick-borne diseases are among the biggest source of economic loss (Uilenberg et al., 2004; Gratz, 2006).



Transmission of the pathogen occurs when an infected tick feeds (Sonenshine 1993; Goodman et al., 2005). Entomological risk for contracting a pathogen is related to the amount of time an individual spends in habitats which are suitable for ticks, the habits of the tick, season, weather, tick density, and infection rate within the tick community (Cavendish, 2003; Stjernberg and Berglund, 2005).

Most tick borne diseases are zoonoses, meaning they can infect humans but humans are dead end hosts for the disease, with the exception of *Borrelia duttonii* which cycles exclusively between humans and ticks (*Ornithodoros moubata*, found in Africa) (Goodman et al., 2005). Transmission within the tick community occurs by a naïve tick feeding on an infected host; depending on the pathogen it can then be transmitted vertically creating thousands of infected larvae; and/or trans-stadially leaving the tick infected to inoculate naïve hosts during the tick's next meal. The varieties of pathogens carried by ticks which can infect humans and animals have diverse syndromes (Goodman et al., 2005). The distribution of endemic ticks is an important factor in establishing the overall epidemiology of tick borne disease.

### **Medically Important Ticks of Southeastern Nebraska-**

Historical data from southeast Nebraska indicates two species of tick which are known to be present and medically important, *Dermacentor variabilis* (Say) (American dog tick) and *Amblyomma americanum* L. (Lone Star tick) (Cortinas, unpublished).

***Dermacentor variabilis* (Say)-**

The American Dog Tick, *D. variabilis* occurs in the Nearctic with a distribution ranging from the Yucatan peninsula northward through suitable habitats to southern Canada (Map 4).

*Dermacentor variabilis* is the most common tick in Nebraska. In Nebraska, the tick is the most commonly found in the forest field ecotone and along trails bordered by tall grass (Oliver, 1989; Goodman et al., 2005). It is a questing tick that attaches to its prey as it passes by (McEnroe and McEnroe, 1973), and is active from early spring into late fall (Oliver, 1989; Sonenshine, 1993).

*Dermacentor variabilis* is a vector of *Francisella tularensis*, *Rickettsia rickettsii*, *Ehrlichia chaffeensis* and tick paralysis (Table 1). The American dog tick is not a competent vector of *Borrelia burgdorferi*, the causative agent of Lyme disease (Piesman and Happ, 1997).

***Amblyomma americanum* L.-**

Lone Star tick distribution in the United States is from Texas north to Nebraska, from Nebraska to the east coast, along the east coast north to New York (Map 5). The Lone Star tick is less widely distributed in southeast Nebraska than the dog tick, but where locally established the tick is much more numerous than the *D. variabilis*. The distribution is concentrated towards the southeast tip of the state and quickly diminishes heading north and west.

The Lone Star tick is found most often in woodland habitats (Schulze et al., 1997; Childs and Paddock, 2003). The tick will quest on vegetation, host seek via ambush strategy, or seek out a host if it senses one close by (hunter strategy). They are aggressive hunters and nondiscriminatory feeders (Kollars et al., 2000; Goodman et al., 2005). White tailed deer (*Odocoileus virginianus*) have been reported as the principal host for all life stages (Apperson et al., 1990; Mount et al., 1993; Kollars et al., 2000; Yabsley, 2010). Other hosts include coyotes, raccoons, white-footed mice, rabbits, foxes, opossums, and squirrels (Oliver, 1989; Kollars et al., 2000; Goodman et al., 2005). Adults and nymphs are active from late winter to late summer. Larvae are typically not seen until mid-summer into late fall (Sonenshine, 1991; 1993; Kollars et al., 2000).

*Amblyomma americanum* is a vector for *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Coxiella burnetti*, *Francisella tularensis*, *Rickettsia amblyommi* and *Borrelia lonestari* the causative agent of Southern Tick Associated Rash Illness (STARI) (Table 1) (Goodman et al., 2005). STARI was initially confused with Lyme disease due to the appearance of erythema migrans known as a bull's eye rash, the presence of which was previously diagnostic for Lyme disease (Blanton et al., 2008). The bacterium *Borrelia lonestari* is the probable causative agent of STARI, while the bacterium *Borrelia burgdorferi* is the causative agent of Lyme disease (Stegall-Faulk et al., 2003). The Lone Star tick is not a competent vector of *B. burgdorferi* (Piesman and Happ, 1997).

## **Chapter II**

### **Ecology of Ticks (Acari: Ixodidae) in Shelterbelts of Southeastern Nebraska**

#### **Introduction**

Shelterbelts not only alter the microclimate but also the ecological dynamics of the agrarian community. They recruit a variety of flora and fauna, increasing species diversity in the agricultural landscape. They provide food, refuge, breeding habitat, and act as travel corridors for aves, mammals and arthropods (Mineau and McLaughlin, 1996), and in many farming landscapes, they are the only habitat suitable for wildlife (Johnson et al., 1994). ). In absence of traditional forest habitats, the shelterbelts effectively concentrate animal inhabitants to these linear arrangements in the agricultural landscape (Yunasa, 2002).

Small mammals and aves present in the shelterbelt community are subject to parasitism from ectoparasites including ticks. Ticks also parasitize humans and are capable of transmitting a variety of disease causing pathogens (Table 1). The intimate relationship between hard ticks and their hosts results in the distribution of ticks correlating with host distribution (Giles, 1978; Bunnell et al., 2003). Increased numbers of ticks will therefore be found along edges and ecotones in areas dominated by farmland (Semtner et al., 1971; Sonenshine, 1993).

Establishing communities where ticks can survive and reproduce has potential consequences on public health. Knowing how ticks survive, reproduce, find hosts and transmit disease is paramount in assessing risk to the human population using the adjacent lands.

Studies of shelterbelts often focus on their benefits to the agricultural landscape and the impact on microclimate. How their establishment affects the microclimate is well known. Research pertaining to tick ecology often focuses on the impact of the microclimate in relation to activity and survival. How the microclimate, in relation to shelterbelts, affects tick ecology and seasonality has not been studied. The purpose of this study is to determine if ticks are present in shelterbelts, which species are present, and to determine if shelterbelt associated factors influence tick ecology

## Materials and Methods

### Study Sites

The study was conducted from May 2010 to September 2011 (May 2010- August 2010, March 2011- September 2011). In 2010 two locations were used for sampling, each with multiple shelterbelts; in 2011, four additional sites were added. Study sites are located in Southeast Nebraska (Map 6).

The Agricultural Research and Development Center (ARDC) of the University of Nebraska (UNL) located in Saunders County Nebraska ( $41.155443^{\circ}$ ,  $-96.491005^{\circ}$  elevation 354 m) has 24 shelterbelts located within the Forestry Research Unit (Fig. 7). All are adjacent to crop fields. In 2010, 24 were sampled; 6 were sampled once, 2 were sampled twice, and 16 were sampled three times; a total distance of 74.22 km was sampled. Shelterbelts that were not sampled three times were because they became inaccessible as planted crops matured. In 2011 the number sampled at ARDC was reduced from 24 to 8, each sampled 4 times; total distance 42.24 Km.

Prairie Pines, a farm owned by the University of Nebraska Foundation and managed by the School of Natural Resources is located in Lancaster County Nebraska ( $40.847376^{\circ}$ ,  $-96.563887^{\circ}$  elevation 362 m) and has six shelterbelts which were sampled in 2010 and 2011 (Fig. 8). In 2010, each line was sampled four times, for a total distance 28.4 km, and in 2011 each line was sampled five times, for a total distance 35.5 km. Shelterbelts at Prairie Pines are adjacent to native grasslands with minimal management practices.

Claire Avenue at Southwest 40th St., is located in Lancaster County, Nebraska (40.763769°, -96.776885° elevation 365 m) on private land. Two shelterbelts were sampled three times, for a total distance of 12.18 km. They are adjacent to crop fields (Fig.9).

The remaining three sites are located in Saunders County Nebraska, were sampled twice and are adjacent to crop fields.

ARDC Farm Operations Unit (41.185079°, -96.482853° elevation 360 m), has two shelterbelts which were sampled twice, for a total distance of 9.04 Km (Fig. 10). They are adjacent to crop fields and county roads.

County Rd. 11 (41.124850°, -96.499307° elevation 349 m) on private land, has a single shelterbelt and was sampled twice; total distance 2.52 Km (Fig. 11). It is adjacent to crop fields.

County Rd. E (41.113910°, -96.511611° elevation 369 m) on private land has a single shelterbelt and was sampled twice; total distance 5.44 km (Fig. 12). The shelterbelt is adjacent to crop fields.

Total distance sampled in 2010 was 102.62 km and in 2011 was 106.92 km for a total of 209.54 km during the study period.

### **Tick Collections and Sampling Methods**

Sites with more than a single shelterbelt had the collection order randomized so as not to follow the same pattern during each visit.

Selected shelterbelts were aligned north-south or east-west. Sampling was conducted on either side in 2 lanes, the proximal lane centered at 0.5 meters from the

edge of the shelterbelt and the distal lane centered at 1.5 meters. The lanes were then divided in half effectively creating quadrants on the aspect of each line (Fig. 13). Each shelterbelt was sampled twice per visit, the first sample occurring between 9am and 12pm and the second sample between 3pm and 6pm. During the first sample two of the four quadrants located diagonally from each other (quadrant 1, 3 or 2, 4) were sampled and the remaining two quadrants sampled during the second sampling period. The process was then reversed each subsequent visit.

Ticks were collected via drags. The drags were constructed of double layered 12oz weight bleached denim and are  $1\text{m}^2$  (Fig. 14). The quadrants were dragged in 20 meter long intervals. At the end of each interval the drag was observed for ticks. Ticks were removed from the drag and placed in vials containing 70% ETOH, labeled with the appropriate date and location and identified in the laboratory. Ticks collected from clothing in each section were placed in a separate vial. When large numbers of larvae (>50) were present, they were collected with lint rollers and placed in sandwich bags to maximize collection efforts and minimize removal time (Fig. 15). Sampling did not occur when vegetation was wet as a result of rain or dew, or when the heat index exceeded  $43^{\circ}\text{C}$ .

Laboratory identification was done on an Olympus dissecting scope model SZ61. Each specimen was identified to species, life stage and gender (adults) using Clifford et al., (1961), Jones et al., (1972), and Keirans and Durden (1988), Keirans and Litwak (1989).



**Environmental Data:**

HOBO® Pro Series data loggers (Onset) were used to collect temperature data and humidity data in 15 minute intervals. HOBOS were placed in select lines at locations ARDC and Prairie Pines at the half-way point of the length of the line and between the two drag lanes (Fig. 16). Select lines at ARDC: Line H, west and east side; Line K, east and west side; Line U north and south side, Line W, north and south side. Select lines at Prairie Pines: Line B, east and west side; Line G, north and south side. The HOBOS were placed at 0.5 meters above the soil surface.

**Data Analysis:**

Collection data were entered into an Excel spreadsheet (Microsoft) including date, species, life stage, and location parameters. Individual shelterbelts were treated as separate units, even if multiples were located at a site. Data were analyzed in a generalized linear mixed model (GLMM) using Proc GLIMMIX in SAS version 9.2 with a negative binomial distribution and a log link function (SAS Institute 2008). GLIMMIX was chosen due to the random distribution of ticks in the environment coupled with the fixed effects of the study. Nymphs and larvae were excluded from the data analyses. Data were standardized by distance dragged when comparing shelterbelts of different lengths. In each analysis the data are presented as P values with significance being  $P < 0.05$ . In some analyses least square means (LSM)  $\pm$  standard error of the mean (SEM) are reported. Type III fixed effects analyzed for adults were: month (March-September), year (2010, 2011), site (1-6), shelterbelt composition, adjacent land use, direction (north,

south, east, west), time of day (morning or afternoon), and sampling methodology.

Additional contrasts were performed for site, direction and distance from shelterbelt.

## Results

Total number of ticks collected during the study period was 1,080, sampled from 209.54 kilometers (102.62 in 2010 and 106.92 in 2011) from 36 shelterbelts (Figs. 7-12) over six separate sites (Table 2).

### Species

Four tick species were collected during the study period (Table 3). One species, *D. variabilis* (The American Dog Tick) was identified at all six locations and comprised 97% of the total number of adults collected. Three of the six locations yielded more than a single species of tick. *Amblyomma americanum* (The Lone Star Tick) was collected from three locations; ARDC, Prairie Pines and Claire Avenue at SW 40th. *Haemaphysalis leporispalustris* P. (The Rabbit Tick) was collected from two sites, ARDC and Prairie Pines. A single female *Ixodes scapularis* (Say) (The Deer Tick) was collected at Prairie Pines.

### Seasonality

In 2010 the total number of lone star ticks collected was 148 (12 adults, 129 larvae, 7 nymphs), in 2011 total number collected was 173 (12 adults, 149 larvae, 12 nymphs). Adults were present from May through July and absent from March, April, August, and September (Table 4). Nymphs were present from May to August. Larvae were only present in August. The graphs show seasonality varies with life stage (Figs. 17, 18, 19).

In 2010 the total number of *D. variabilis* collected was 353 (352 adults, 1 nymph), in 2011 the total number collected was 401, all adults. Adults were present from March through August and absent in September (Table 4). A single nymph was collected in August of 2010. Larvae were not present in the study for either year. The graphs show the highest number of adults were present in May, decreasing into August, then becoming absent in September (Fig. 20).

### **Sites**

ARDC Forestry Research Unit and Prairie Pines comprise most of the sampling; both sites contain multiple shelterbelts (24 and 6 respectively) of similar orientation (N, S or E, W) and were sampled in 2010 and 2011. The four remaining sites had one or two shelterbelts each and were sampled in 2011. Sites were analyzed to see if a significant difference in tick densities existed among the sites. Analysis included all six locations. There was a significant difference in density of adults collected between site locations ( $P=.0058$ ). Differences between sites occurred between the smaller sites added in 2011 and the sites used in 2010 and 2011 (Table 5). Prairie Pines and ARDC, which comprise the majority of samples, were not significant ( $P=.0683$ ) when compared to each other.

Absolute number of adult ticks collected between 2010 and 2011 was not significantly different ( $P=.5519$ ).

### **Shelterbelt Composition**

Shelterbelt composition analysis was limited to the ARDC for 2010 and 2011. The ARDC has three types of plantings; eastern red cedar (*Juniperus virginiana*); eastern

red cedar and Austrian pine (*Pinus nigra*); eastern red cedar, Austrian pine, and green ash (*Fraxinus pennsylvanica*) (Table 6). Association of tick numbers with different shelterbelt compositions was not significantly different.

### **Agriculture**

Crop type in the adjacent fields was analyzed to see if specific planted crops influence tick numbers. Types of adjacent land use analyzed were corn, soybeans, wheat, alfalfa, barley, and access roads (Fig. 21, 22). Analysis was limited to the ARDC for 2010 and 2011. Association of tick numbers with adjacent land use/ crop type was not significantly different.

### **Aspect**

Aspect refers to the cardinal directions: north, south, east, and west. Distance sampled for north-south facing shelterbelts was 92.78 km (2010=50.76; 2011=42.02) and east-west facing lines was 116.76 km (2010=51.8; 2011=64.90).

*Amblyomma americanum* adults represent three percent of the total adult ticks collected. Twelve of the 24 adults collected (2010=12; 2011=12) were found on the east side (Table 7, Fig. 23). *Amblyomma americanum* larvae represent 99% of the larval ticks collected. Larval numbers were dominant on the east side; 251 of the 278 collected (2010=129; 2011=149) were present on the east side (Table 7, Fig. 24).

*Dermacentor variabilis* accounted for the majority of the ticks collected. Total number of *D. variabilis* adults collected from both years was 753 (2010=352; 2011=401), 257 (.34) were collected on the east side (Table 7, Fig. 25).

Aspect had a significant relationship to adult tick presence ( $P=.0106$ ). Further analysis was conducted in paired aspects (N, S or E, W) with each individual shelterbelt considered to be a single unit. North vs. south was not significant ( $P=.8733$ ,  $LSM=.9713$ ,  $Se=.1763$ ). East vs. west was significant ( $P=.0009$ ,  $LSM=1.8683$ ,  $Se=.3308$ ).

### **Time of Day**

Ticks were collected during two separate time parameters on each shelterbelt during each visit; 9am-12pm (morning) and 3pm-6pm (afternoon). There was no significant difference between morning and afternoon collections among all adults ( $P=.7725$ ). Aspect was combined with collecting time to see if differences occurred in individual aspects (Table 8). There was no significant difference in number of adults between collecting times per direction ( $P=.44$ ).

### **Sampling Methodology**

Sampling methodology refers to the division of each aspect into quadrants. The first half refers to either the northern portion of a north-south shelterbelt or the western portion of an east-west shelterbelt; the second half being southern, eastern respectively. Analysis of first half vs. second half is not significant ( $P=.2807$ ).

Shelterbelts were sampled at one meter and two meters from the edge. Sampling distance was significant ( $P=.0266$ ,  $LSM=.3441$ ,  $Se=.1604$ ). A significantly greater number of ticks were found two meters from the edge compared to one meter.

## Discussion

Shelterbelts are the focal point in this study as past studies have shown that tick hosts use them for food, refuge and breeding. Host presence in shelterbelts increases the probability of tick presence. Understanding the influence of biotic and abiotic factors on questing populations of endemic ticks is essential to understanding their distribution in the agricultural landscape. Knowledge of tick distribution is necessary for predicting likely areas for transmission of tick-borne infections to humans and livestock.

The four species collected during the study represent three species known to be found in Nebraska (*D. variabilis*, *A. americanum*, and *H. leporispalustris*) and one species that was not previously recorded in a field collection (*Ixodes scapularis*).

In southeast Nebraska, *D. variabilis* is the most common tick, with a notable exception occurring in the few counties at the extreme southeastern point of the state where *A. americanum* is dominant (Cortinas, unpublished). *Dermacentor variabilis* accounted for 97% of adult ticks collected and 70% of the collection overall; *A. americanum* accounted for 3% of adults and 30% of the collection. *Haemaphysalis leporispalustris* and *Ixodes scapularis* represented less than 1% of the collection combined.

Immature *D. variabilis* are rarely collected from drags (Sonenshine, 1993; Cilek and Olsen, 2000) and that was confirmed in this study. Although 70% of the collection was *D. variabilis*, not a single larva was collected and only a single nymph. The habits of immature *D. variabilis* are to remain at the soil level or quest at minimal height as the principal host is the white footed mouse and meadow vole (Sonenshine, 1993). Presence of larval *D. variabilis* on hosts at Prairie Pines and ARDC has been confirmed in a

current ongoing study (Hotaling, unpublished). In areas of established vegetation the drag will not make contact with the lower portions of the vegetation therefore bypassing any questing immature *D. variabilis*. In contrast, *D. variabilis* adults will feed on a wide variety of hosts including humans. Adults are regularly collected from drags as was the case in this study. The tick can transmit a wide array of pathogens (Table 1), many of which can be life threatening.

The presence of *A. americanum* in the study is not surprising as it has been commonly reported in Nebraska since the early 1990's. However on most maps the tick is absent from Nebraska. The CDC updated their maps in August of 2011 to reflect proper distribution in Nebraska (Map 5). The presence of *A. americanum* is significant in terms of public health. It is hypothesized that *B. lonestari* is the causative agent of STARI due to similarities with Lyme disease, which is caused by a bacterium in the same genus (Table 1). Erythema migrans (bull's-eye rash) was diagnostic for Lyme disease prior to the discovery of *B. lonestari*, which also causes erythema migrans but not Lyme disease. The long term effects of STARI are not known (Blanton et al., 2008).

*Haemaphysalis leporispalustris* is found throughout the United States and parasitizes small mammals and is often found on rabbits. *Haemaphysalis leporispalustris* is rarely collected due to their habits. They are considered a nesting tick as their photoperiodic entrainment causes them to drop off their host during the day when the host is at rest (Burgdorfer, 1969; Sonenshine, 1993). The collection of one larva and two nymphs could have occurred either as a result of contact with a rabbit nest or after being recently dislodged from a host. The tick does not feed on humans (Sonenshine, 1993). However the presence of the tick is worthy of mentioning as it can harbor and transmit



several pathogens (Table 1) to hosts which can be hosts to other tick species, consequently infecting other naïve tick species which can feed on humans (Sonenshine, 1993).

The single female specimen of *Ixodes scapularis* was the first field collected deer tick in Nebraska. The specimen was sent to the National Tick Collection at Georgia Southern University for confirmation and currently resides in their collection under voucher number RML124926. *Ixodes scapularis* has been commonly reported in states bordering Nebraska to the east and south (Map 7)(CDC).

*Ixodes scapularis* is a vector of several disease causing pathogens, most notably *Borrelia burgdorferi*, the causative agent of Lyme disease (Table 1). Lyme disease is the most common vector-borne disease in the United States. In 2010, eight cases of Lyme disease were reported from Nebraska (CDC).

Site analysis showed that there are differences between the six study locations. The differences were significant between the sites added in 2011 and the already established study sites (ARDC, Prairie Pines). The sites added in 2011 were sampled two to three times each. The sites contrast to ARDC and Prairie Pines in that the ARDC and Prairie Pines have multiple shelterbelts in close proximity to each other whereas the other sites stand alone in the landscape. The aggregation of shelterbelts causes a dilution effect as there are multiple areas of refuge for hosts to use compared to a solitary shelterbelt (Yahner 1983). This could explain the decreased tick numbers seen in aggregated versus stand-alone.

Comparison of the two larger sites with multiple shelterbelts (ARDC, Prairie Pines) were not significant ( $P=0.068$ ). The ARDC is a University of Nebraska research site

which is actively engaging crop growth and crop rotation. Shelterbelt composition and adjacent land use were analyzed for the ARDC and were not significant. The data may be too diluted at this level to properly determine if adjacent land use influences tick numbers.

Composition was not a factor; this may be due to limited variation in composition between each line. All shelterbelts (24) had the same species, *J. virginiana*, 19 of the 24 contained two of the same species, *J. virginiana* and *P. nigra* and 11 of the 24 contained *J. virginiana*, *P. nigra*, and *F. pennsylvanica* (Table 6).

The landscape at Prairie Pines is homogenous grassland with limited interaction in terms of land management. Prairie Pines was the only location in the study to contain all four species of ticks and larval *A. americanum*.

A significantly greater number of ticks were found two meters from the shelterbelts compared to one meter. Two interpretations of this data could be made, that hosts are present at two meters in greater numbers hence greater tick presence or more likely that at two meters host presence is limited compared to one meter and the ticks collected were those that have failed to find hosts. Immature ticks often use smaller mammals as hosts (Sonenshine, 1993). Smaller hosts such as voles and mice tend to be in the shelterbelt and close to the edge to avoid predation (Johnson et al., 1991). Large aves of prey were noticed at several locations during both years. Current studies provide evidence of small mammals at each site (Hotaling, unpublished). It is within this edge or perhaps one meter from the edge that the hosts are active and encountering questing ticks. At two meters there may be less host activity and/ or higher predation of hosts therefore a higher presence of questing ticks.

Analysis of direction provides strong evidence that questing ticks are present in greater numbers on the east side of shelterbelts. This was true for the adults and larvae. At Prairie Pines 90% of the larvae were collected on the east side. During each month in the 2010 and the 2011 season the east side had higher numbers compared to the north, south and west.

On the north side the presence of questing ticks peaked in May of 2010 and June of 2011 (Fig. 26). The south had greater presence of questing ticks, peaking in May of 2010 March of 2011. The west and east sides saw the presence of questing ticks peak in May for 2010 and 2011. In June through August of 2010 and July through September of 2011 the numbers of questing ticks collected were similar on the north, south, east and west. However when peaks were recorded in 2010 they may have obscured the true seasonal abundance of questing ticks. The explanation is that the study occurred from May to August in 2010 which did not account for tick activity from March, April and September. This was corrected in 2011 as the collection season was expanded and the seasonal patterns of questing ticks become clear.

North versus south had the least amount of variability in tick numbers for the year in total with the total number of ticks collected being almost equal (LSM= .9713, SE=.1763). East had a greater presence of questing ticks throughout the year in both years. Temperatures on the west and north side remain close to the dew point resulting in moist vegetation during the morning hours. Ticks will not quest on moist vegetation (Oliver, 1989) and attempts to drag moist vegetation will soak the equipment rendering it ineffective. As a result an analysis was done to see if differences occurred among collection times on each aspect. The difference between collecting times per direction

was not significant ( $P > .05$ ). This indicates that moist vegetation as result of dew point and ambient temperature interactions did not bias the collection numbers.

The similarity in numbers when comparing the north versus south appears unusual and the consistently higher numbers on the east suggests either increased survivability or greater abundance of ticks. This can be explained by seasonality, microclimate and cohorts. Two adult cohorts occur each year; the first are those that emerge from diapause in the spring and the second are those that fed in the spring as nymphs and have molted to the next life stage or newly hatched. The first cohort of the year consists of those ticks currently in diapause awaiting the proper accumulation of degree days (ADD) to emerge and begin questing. Once the initial cohort has emerged, tick presence drops going into the summer months. Fed ticks are molting into the next life stage and will either begin questing again in late summer, early fall or enter diapause to emerge next spring. This explains the bimodal seasonality of ticks (Oliver, 1989; Sonenshine, 1993) which was present in this study on two of the four aspects. Microclimatic differences do occur between aspects and may explain the observed differences of seasonality in relation to aspect. Changes in temperature and relative humidity data are similar throughout the year during both years and all aspects (Figs. 27, 28). Ambient temperature and relative humidity therefore are not indicators of the presence of questing ticks as there was little variation among the different aspects while tick numbers varied significantly. However air temperature and humidity are factors in tick survival and work in concert with other abiotic and biotic factors to determine environmental variation. Different factors must therefore be responsible for the observed variation.

At Prairie Pines, in March of 2011, the south side of lines F and G were warm and dry compared to the north side which was still covered in snow and ice. In late winter early spring when the temperatures are steadily increasing it has an effect on the ticks currently in diapause. In 2011 on the south side the peak activity occurs in March while on the north side the peak activity is not reached until June. The HOBO data loggers were deployed on February 16<sup>th</sup> 2011 at Prairie Pines. Average daily temperatures were used to calculate degrees above freezing. On the south side when collections peaked in March the total Celsius degrees accumulated above freezing were 74. When collections peaked in June on the north side the numbers of accumulated degrees above freezing were 908. Similar variations were observed for lines with HOBO data loggers. The temperatures are not similar further supporting the hypothesis that ambient air temperature is not a good predictor of tick questing activity. It is important to note the snow cover was still present in February and the data loggers were 0.5 meters above the ground, above the snow and ice. The data loggers were reading the ambient air temperature not the ground temperature, where the ticks are in diapause.

Snowfall for 2009-2010 was 41.6 inches with 24.3 of the total occurring in December, in 2010-2011 total snowfall was 29.3 inches with the majority occurring in January and February. Peak activity in 2010 occurred in May after a season of higher than average snowfall; in 2011 peak activity was variable depending on aspect. In early spring of 2010 snowfall was less than the spring of 2011. Even though 2010 had a higher snowfall accumulation the majority of it occurred in the early part of the winter leaving less snow on the ground in early spring of 2010 compared to 2011.

Snow acts as an insulator, increasing tick survival through the winter (Oliver, 1989). Snow retains heat given off by the soil thereby creating a stable environment, while bare soil provides little to no insulation (Brady and Weil, 2004) and rapidly increases tick mortality. Snow cover may contribute to a lag in ending diapause as the soil can remain covered keeping the litter layer cooler than the ambient air temperature and obstructing the ticks. Also as the snow cover melts the soil moisture increases; consequently the amount of energy required to increase soil temperatures to the parameters required for tick activity (ADD) therefore increase. Specific heat of dry soil is approximately 0.8J/g; the specific heat of water is 4.18J/g (Brady and Weil, 2004) therefore wet soil will have specific heat higher than that of dry soil. Snow melting, ground thawing, and evaporation rates occur at an increased rate in areas of increased solar radiation (Brady and Weil, 2004). The south side receives solar radiation throughout the year from dusk till dawn, east and west receive solar radiation in the morning and afternoon, respectively. The north side receives limited solar radiation for most of the year. As the summer solstice approaches the amount of solar radiation received on the north side gradually increases. In 2010 and 2011 summer solstice occurred on June 21<sup>st</sup>. Peak activity on the north side occurred in May of 2010 and June of 2011. The amount of solar radiation received on each aspect offers an explanation as to the observed differences in seasonality. Snowfall and retained snow cover acting in concert with solar radiation influence the emergence of the first cohort of the year.

With snowfall and solar radiation offering an explanation of variation in tick activity it is important to revisit the increased tick numbers on the east side and how the north and south could have similar numbers with differences in seasonal duration. The

ARDC was visited on January 3<sup>rd</sup> 2011 to observe recent snowfall and the accumulation on individual aspects (Figs. 29, 30). On the south aspect the snow accumulation varies from bare to several inches deep, determined by wind patterns through the shelterbelt. This creates a saw tooth effect of snowdrifts. Snow deposition on the north side was even and consistently several inches deep. What this implies is that the bare areas on the south side result in tick mortality, bare areas were not present on the north side. This furthers the hypothesis that snow cover increases tick survival as similar numbers of ticks were collected from the north and south side, even though seasonal duration of activity is different. The east side dominates in tick numbers throughout the year. Comparing snow cover on the east and west side again may offer some explanation. On the west side the snow cover is thin to nonexistent, conversely on the east side the snow cover is uniform and several inches deep. Variation in solar radiation is not as severe compared to north – south aspects as the sun is present on both sides each day. The snow cover variation may explain the increased numbers on the east side. The uniform snow cover on the east side increases tick survival along the entire shelterbelt. The variable to nonexistent snow cover on the west side creates patches of increased tick mortality, similar to the south side. The increased numbers of questing ticks on the east side can be a result of increased survival during diapause compared to the west and south; and a greater duration of seasonality resulting from solar radiation compared to the north.

Reviewing the earlier studies of Harlan and Foster (1990) and Atwood and Sonenshine (1967) the differences in their outcome may be a result of their methodology. Harlan and Foster (1990) conducted their study over a period of 14 days from late June to early July. The study was conducted on the east side of a forest edge in the Alum Creek

State Park. Temperatures averages were in the 16-27° C range. Six days of collecting occurred in the 14 day window of the study. During those 14 days, 4 days had rainfall between 0.5-1.0 inches. The study measured a suite of environmental parameters and concluded that temperature is the most important predictor of tick questing activity. The results could be skewed as the activity of questing ticks depends on a variety of factors. Harlan and Foster did not disclose which days they sampled but did mention that collections were reduced after rainfall. The study was conducted in a minuscule time period from which the temperature should be consistent. Rainfall records show that it was raining during the study period thereby affecting many of the environmental parameters that were measured resulting in a lack of consistency. With temperature remaining stable and several other parameters in flux the only conclusion to be drawn from the data is that temperature is strongly correlated with questing activity. However as mentioned the study was not long term, rather a snapshot over six days.

In contrast, the study done by Atwood and Sonenshine (1967) was conducted from March 1963 through February 1965. Field drags were conducted weekly and the results correlated with multiple environmental factors. Interestingly, the number of questing ticks was reduced during periods of overcast sky, confirmed from one drag to the next with a significant difference in tick numbers when sky cover was different. This may further explain bias in the Harlan and Foster study as it had rained for 29% of their collection period. Atwood and Sonenshine concluded the predictor for seasonal activity and emergence is dependent on solar radiation and snow cover, a conclusion which is supported by this study.



## Conclusion

Shelterbelts in the agricultural landscape not only provide benefits to landowners, they also function as ecological communities. It is within these communities that interactions between flora, fauna, and the microclimate create a contrasting environment compared to the adjacent monoculture field or pasture land.

The goal of this study was to determine how ixodid ticks interacted within these communities. Ticks were collected throughout their active season and were categorized by several means. Collections yielded four species of ticks, two of which were known to be active in the area (*D. variabilis*, *A. americanum*), one suspected to be active (*H. leporispalustris*), and one species not previously recorded in Nebraska (*I. scapularis*). Sampling was determined to be free of bias by way of statistical analysis. Sites were chosen which utilized different land management practices and uses. The studies main sites (Prairie Pines and ARDC) had little variation between them while the smaller sites had a higher abundance of ticks.

Sampling distance away from the shelterbelt was statistically significant indicating that there are factors which influence tick survival and the ability to find hosts as the distance from the shelterbelt increases. At two meters the number of ticks was significantly higher than at one meter. The difference in distance is nominal, however the study showed that it had an impact on the number of questing ticks.

The study provides strong evidence that abiotic or biotic factors do influence tick numbers in relation to aspect. The east side had a greater presence of ticks. The north side had a shorter season of tick activity than the south; surprisingly the numbers of ticks were almost equal. Temperature and relative humidity did not determine peak activity in

relation to aspect. The north side remains shaded and as a result the winter snow takes longer to melt and the subsequent moisture in the soil requires more solar energy to increase soil temperature. Accumulated degree days required for the development of diapausing ticks on the north therefore accumulate at a slower rate compared to the other aspects explaining the apparent lag in tick activity on the north side. Coincidentally the increase in activity of the north side increases as the summer solstice approaches which increases solar radiation. Snowfall accumulation also influences tick survival.

Observations at the ARDC in the winter 2010-2011 show that the south and west side have an uneven distribution of snow and several bare spots. In contrast the north and east side had a consistently even depth of snow along the length of the shelterbelt.

Microclimate influences tick ecology in shelterbelts. Previous studies have attempted to determine what the predicting factors are for questing ticks. According to the data, temperature and relative humidity are not good predictors of tick activity; however they are a result of factors which do influence tick activity. In order to elucidate a complete understanding of the interaction between ticks and the diverse microclimates present in fragmented landscapes; further studies should include, depth of snow cover, daily soil temperature and soil moisture, and radiation load measurements in conjunction with tick collecting throughout the year.

Animal trapping should be done to determine which hosts are present and which species of tick are utilizing the hosts. They may be more species present than recorded in this study as some species are difficult to collect by dragging. Host collection and examination may also yield different results in term of tick abundance and species numbers.

Field collected ticks should also be examined to determine if they are infected with disease causing pathogens. The variety of pathogens carried by the various species of ticks is a risk to human and animal populations. Knowing which pathogens are present in current tick populations and what the prevalence is will augment current public health policies and control strategies.

Tick species, seasonality, host use and abundance, and pathogen prevalence are all factors which can be used to determine the entomological risk, from ticks, for farmland use in southeast Nebraska.

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### Open Wind Speed 20 mph Deciduous 25-35% Density

| H distance from windbreak | 5H  | 10H | 15H | 20H | 30H  |
|---------------------------|-----|-----|-----|-----|------|
| miles per hour            | 10  | 13  | 16  | 17  | 20   |
| % of open wind speed      | 50% | 65% | 80% | 85% | 100% |



### Open Wind Speed 20 mph Conifer 40-60% Density

| H distance from windbreak | 5H  | 10H | 15H | 20H | 30H |
|---------------------------|-----|-----|-----|-----|-----|
| miles per hour            | 6   | 10  | 12  | 15  | 19  |
| % of open wind speed      | 30% | 50% | 60% | 75% | 95% |



### Open Wind Speed 20 mph Multi Row 60-80% Density

| H distance from windbreak | 5H  | 10H | 15H | 20H | 30H |
|---------------------------|-----|-----|-----|-----|-----|
| miles per hour            | 5   | 7   | 13  | 17  | 19  |
| % of open wind speed      | 25% | 35% | 65% | 85% | 95% |



### Open Wind Speed 20 mph Solid Fence 100% Density

| H distance from windbreak | 5H  | 10H | 15H | 20H | 30H  |
|---------------------------|-----|-----|-----|-----|------|
| miles per hour            | 5   | 14  | 18  | 19  | 20   |
| % of open wind speed      | 25% | 70% | 90% | 95% | 100% |

Figure 1  
Wind speed reductions via type, density, height and number of rows on the lee side of the shelterbelt, H represents the height of the shelterbelt  
From Brandle et al., 2009

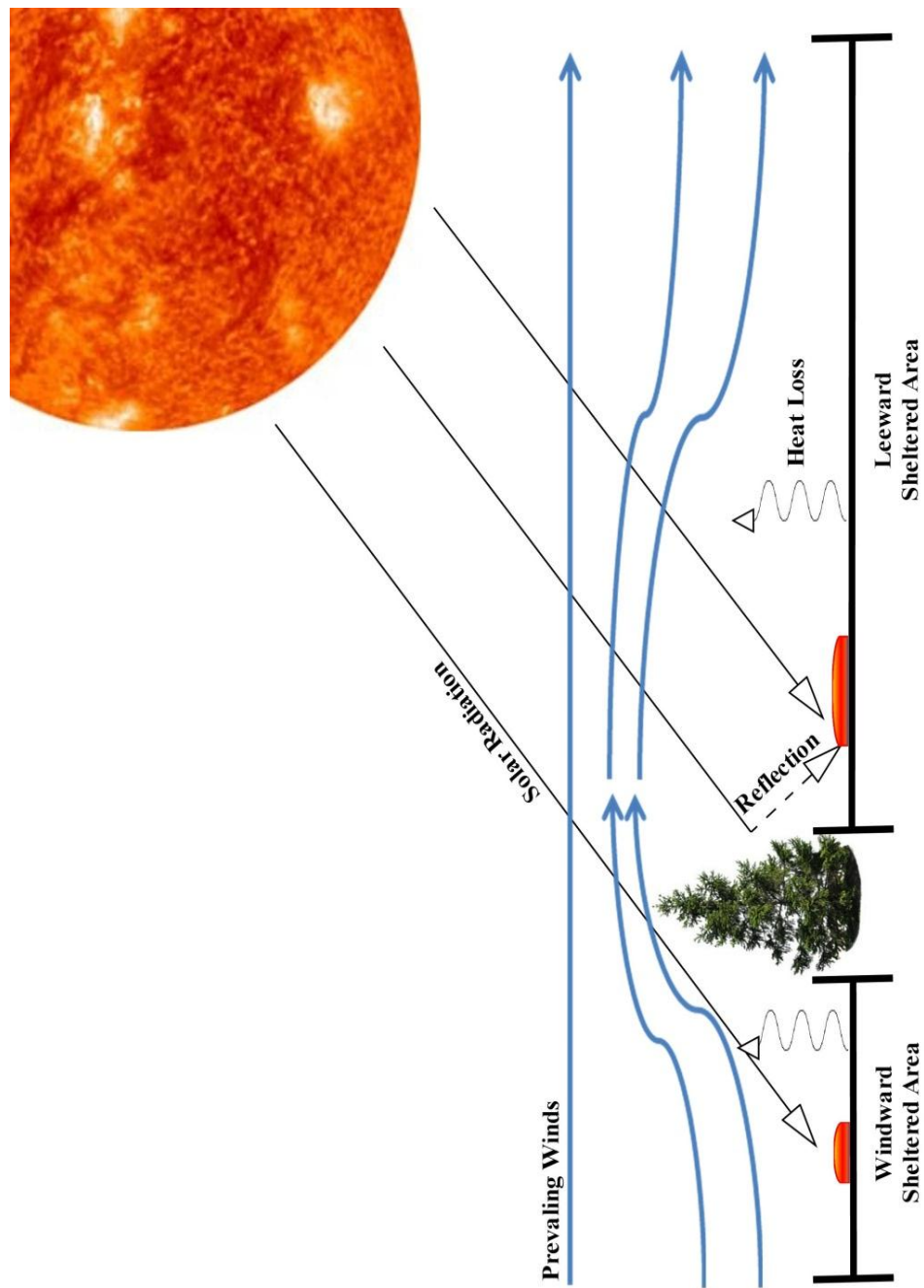


Figure 2  
 Factors creating the microclimate associated with windbreaks. Solar radiation warms the soil, reflected radiation adds to the soil temperature. Prevailing wind creates a pressure difference which sequesters the air in the sheltered area.

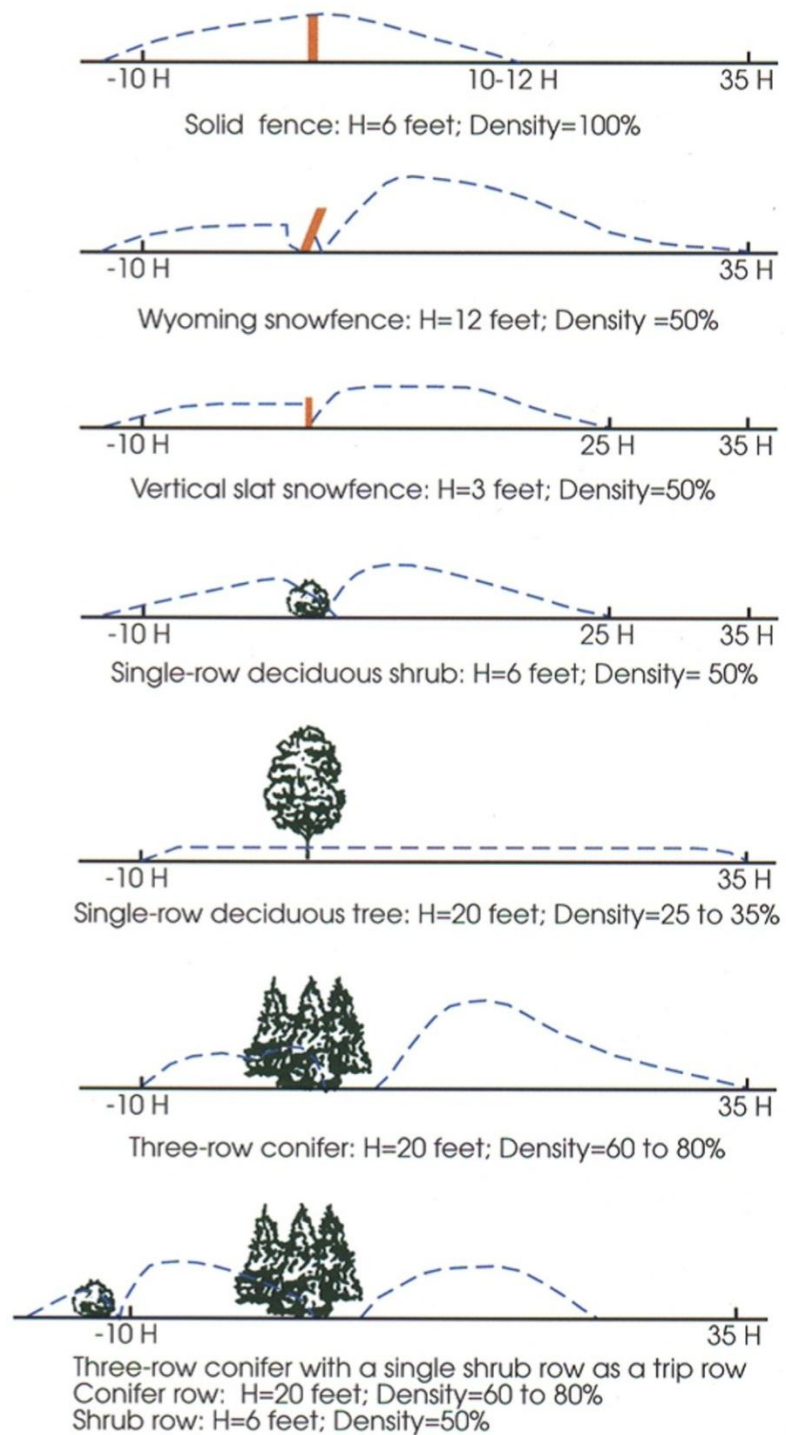


Figure 3  
 Differences in snow distribution in relation to windbreak design.  
 From Brandle et al., 2009



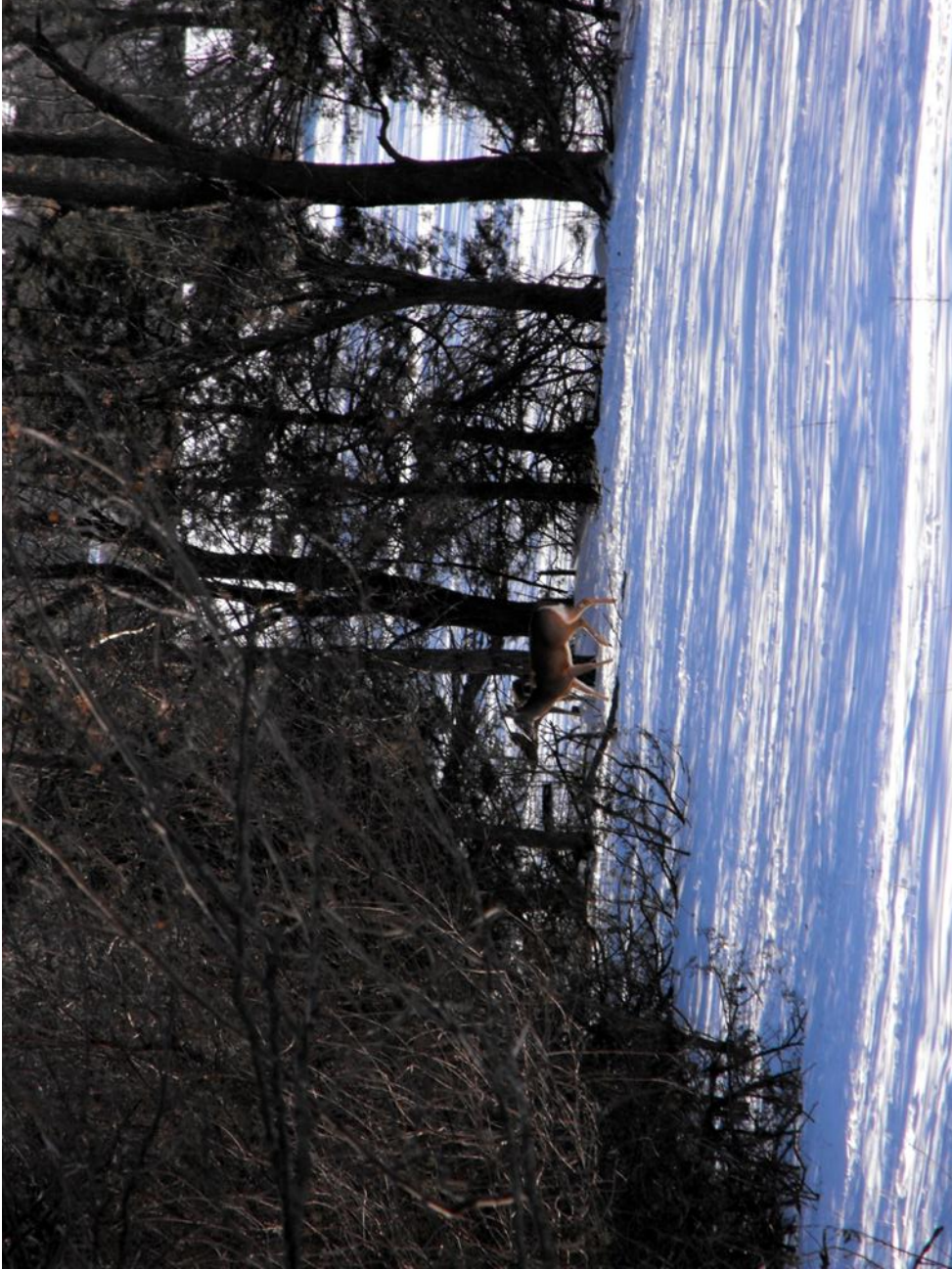


Figure 4  
Deer using shelterbelts at the University of Nebraska Agricultural Research and Development Center (ARDC), Saunders County, Nebraska. Photo taken January 3rd 2011 by M. W. Yans



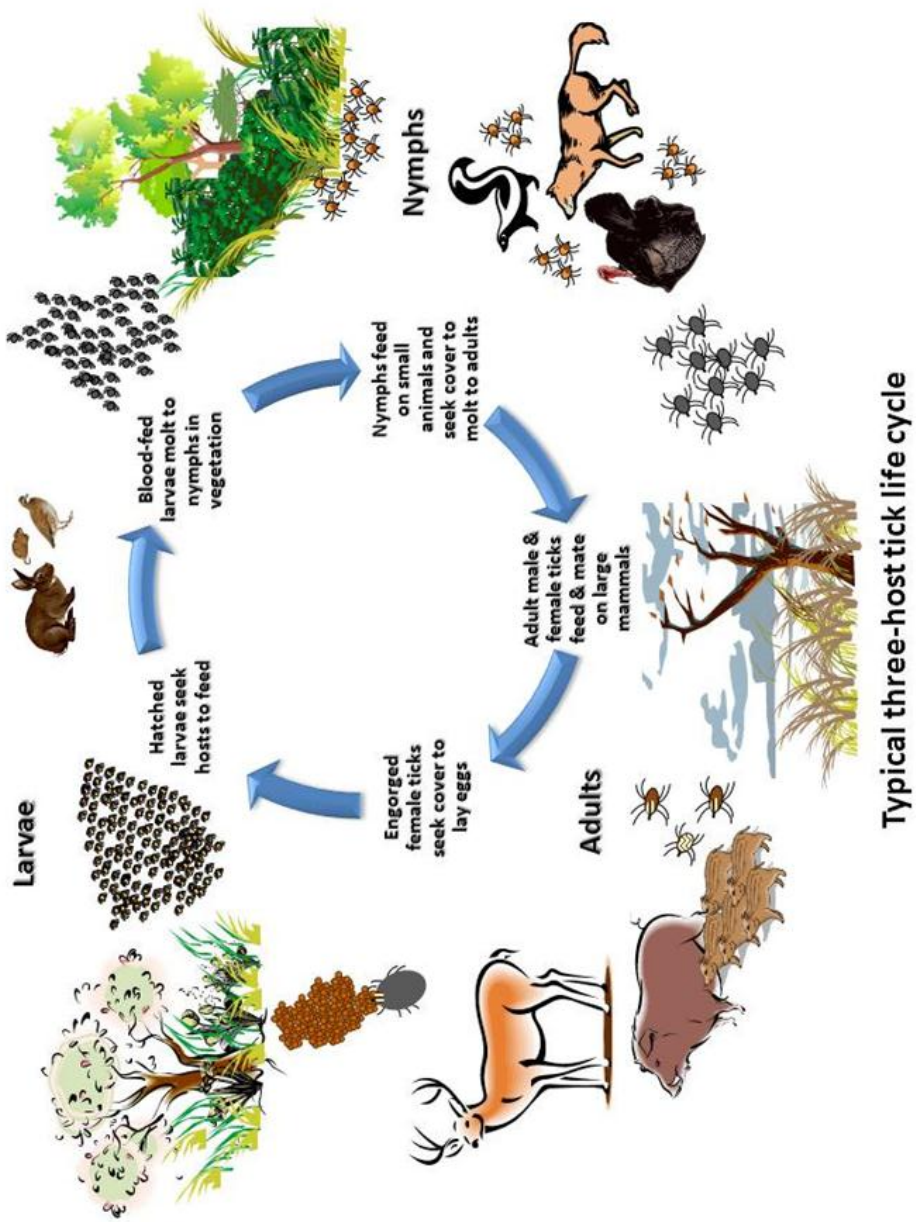


Figure 5  
Three host tick life cycle.

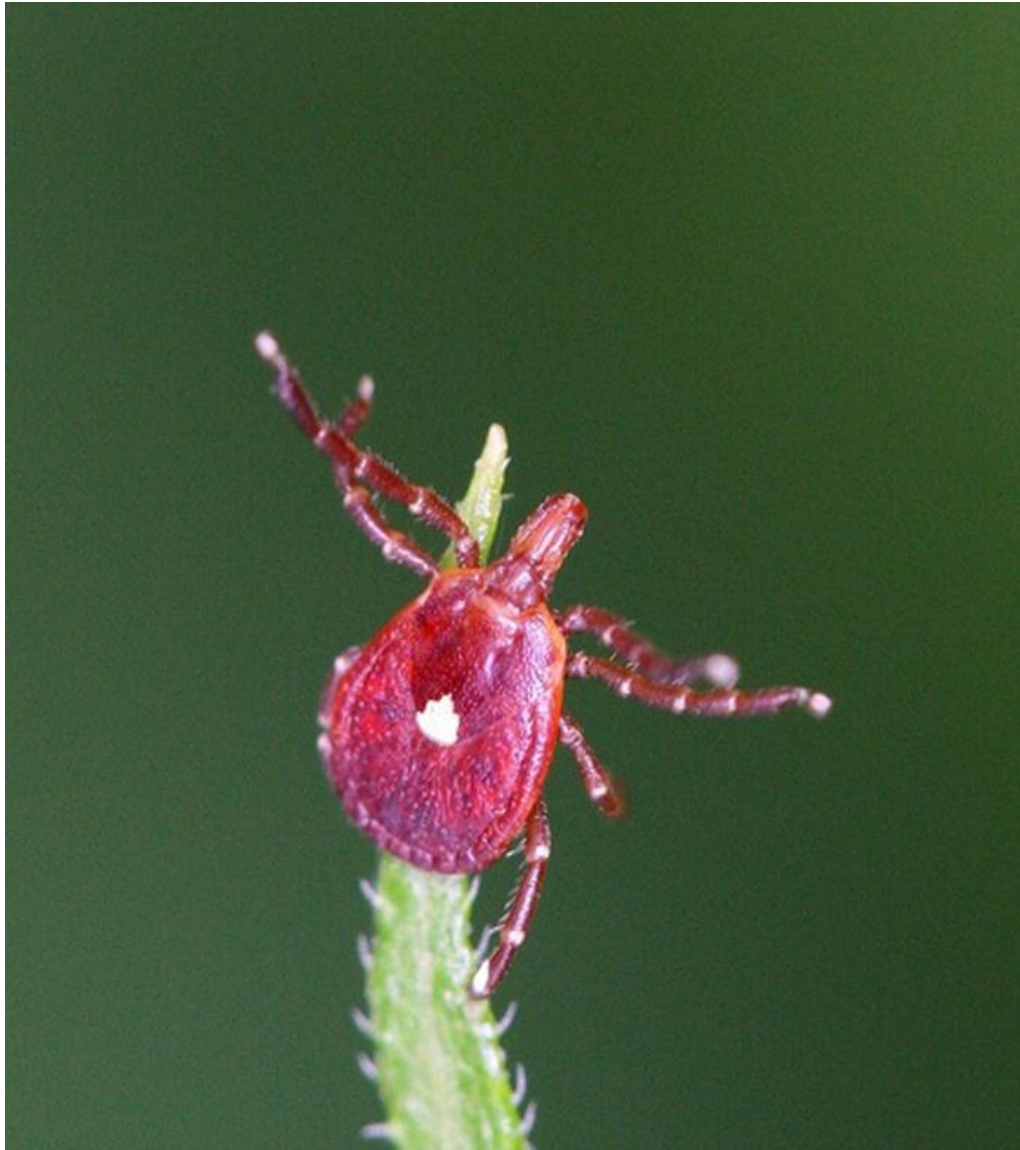


Figure 6  
Female Lone Star tick questing on vegetation.

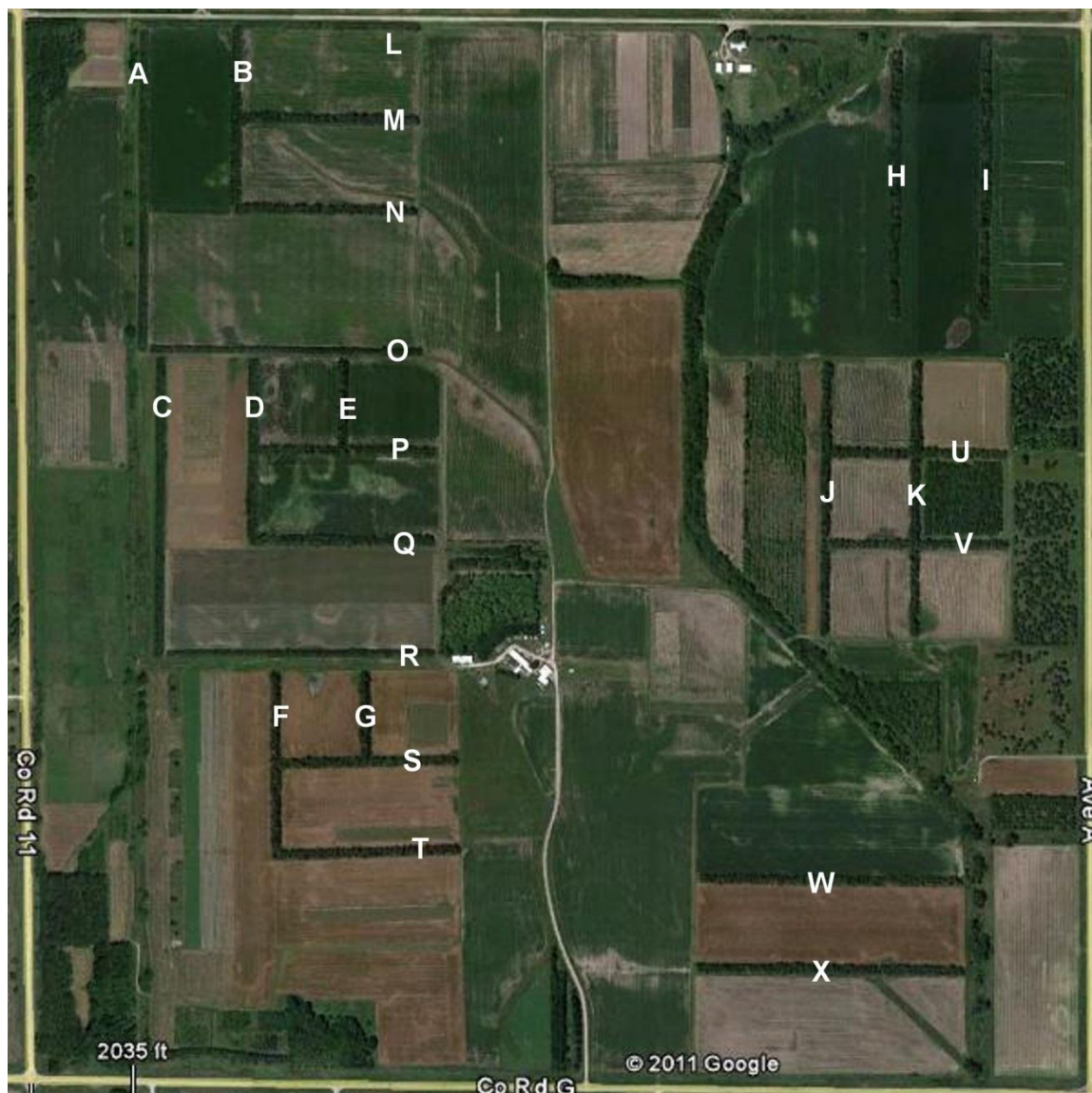


Figure 7  
Shelterbelts at the Agricultural Research and Development Center of the University of  
Nebraska Forestry Research Unit.  
Saunders County, Nebraska.



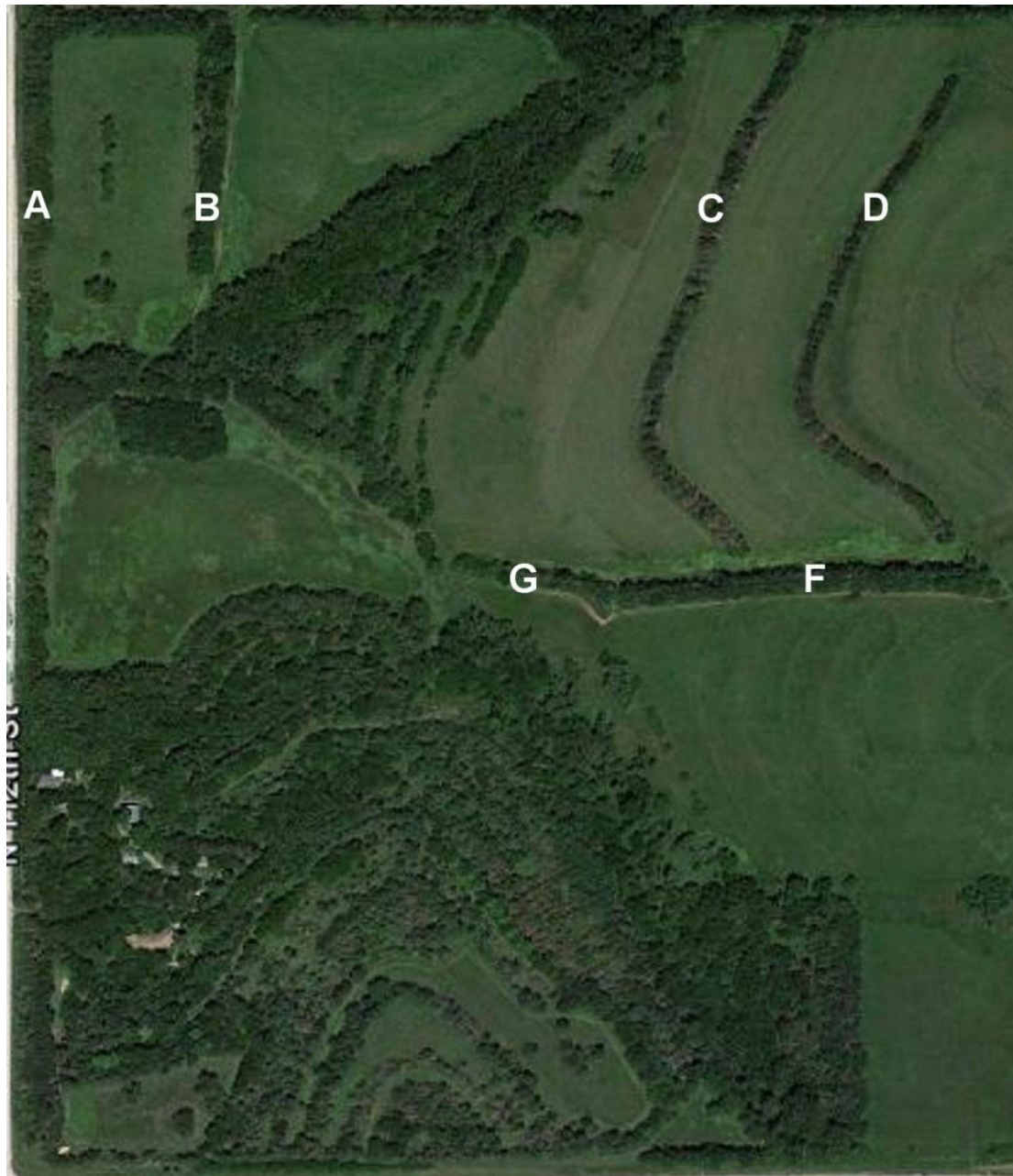


Figure 8  
Shelterbelts at Prairie Pines.  
Lancaster County, Nebraska.

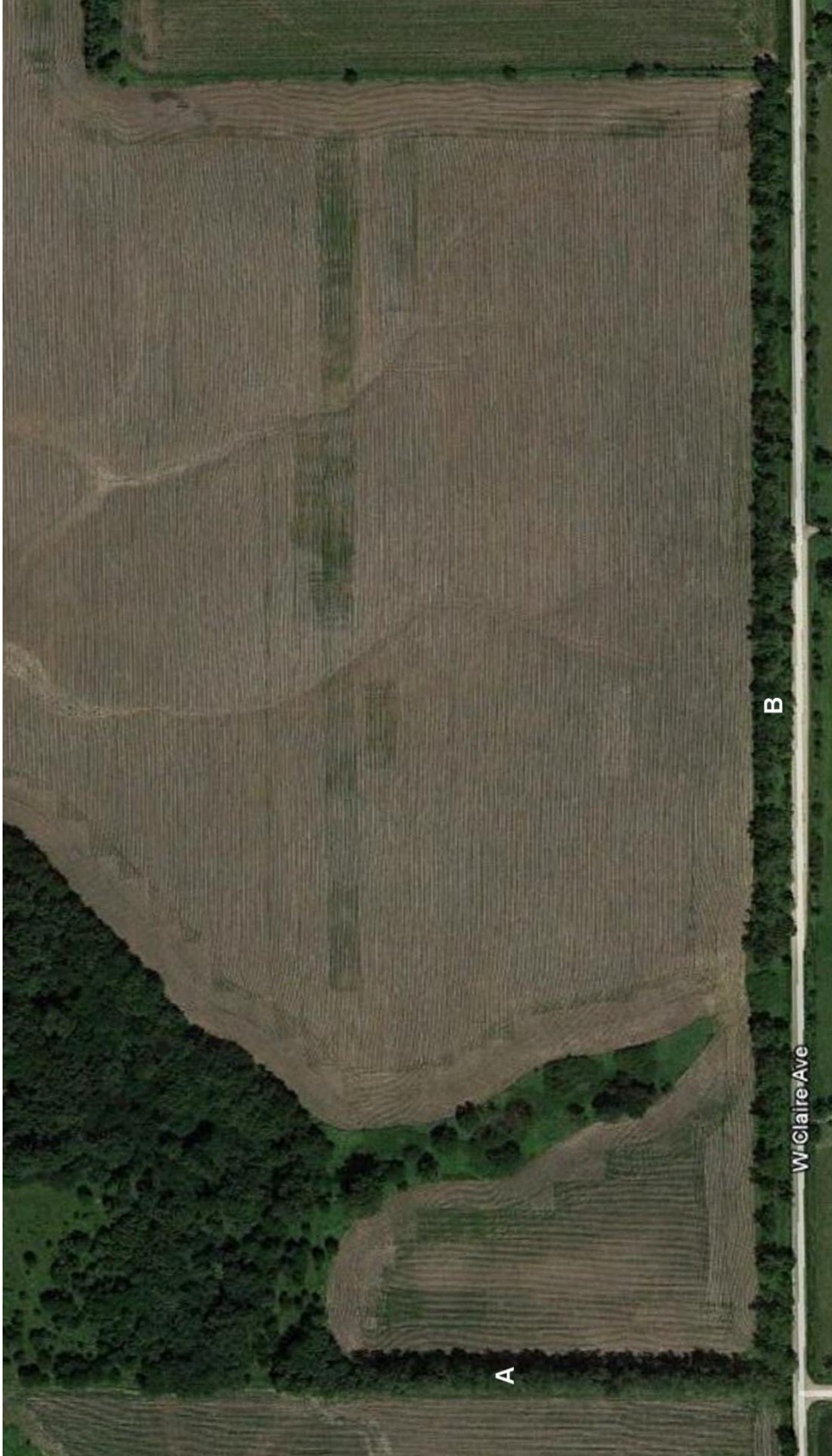


Figure 9  
Shelterbelts at West Claire Avenue and SW 40<sup>th</sup> St.  
Lancaster County, Nebraska.





Figure 10  
Shelterbelts at the Agricultural Research and Development Center of the University of  
Nebraska Farm Operations Unit.  
Saunders County, Nebraska.



Figure 11  
Shelterbelt located on county road 11.  
Saunders County, Nebraska



Figure 12  
Shelterbelt located on county road E.  
Saunders County, Nebraska.



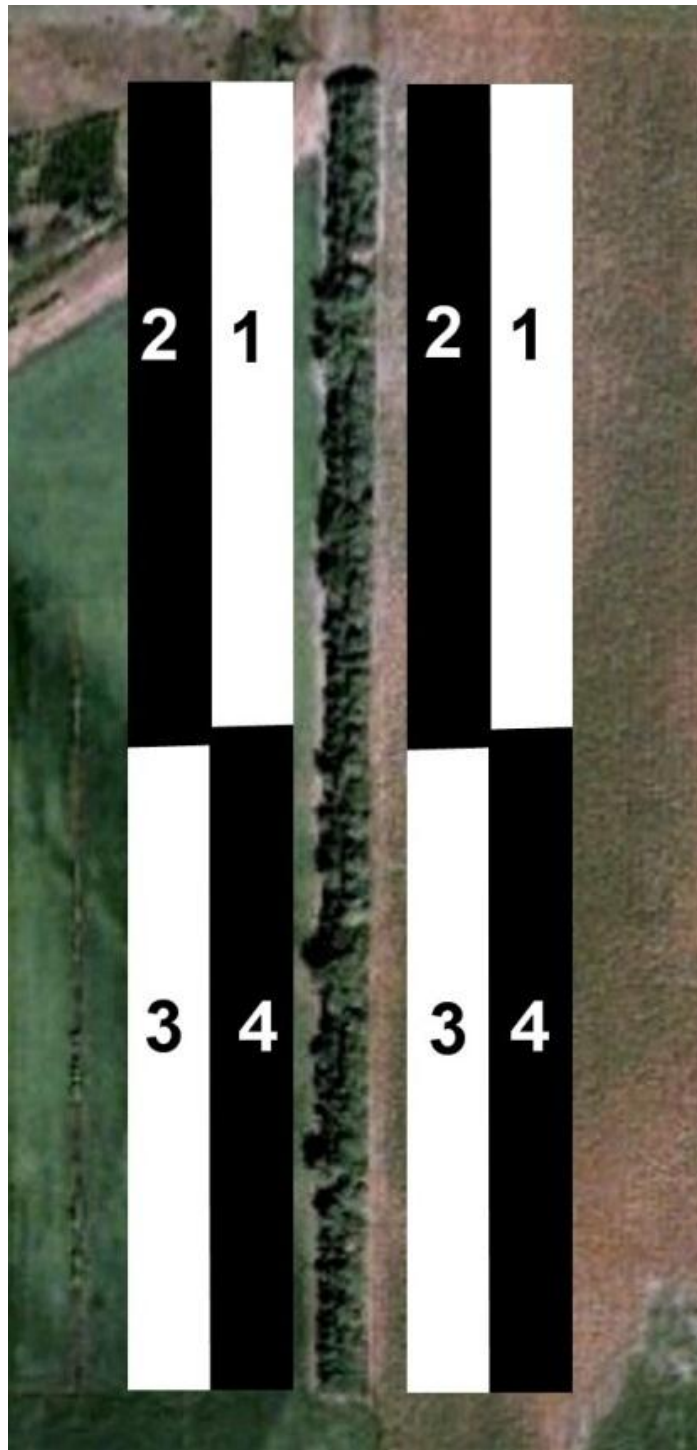


Figure 13  
Quadrants used in sampling methodology. Not to scale.



Figure 14  
Tick drag made of double layered 12oz bleached denim; 1m<sup>2</sup>.

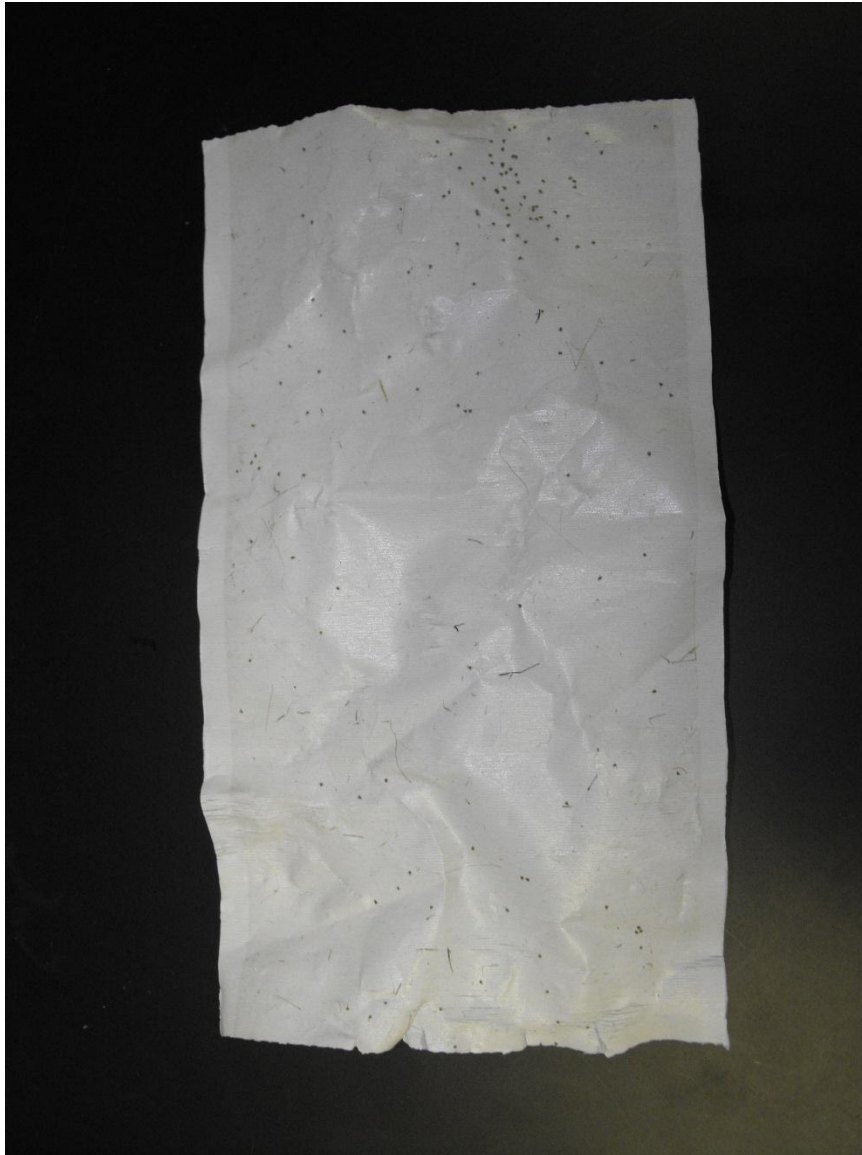


Figure 15  
Larval ticks removed with a lint roller.





Figure 16  
HOBO Pro Series data logger placed at 1/2 meter above the ground on the south  
side of tree line G at Prairie Pines.

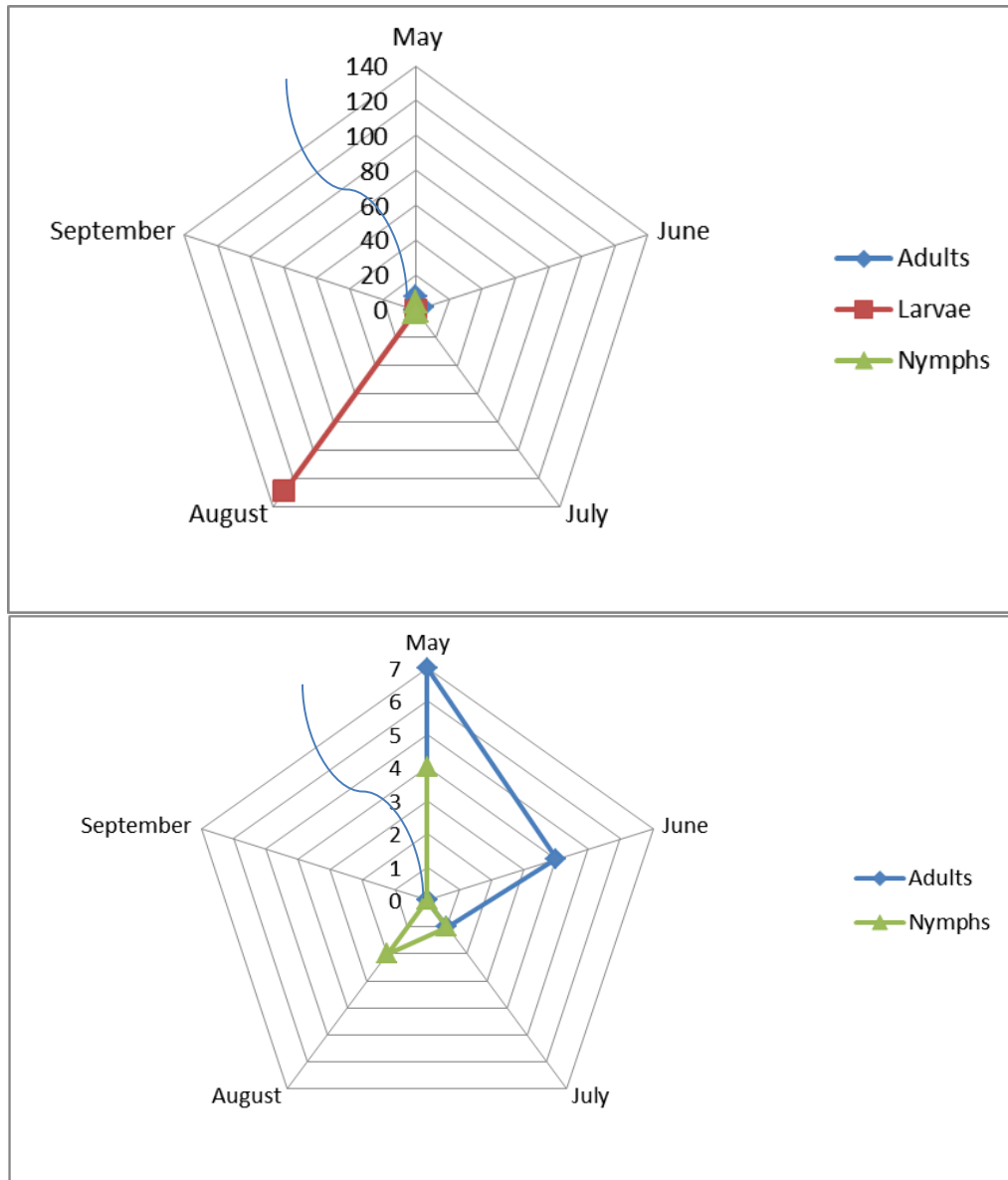


Figure 17  
*A. americanum* seasonality radar graphs for 2010. Top graph includes larvae, bottom graph omits larva.

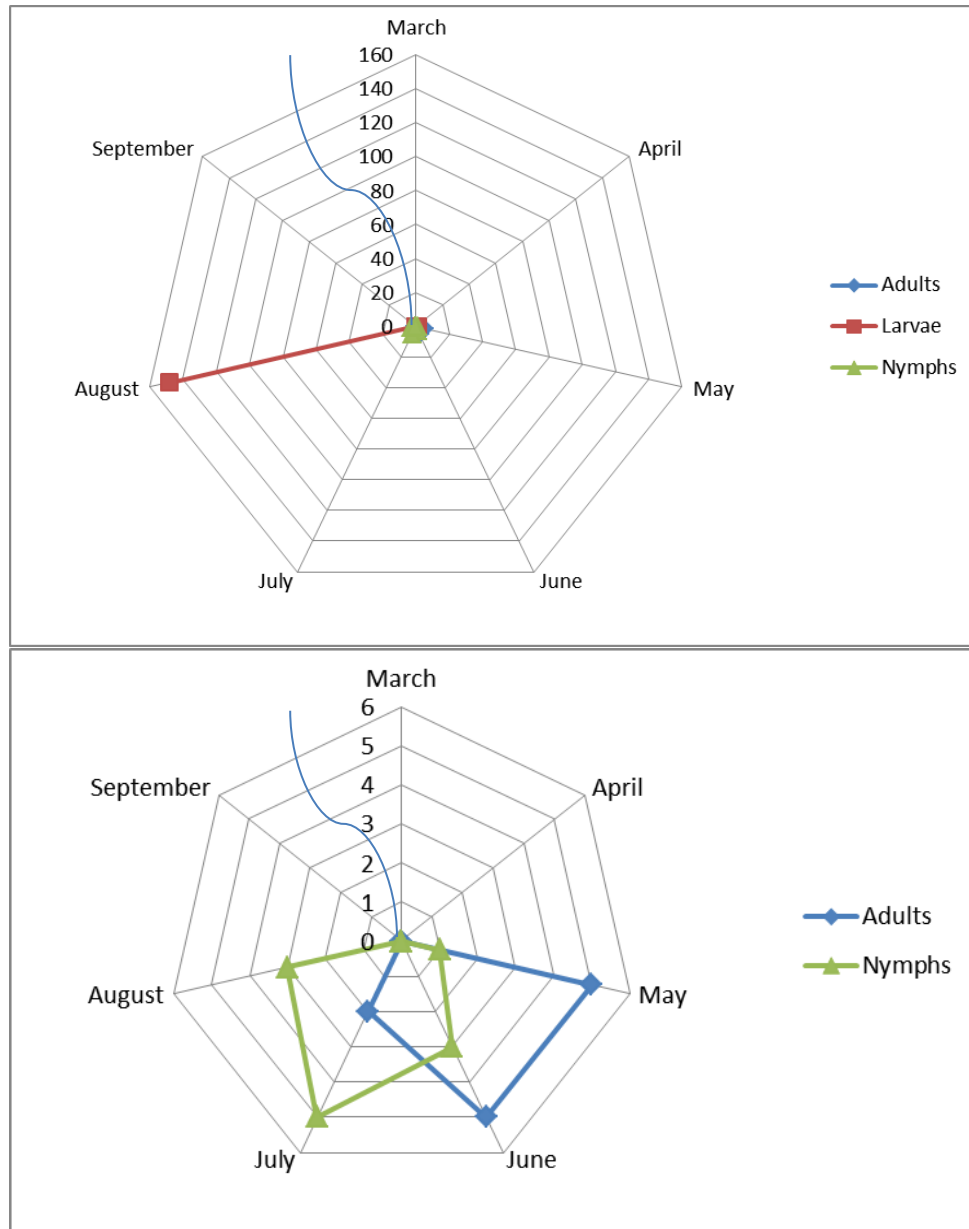


Figure 18  
*A. americanum* seasonality radar graphs for 2011. Top graph includes larvae, bottom graph omits larvae.

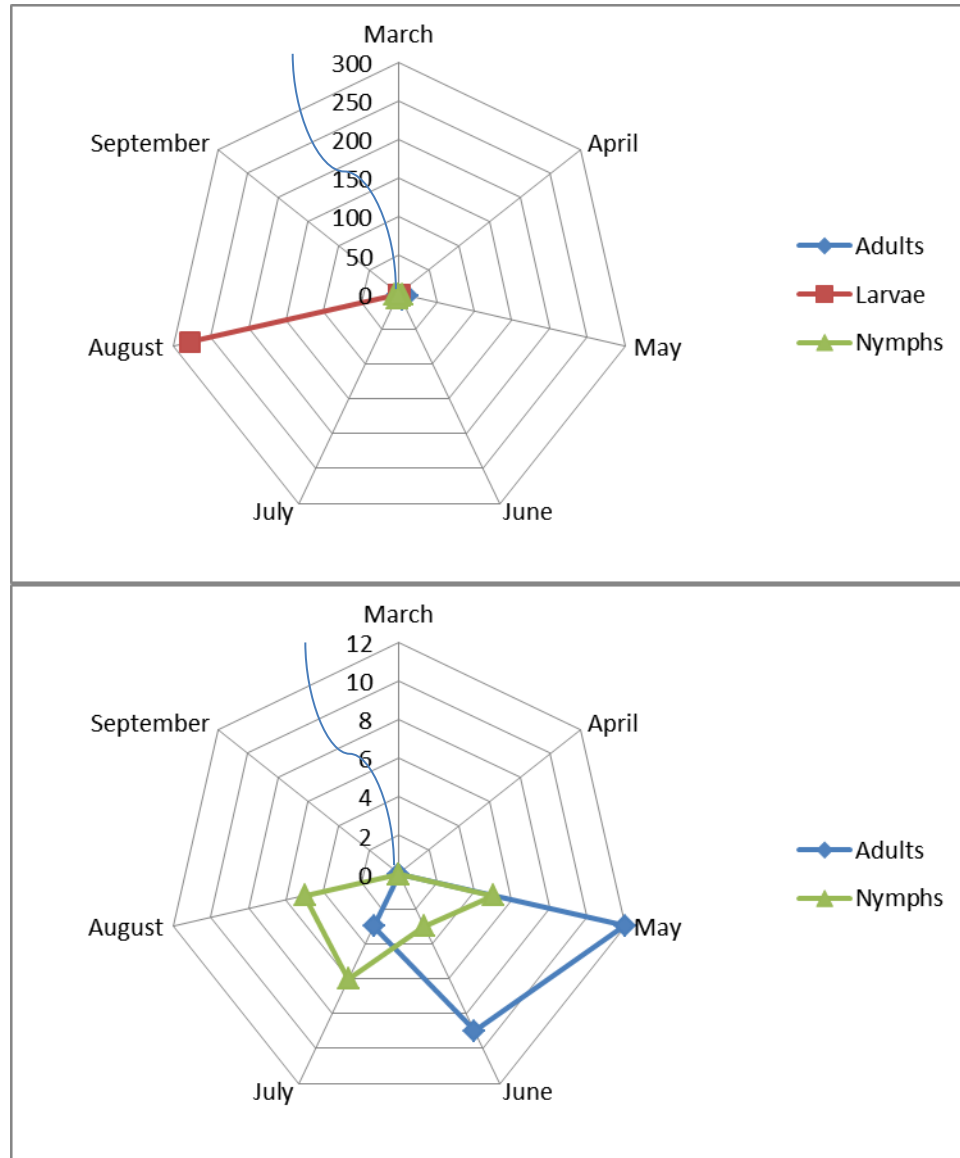


Figure 19  
*A. americanum* seasonality radar graphs for 2010 and 2011 combined. Top graph includes larvae, bottom graph omits larvae.

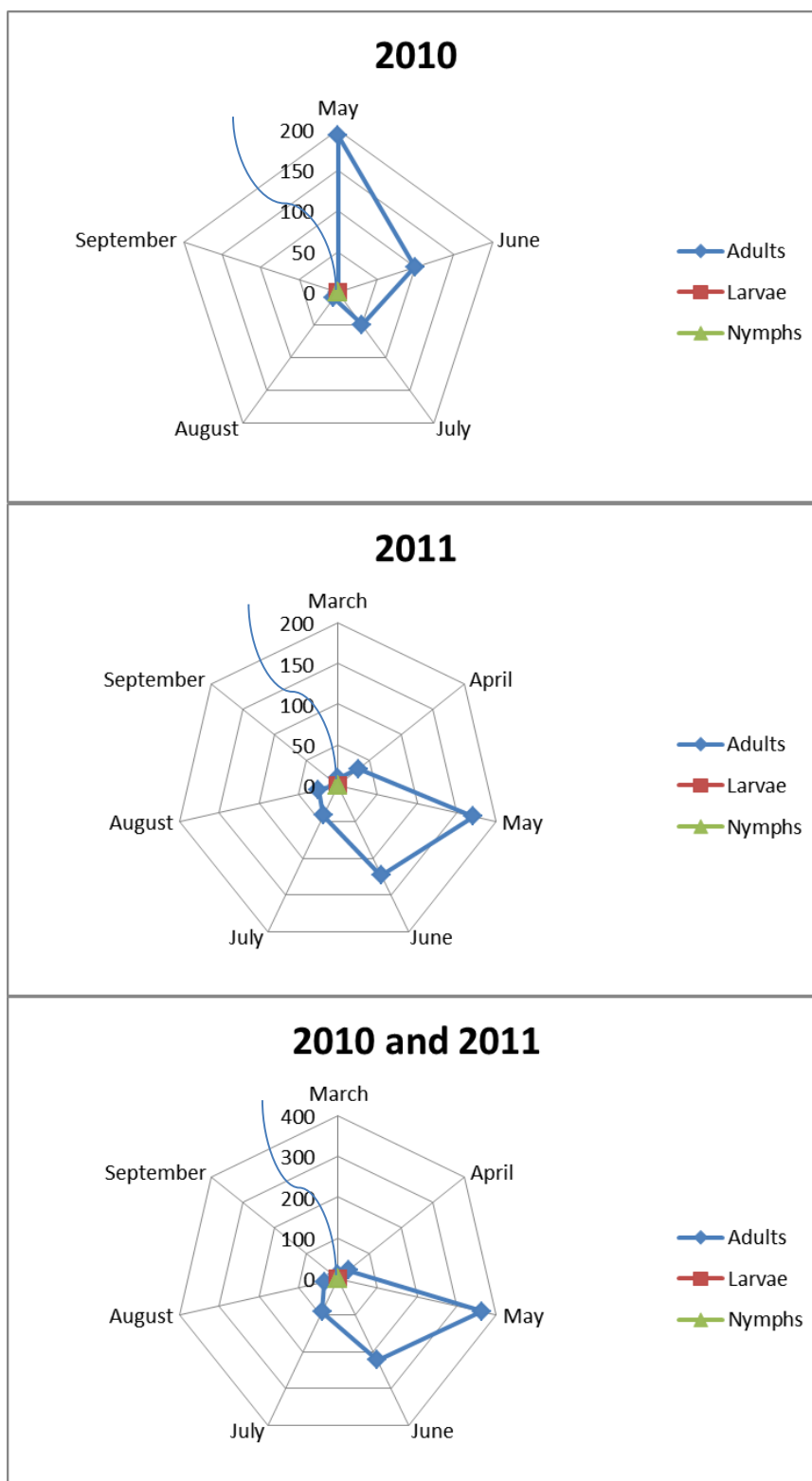


Figure 20  
*D. variabilis* seasonality radar graphs. Top 2010; middle 2011; bottom 2010 and 2011 combined





Figure 21  
2010 Crop map for the Agricultural Research and Development Center.  
Saunders County, Nebraska.

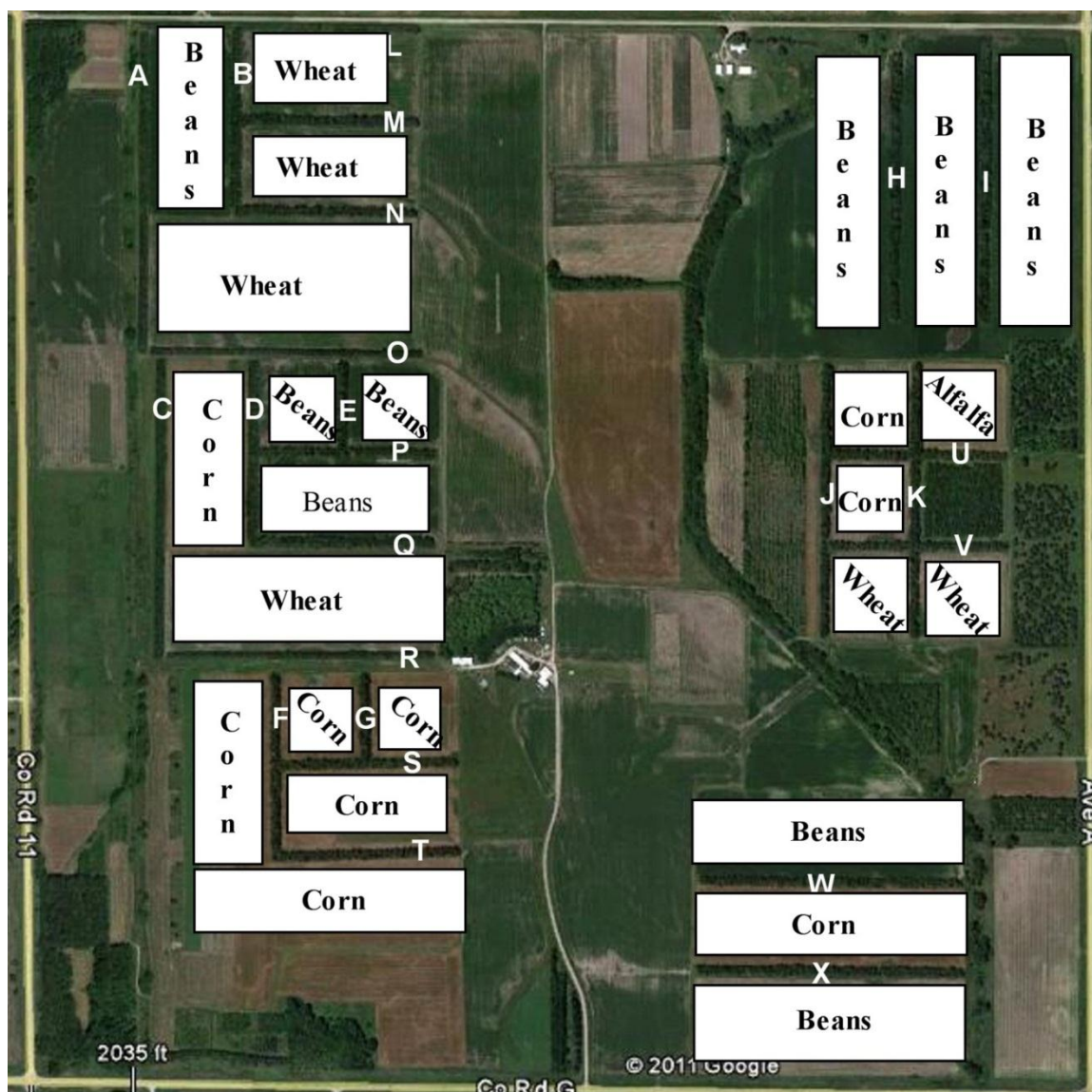


Figure 22  
2011 Crop map for the Agricultural Research and Development Center.  
Saunders County, Nebraska.

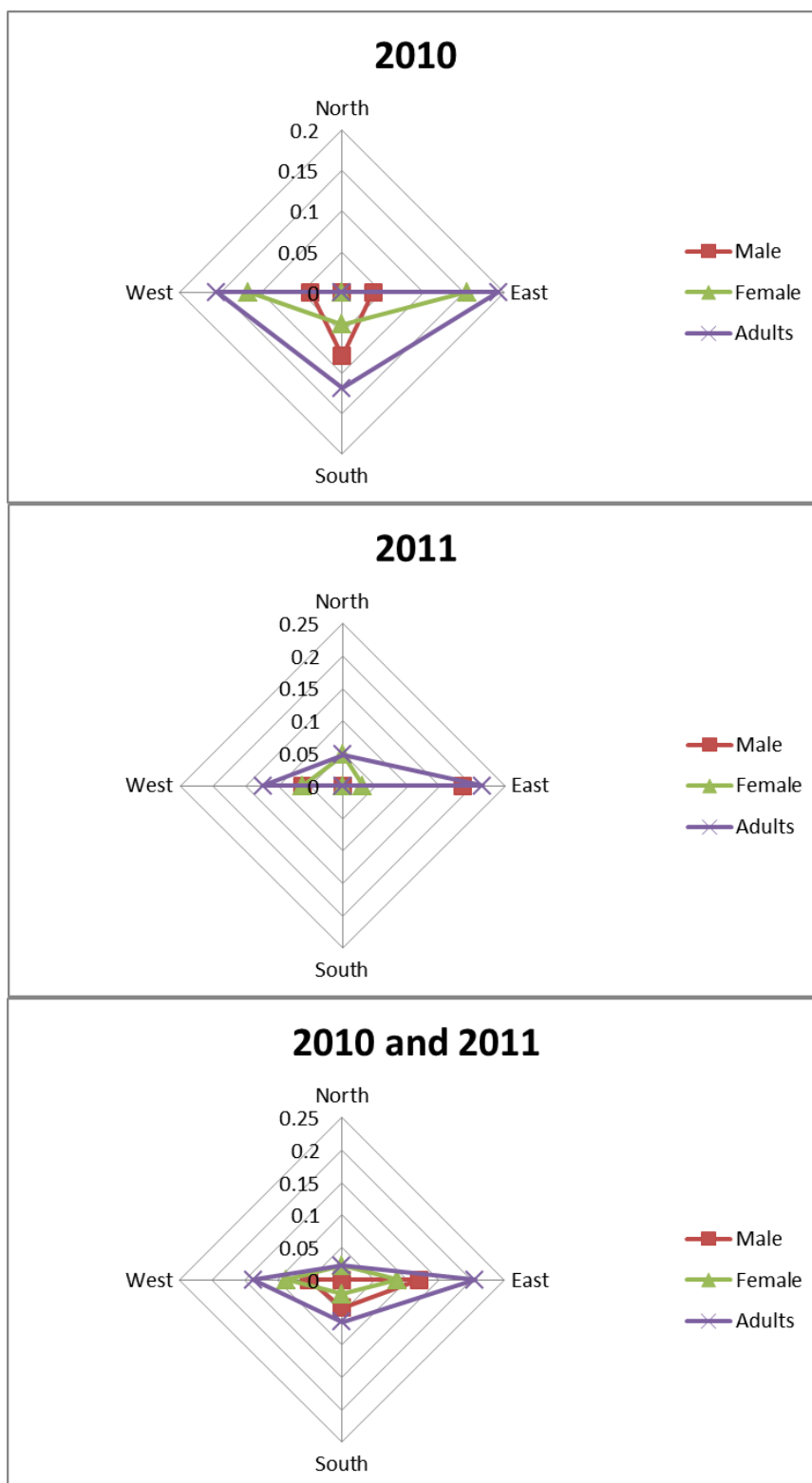


Figure 23  
*A. americanum* distribution of adults, males, and females by aspect. Expressed as ticks per Kilometer. Top 2010; middle 2011; bottom 2010 and 2011 combined.

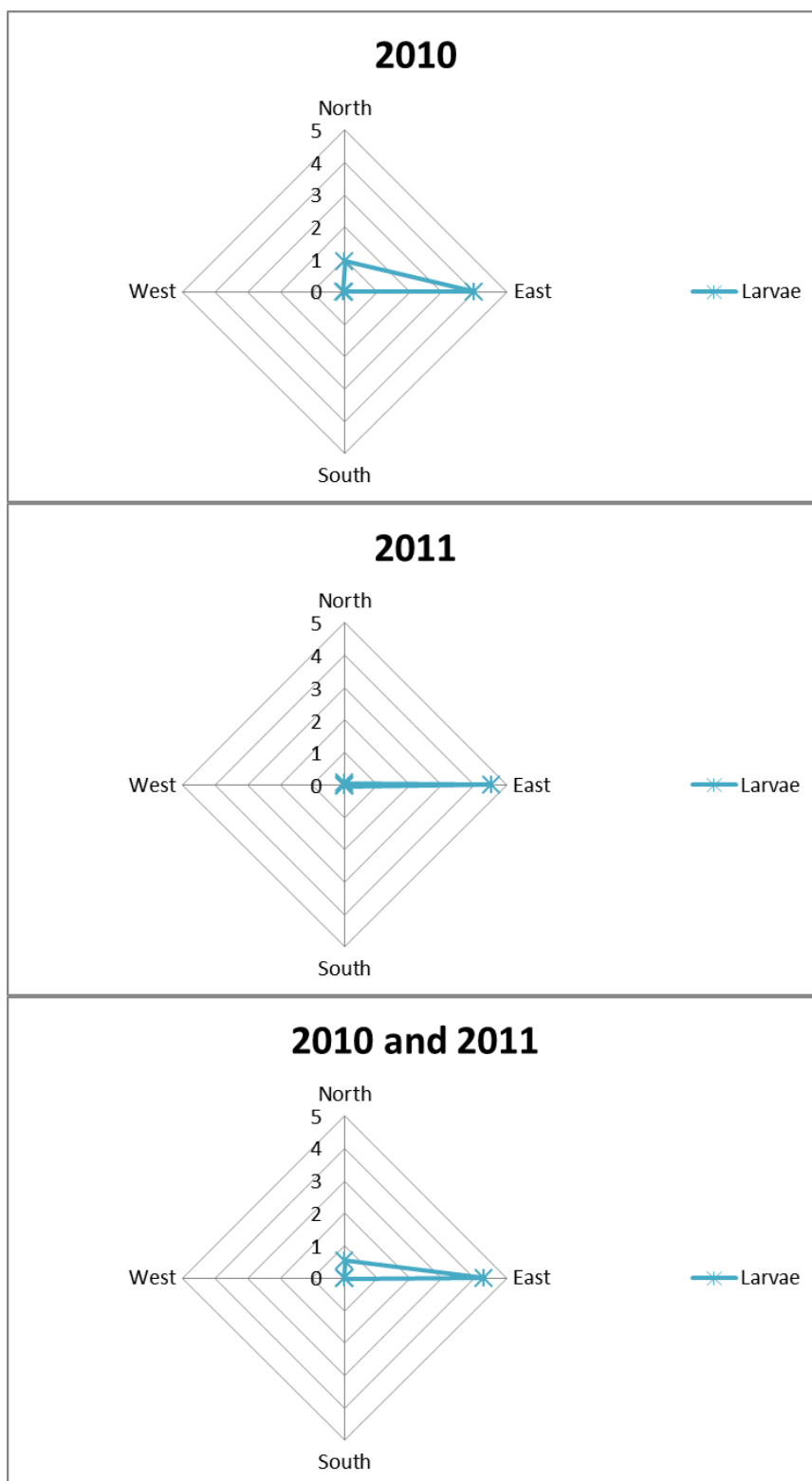


Figure 24  
*A. americanum* larval distribution by aspect. Expressed as ticks per Kilometer. Top 2010; middle 2011; bottom 2010 and 2011 combined.

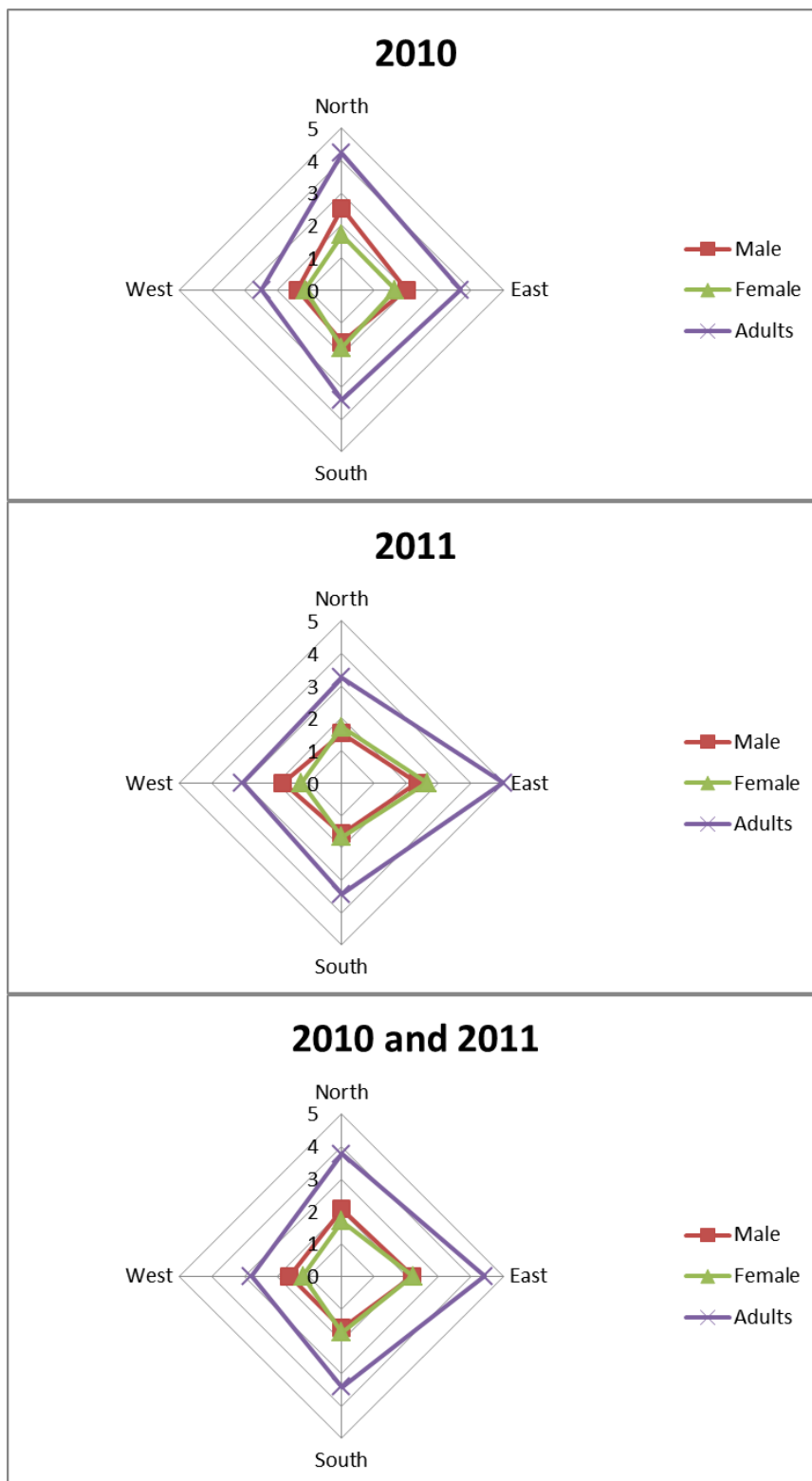


Figure 25  
*D. variabilis* distribution by aspect. Expressed as ticks per Kilometer. Top 2010; middle 2011; bottom 2010 and 2011 combined.

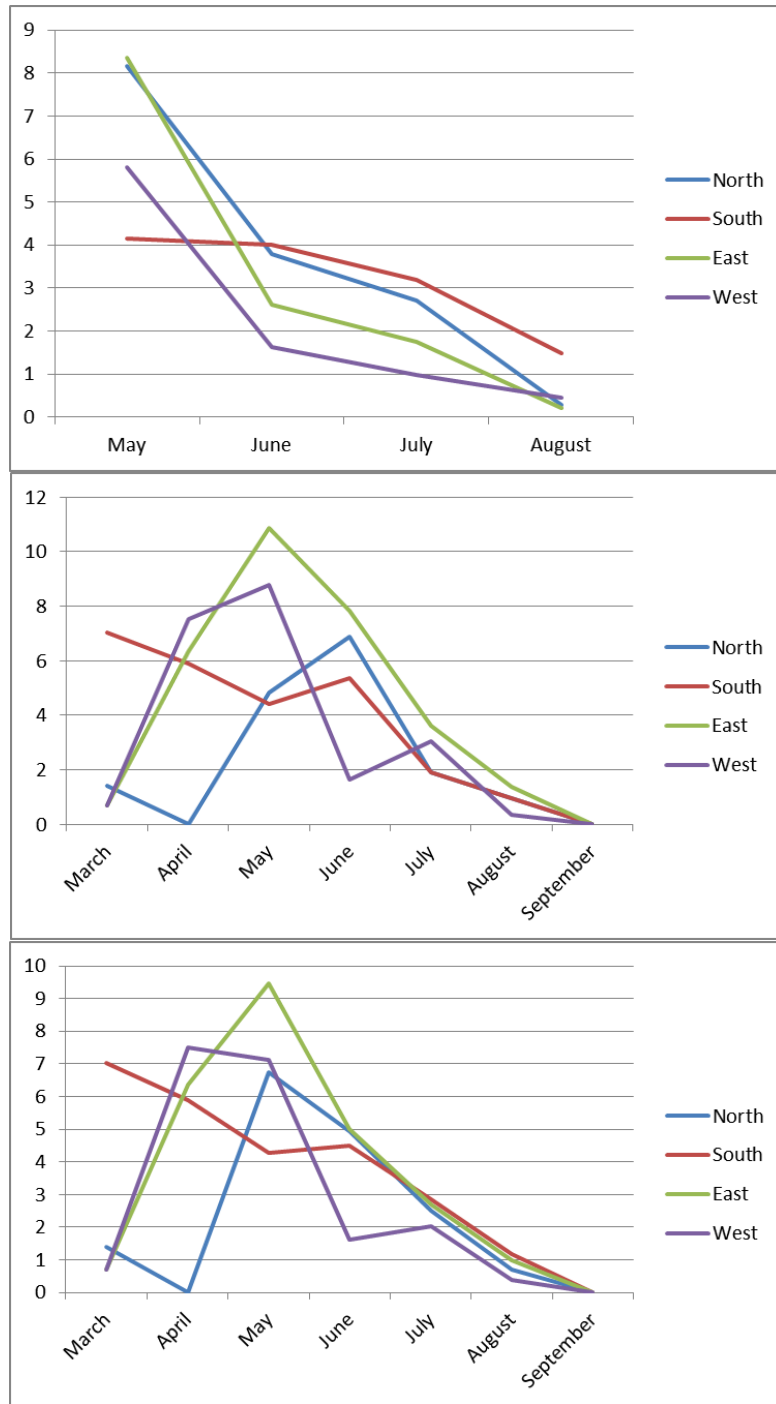


Figure 26  
 Monthly distribution graph of adults by aspect. Expressed as ticks per kilometer. Top, 2010; middle, 2011; bottom 2010 and 2011.

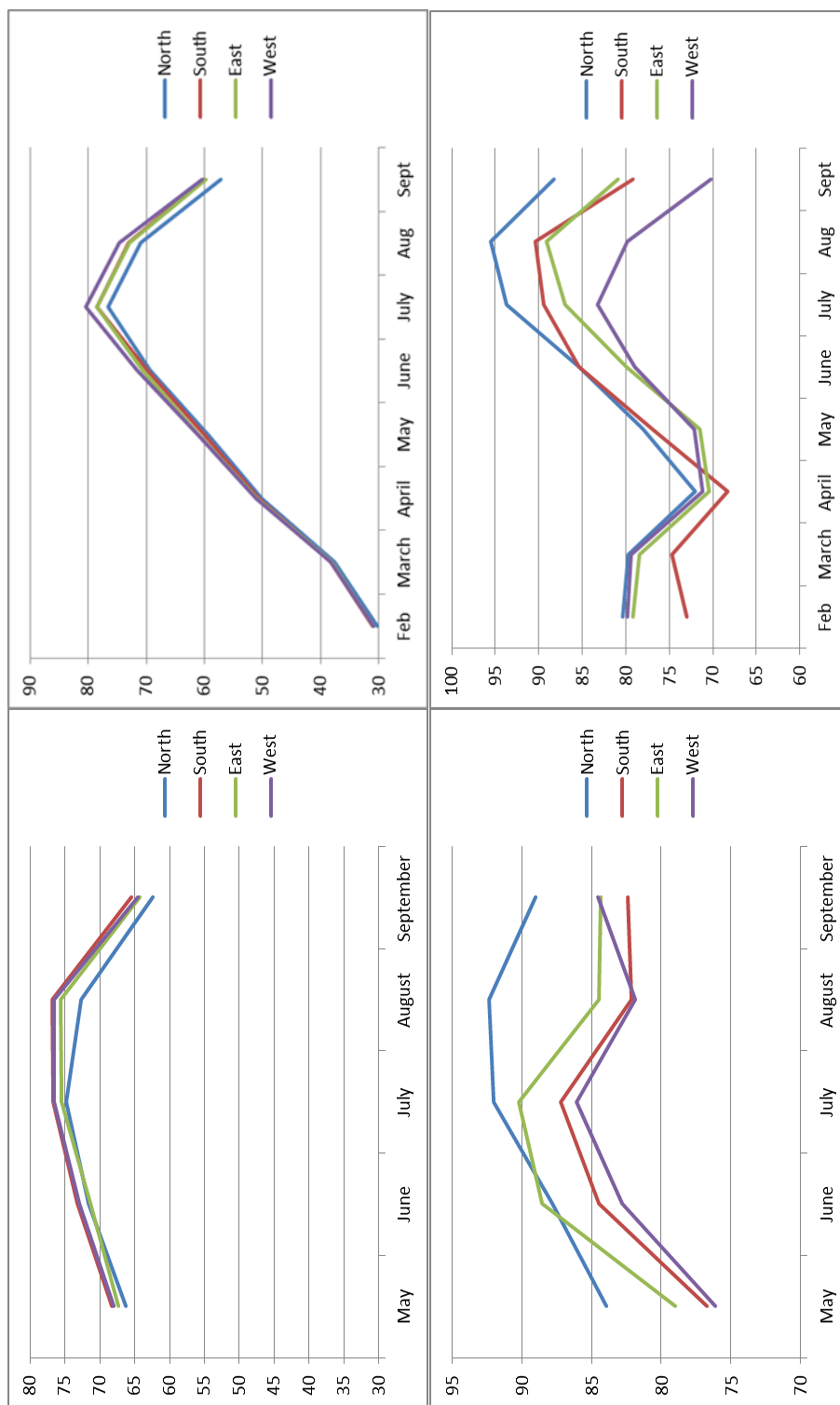


Figure 27  
 Temperature and relative humidity data for Prairie Pines in 2010 and 2011. Upper left, temperature data for 2010; upper right, temperature data for 2011; lower left, RH data for 2010; lower right, RH data for 2011



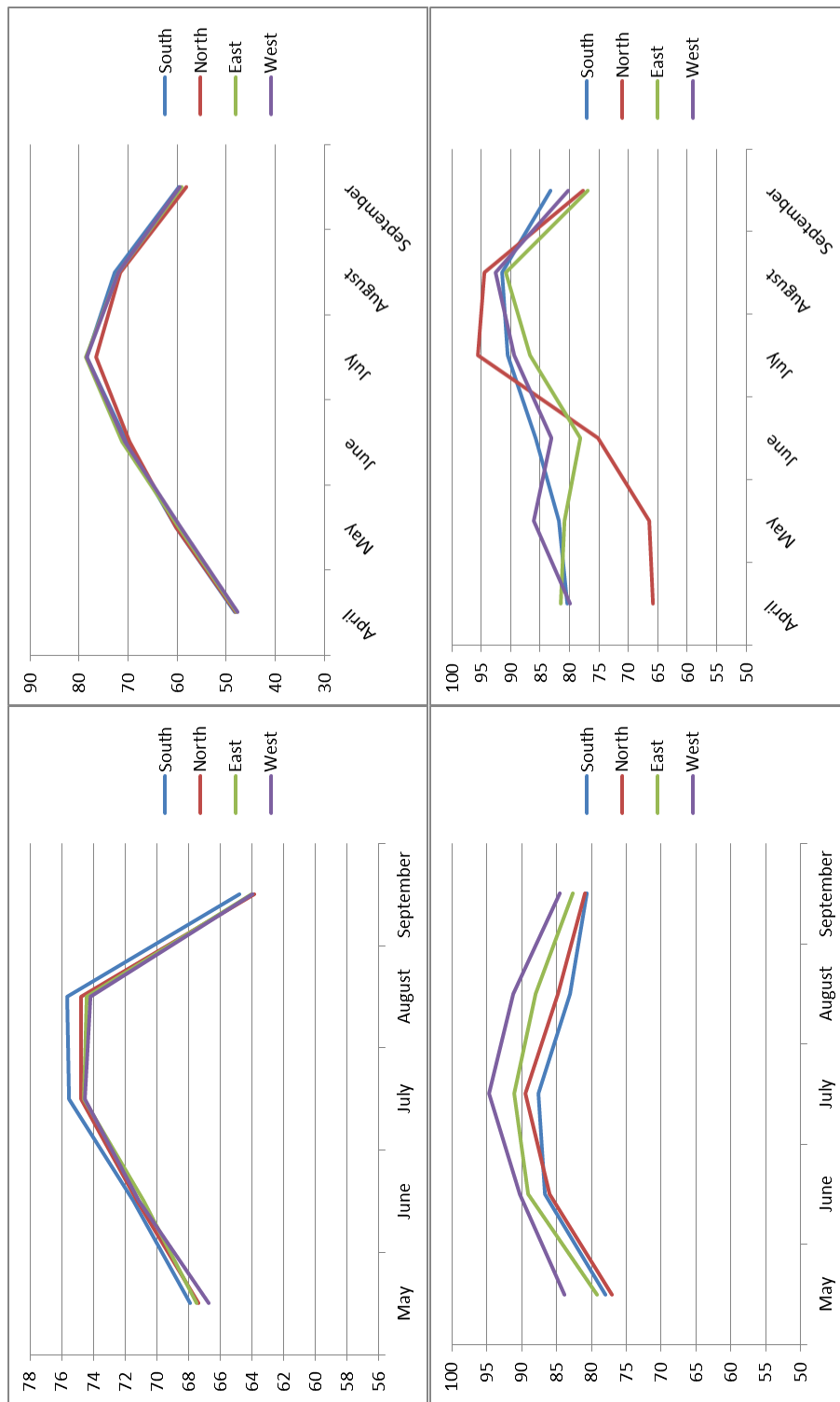


Figure 28  
 Temperature and relative humidity data for ARDC in 2010 and 2011. Upper left, temperature data for 2010; upper right, temperature data for 2011; lower left, RH data for 2010; lower right, RH data for 2011





Figure 29  
Snow cover in shelterbelts at the ARDC. Top, line O north; bottom, line P south.  
Photos taken January 3<sup>rd</sup> 2011 by M. W. Yans



Figure 30  
Snow cover in shelterbelts at the ARDC. Top, line A east; bottom, Line H west.  
Photos taken January 3<sup>rd</sup> 2011 by M. W. Yans

**Table 1.** List of pathogens and resulting disease per tick species.

| <b>Species</b>       | <b>Pathogen</b>               | <b>Disease</b>                                |
|----------------------|-------------------------------|---|
| <i>A. americanum</i> | <i>Borrelia lonestari</i>     | Southern Tick Associated Rash Illness (STAR)I |
|                      | <i>Coxiella burnetii</i>      | Q Fever                                       |
|                      | <i>Ehrlichia</i>              | Panola Mountain Ehrlichia                     |
|                      | <i>Ehrlichia chaffeensis</i>  | Human Monocytotropic Ehrlichiosis (HME)       |
|                      | <i>Ehrlichia ewingii</i>      | Canine Granulocytic Ehrlichiosis (CGE)        |
|                      | <i>Francisella tularensis</i> | Tularemia                                     |
|                      | <i>Rickettsia amblyommi</i>   | Unknown                                       |
| <i>D. variabilis</i> | <i>Ehrlichia chaffeensis</i>  | Human Monocytotropic Ehrlichiosis (HME)       |
|                      | <i>Francisella tularensis</i> | Tularemia                                     |
|                      | <i>Rickettsia rickettsia</i>  | Rocky Mountain Spotted Fever (RMSF)           |
|                      | Unknown                       | Tick Paralysis                                |

**Table 1 continued.** List of pathogens and resulting disease per tick species.

| <b>Species</b>             | <b>Pathogen</b>                  | <b>Disease</b>                        |
|----------------------------|----------------------------------|---------------------------------------|
| <i>H. leporispalustris</i> | <i>Borrelia burgdorferi</i>      | Lyme Disease                          |
|                            | <i>Coltivirus</i>                | Colorado Tick Fever (CTF)             |
|                            | <i>Francisella tularensis</i>    | Tularemia                             |
|                            | <i>Rickettsia rickettsia</i>     | Rocky Mountain Spotted Fever (RMSF)   |
| <i>I. scapularis</i>       | <i>Anaplasma phagocytophilum</i> | Human Granulocytic Anaplasmosis (HGA) |
|                            | <i>Babesia microti</i>           | Babesiosis                            |
|                            | <i>Borrelia burgdorferi</i>      | Lyme Disease                          |
|                            | <i>Flavivirus</i>                | Powassan encephalitis                 |
|                            | <i>Francisella tularensis</i>    | Tularemia                             |

**Table 2.** Distance sampled and number of ticks collected per location per year.

| Site          | Distance Sampled Km |       | Number of Ticks 2010/2011 |        |        |         |         |       |
|---------------|---------------------|-------|---------------------------|--------|--------|---------|---------|-------|
|               | 2010                | 2011  | Total                     | Male   | Female | Adult   | Larva   | Nymph |
| ARDCF.U.      | 74.22               | 42.24 | 255/82                    | 136/43 | 119/37 | 255/80  | 0/0     | 0/2   |
| ARDCF.O.      | N/A                 | 9.04  | 39                        | 19     | 20     | 39      | 0       | 0     |
| Claire Avenue | N/A                 | 12.18 | 14                        | 6      | 8      | 14      | 0       | 0     |
| County rd. 11 | N/A                 | 2.52  | 11                        | 5      | 6      | 11      | 0       | 0     |
| County rd. E  | N/A                 | 5.44  | 14                        | 9      | 5      | 20      | 0       | 0     |
| Prairie Pines | 28.4                | 35.5  | 246/418                   | 60/128 | 49/128 | 109/256 | 129/151 | 8/11  |

**Table 3.** Tick species by location

| Species                       | Location             | Number of Ticks 2010/2011 |        |        |         |         |       |
|-------------------------------|----------------------|---------------------------|--------|--------|---------|---------|-------|
|                               |                      | Total                     | Male   | Female | Adult   | Larva   | Nymph |
| <i>Dermacentor variabilis</i> | ARDC F.U.            | 252/79                    | 135/43 | 117/36 | 252/79  | 0       | 0     |
|                               | ARDC F.O. (2011)     | 39                        | 19     | 20     | 39      | 0       | 0     |
|                               | Claire Avenue (2011) | 13                        | 6      | 7      | 13      | 0       | 0     |
|                               | County rd. 11 (2011) | 11                        | 5      | 6      | 11      | 0       | 0     |
|                               | County rd. E (2011)  | 14                        | 9      | 5      | 14      | 0       | 0     |
|                               | Prairie Pines        | 103/245                   | 58/120 | 44/125 | 102/245 | 0/0     | 1/0   |
|                               | ARDC F.U.            | 3/2                       | 1/0    | 2/1    | 3/1     | 0/0     | 0/1   |
| <i>Amblyomma americanum</i>   | Claire Avenue (2011) | 1                         | 0      | 1      | 1       | 0       | 0     |
|                               | Prairie Pines        | 145/170                   | 3/8    | 6/2    | 9/10    | 129/149 | 7/11  |

Table 3 continued. Tick species by location

| Species                               | Location             | Number of Ticks 2010/2011 |      |        |       |       |       |
|---------------------------------------|----------------------|---------------------------|------|--------|-------|-------|-------|
|                                       |                      | Total                     | Male | Female | Adult | Larva | Nymph |
| <i>Haemaphysalis leporispalustris</i> | ARDC F.U. (2011)     | 1                         | 0    | 0      | 0     | 0     | 1     |
|                                       | Prairie Pines (2011) | 2                         | 0    | 0      | 0     | 2     | 0     |
| <i>Ixodes scapularis</i>              | Prairie Pines (2011) | 1                         | 0    | 1      | 1     | 0     | 0     |

**Table 4.** Monthly collection numbers by species

| Species                    | Year | Number of Ticks: Adult/ Larva/ Nymph |        |         |         |        |         |           |  |  |
|----------------------------|------|--------------------------------------|--------|---------|---------|--------|---------|-----------|--|--|
|                            |      | March                                | April  | May     | June    | July   | August  | September |  |  |
| <i>D. variabilis</i>       | 2010 | N/A                                  | N/A    | 193/0/0 | 100/0/0 | 50/0/0 | 9/0/1   | 0/0/0     |  |  |
|                            | 2011 | 9/0/0                                | 32/0/0 | 171/0/0 | 123/0/0 | 41/0/0 | 25/0/0  | 0/0/0     |  |  |
| <i>A. americanum</i>       | 2010 | N/A                                  | N/A    | 7/0/4   | 4/0/0   | 1/0/1  | 0/129/2 | 0/0/0     |  |  |
|                            | 2011 | 0/0/0                                | 0/0/0  | 5/1/1   | 5/0/3   | 2/0/5  | 0/148/3 | 0/0/0     |  |  |
| <i>H. leporispalustris</i> | 2011 | 0/0/0                                | 0/1/0  | 0/0/0   | 0/0/0   | 0/0/0  | 0/0/2   | 0/0/0     |  |  |
| <i>I. scapularis</i>       | 2011 | 1/0/0                                | 0/0/0  | 0/0/0   | 0/0/0   | 0/0/0  | 0/0/0   | 0/0/0     |  |  |



**Table 5.** Differences of LSM between sites

---

| <b>Site</b>                     | <b>Pr &gt;  t </b> | <b>LSM</b> | <b>SEM</b> |
|---------------------------------|--------------------|------------|------------|
| ARDC F.O. vs. ARDC F.U.         | .0021              | 8.3958     | 5.4437     |
| County Rd 11 vs. ARDC F.U       | .0270              | 6.8971     | 5.7993     |
| County Rd. E vs. ARDC F.U       | .0201              | 7.3809     | 6.0929     |
| ARDC F.U. vs. Claire Avenue     | .8982              |            |            |
| ARDC F.U. vs. Prairie Pines     | .0683              |            |            |
| ARDC F.O. vs. County Rd. 11     | .8259              |            |            |
| ARDC F.O. vs. County Rd. E      | .8840              |            |            |
| ARDC F.O. vs. Claire Avenue     | .0072              | 9.0646     | 7.0551     |
| ARDC F.O. vs. Prairie Pines     | .0306              | 3.4106     | 1.1683     |
| County Rd. 11 vs. County Rd. E  | .9482              |            |            |
| County Rd. 11 vs. Claire Avenue | .0399              | 7.4465     | 7.0389     |
| County Rd. 11 vs. Prairie Pines | .1287              |            |            |
| County Rd. E vs. Claire Avenue  | .0318              | 7.9688     | 7.4363     |
| County Rd. E vs. Prairie Pines  | .1007              |            |            |
| Claire Avenue vs. Prairie Pines | .3163              |            |            |

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**Table 6.** Shelterbelt composition at ARDC

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| <b>Shelterbelt Composition</b>   | <b>Line</b>           |
|--|-----------------------|
| <i>Juniperus virginiana</i>  | A C L O R             |
| <i>Juniperus virginiana</i> ,<br><i>Pinus nigra</i>                                    | D E H I P Q W X       |
| <i>Juniperus virginiana</i> ,<br><i>Pinus nigra</i> ,<br><i>Fraxinus pennsylvanica</i> | B F G J K M N S T U V |

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**Table 7.** Species numbers by aspect.

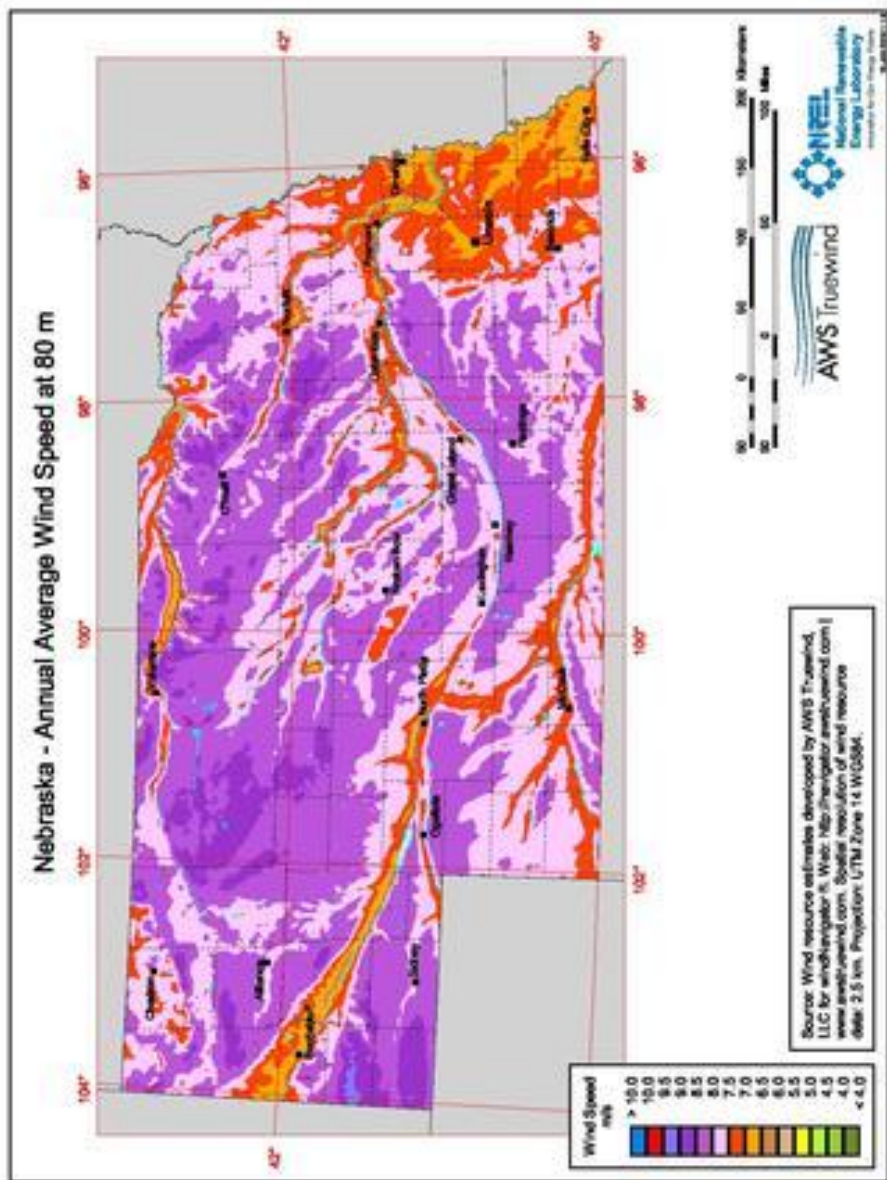
| Species                    | Year | Number of Ticks: Adult/ Larva/ Nymph per Kilometer |           |              |             |
|----------------------------|------|--|-----------|--------------|-------------|
|                            |      | North  | South     | East         | West        |
| <i>D. variabilis</i>       | 2010 | 4.22/0/0   | 3.39/0/0  | 3.66/0/.04   | 2.47/0/0    |
|                            | 2011 | 3.24/0/0   | 3.43/0/0  | 4.99/0/0     | 3.55/0/0    |
| <i>A. americanum</i>       | 2010 | 0/.95/0  | .12/0/0   | .19/4.01/.19 | .15/.04/.08 |
|                            | 2011 | .05/.05/.14  | 0/.05/.05 | .22/4.53/.22 | .12/0/.03   |
| <i>H. leporispalustris</i> | 2011 | 0/.08/0  | 0/0/.04   | 0/0/0        | 0/0/0       |
| <i>I. scapularis</i>       | 2011 | 0/0/0  | .03/0/0   | 0/0/0        | 0/0/0       |

**Table 8.** Aspect vs. time.

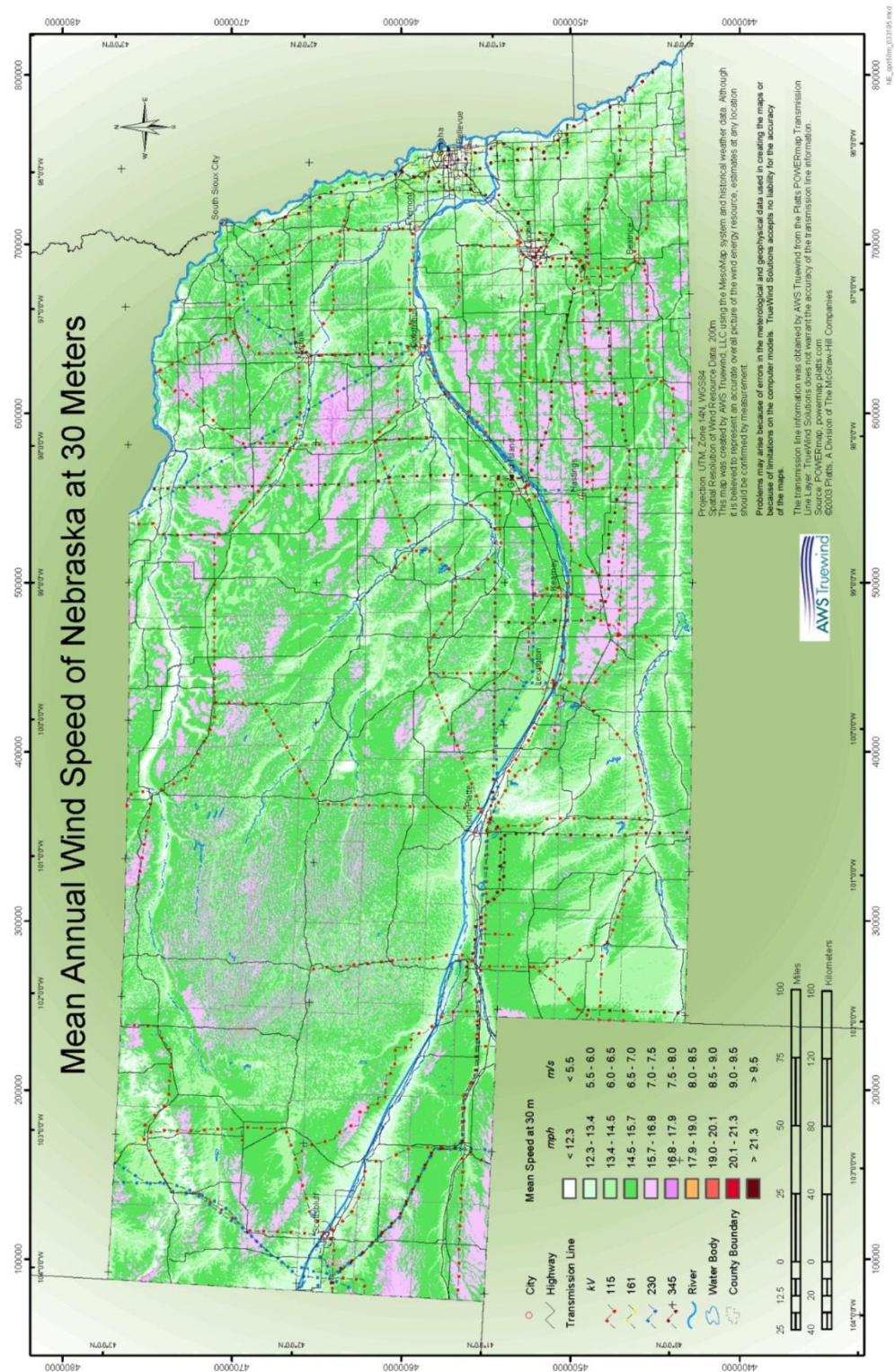
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| <b>Direction</b> | <b>Time</b> | <b>Pr &gt;  t </b> | <b>Mean</b> | <b>SEM</b> |
|------------------|-------------|--------------------|-------------|------------|
| North            | Morning     | 0.4339             | 0.4859      | 0.4479     |
| North            | Afternoon   | 0.4277             | 0.4813      | 0.4434     |
| South            | Morning     | 0.5488             | 0.5756      | 0.5300     |
| South            | Afternoon   | 0.3556             | 0.4266      | 0.3931     |
| East             | Morning     | 0.6491             | 0.6589      | 0.6038     |
| East             | Afternoon   | 0.5032             | 0.5413      | 0.4960     |
| West             | Morning     | 0.2159             | 0.3188      | 0.2942     |
| West             | Afternoon   | 0.3272             | 0.4063      | 0.3732     |

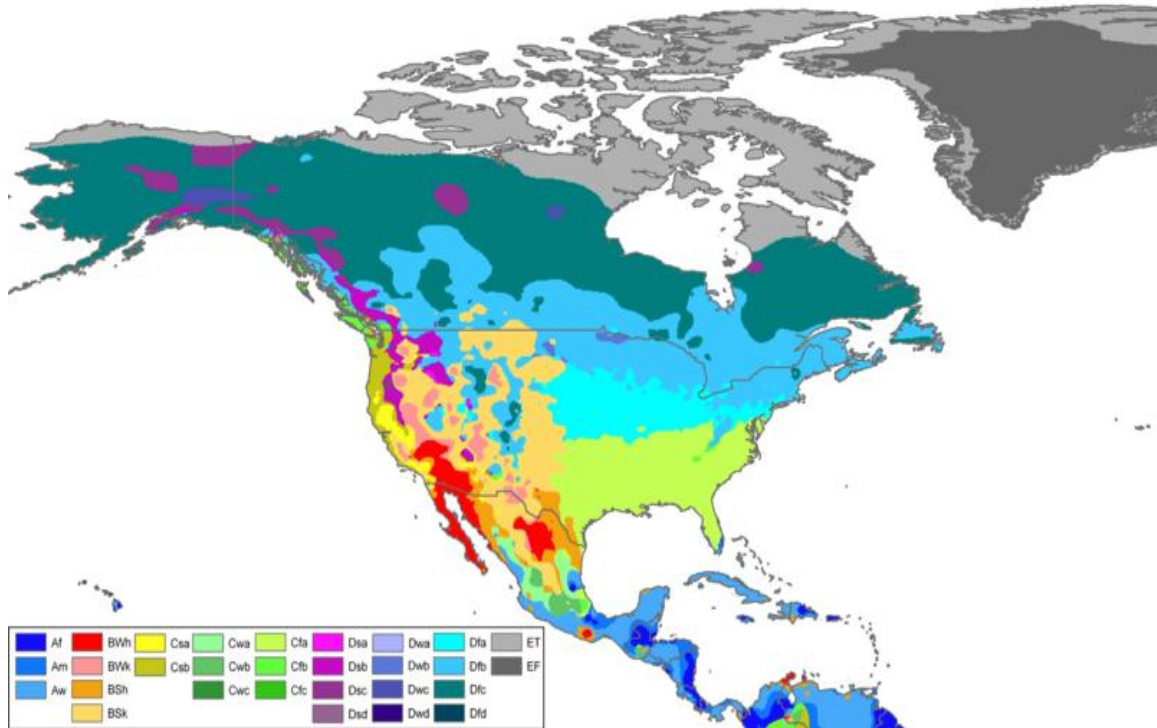
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Map 1  
Nebraska Annual Average Wind Speed at 80M  
Source: <http://www.neo.ne.gov/renew/windmapresource.pdf>



Map 2  
 Mean Annual Wind Speed of Nebraska at 30M  
 Source: [http://www.neo.ne.gov/renew/windresources/NE\\_spd30m\\_0408052.pdf](http://www.neo.ne.gov/renew/windresources/NE_spd30m_0408052.pdf)



|             |   |
|-------------|---|
| <b>Af:</b>  | <a href="#">equatorial climate</a>  |
| <b>Am:</b>  | <a href="#">monsoon climate</a>   |
| <b>Aw:</b>  | <a href="#">tropical savanna climate</a>  |
| <b>BWh:</b> | <a href="#">warm desert climate</a>   |
| <b>BWk:</b> | <a href="#">cold desert climate</a>   |
| <b>Bsh:</b> | <a href="#">warm semi-arid climate</a>  |
| <b>BSk:</b> | <a href="#">cold semi-arid climate</a>  |
| <b>Csa:</b> | <a href="#">warm mediterranean climate</a>                                      |
| <b>Csb:</b> | <a href="#">temperate mediterranean climate</a>                                 |
| <b>Cwa:</b> | <a href="#">humid subtropical climate</a>                                       |
| <b>Cwb:</b> | <a href="#">humid subtropical climate/subtropical oceanic highland climate</a>  |
| <b>Cwc:</b> | <a href="#">oceanic subpolar climate</a>  |
| <b>Cfa:</b> | <a href="#">warm oceanic climate/humid subtropical climate</a>                  |
| <b>Cfb:</b> | <a href="#">temperate oceanic climate</a>                                       |
| <b>Cfc:</b> | <a href="#">cool oceanic climate</a>  |
| <b>Dsa:</b> | <a href="#">warm continental climate/mediterranean continental climate</a>      |
| <b>Dsb:</b> | <a href="#">temperate continental climate/mediterranean continental climate</a> |
| <b>Dsc:</b> | <a href="#">cool continental climate</a>  |
| <b>Dsd:</b> | <a href="#">cold continental climate</a>  |
| <b>Dwa:</b> | <a href="#">warm continental climate/humid continental climate</a>              |
| <b>Dwb:</b> | <a href="#">temperate continental climate/humid continental climate</a>         |
| <b>Dwc:</b> | <a href="#">cool continental climate/subarctic climate</a>                      |
| <b>Dwd:</b> | <a href="#">cold continental climate/subarctic climate</a>                      |
| <b>Dfa:</b> | <a href="#">warm continental climate/humid continental climate</a>              |
| <b>Dfb:</b> | <a href="#">temperate continental climate/humid continental climate</a>         |
| <b>Dfc:</b> | <a href="#">cool continental climate/subarctic climate</a>                      |
| <b>Dfd:</b> | <a href="#">cold continental climate/subarctic climate</a>                      |
| <b>ET:</b>  | <a href="#">tundra climate</a>  |
| <b>EF:</b>  | <a href="#">ice cap climate</a>   |

Map 3

Koppen-Geiger climate classification system of North America.



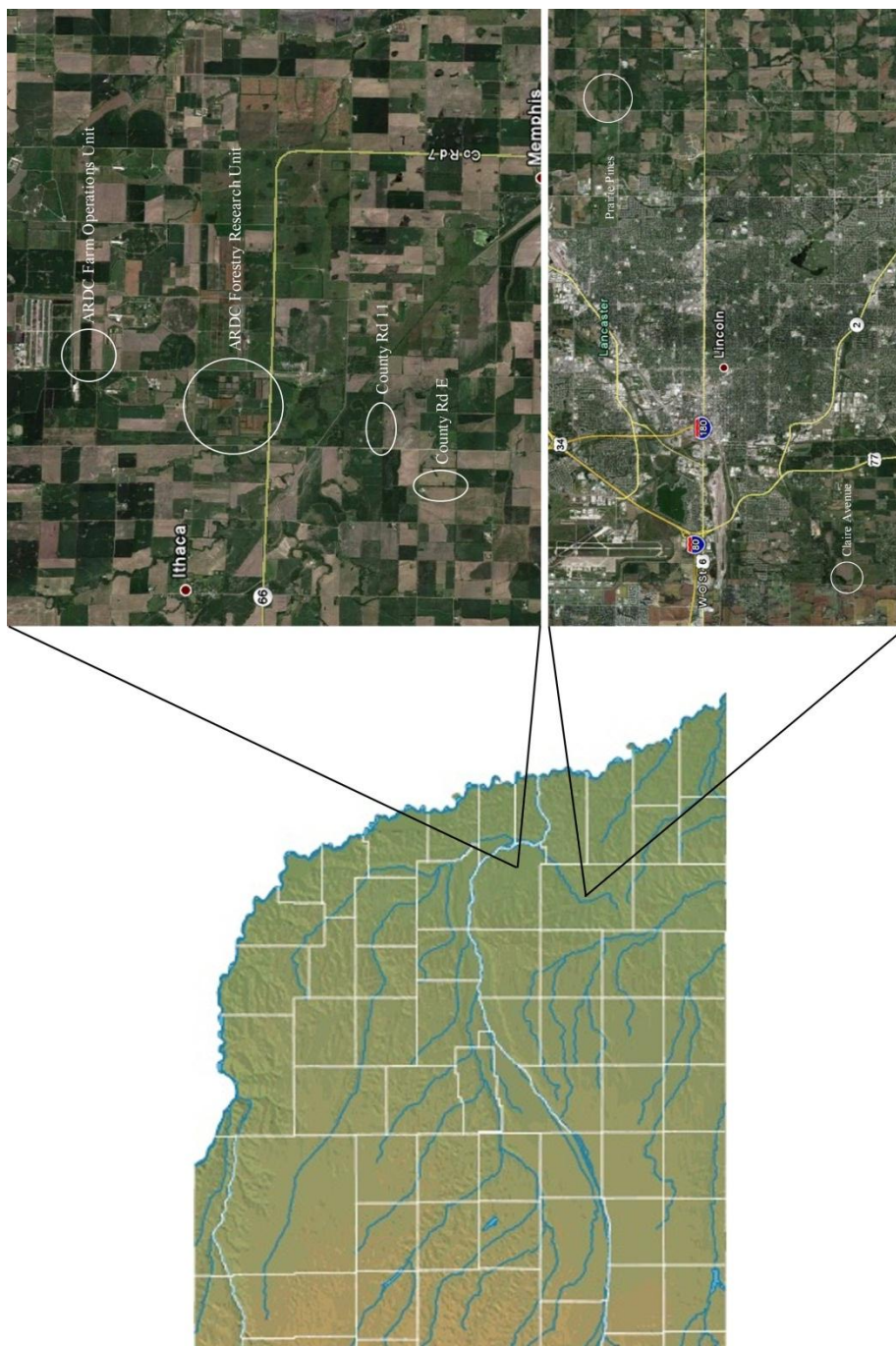


Map 4  
Distribution of *Dermacentor variabilis* in the United States.  
Map courtesy of the Center for Disease Control, updated September 9<sup>th</sup>, 2010.





Map 5  
Distribution of *Amblyomma americanum* in the United States.  
Map courtesy of the Center for Disease Control, updated August 2<sup>nd</sup>, 2011.



Map 6. Study Sites in Saunders and Lancaster County. Top, Saunders County; Bottom, Lancaster County.



Map 7  
Distribution of *Ixodes scapularis* in the United States.  
Map courtesy of the Center for Disease Control, updated September 9<sup>th</sup>, 2010.





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