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IMPACT OF FOREST CONVERSION ON THE ABUNDANCE OF *IXODES RICINUS* TICKS

Thesis submitted in fulfillment of the requirements

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Illustrations on the cover:
Front: Host-seeking female *Ixodes ricinus* [Photograph Gert Arijs, 2011]
Back: Homogeneous pine stand in the Campine region [Photograph Wesley Tack, 2010]

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Woord vooraf

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Contents

List of abbreviations and symbols

Abbreviations and symbols

AIC	Akaike’s Information Criterion
df	degrees of freedom
GLM	generalized linear model
GLMM	generalized linear mixed model
HGA	human granulocytic anaplasmosis
n	number of samples or replications
p	significance of statistical test
SD	standard deviation
SE	standard error
sl	sensu lato
ss	sensu stricto
TBEV	tick-borne encephalitis virus
w	Akaike weight

Definitions of terms used in this thesis

arbovirus	a virus transmitted to humans by arthropods such as mosquitoes and ticks
argasid	a member of the family Argasidae
ectoparasite	a parasite, e.g., a tick, that lives on the outside of its host rather than within its host’s body
encephalitis	inflammation of the brain
endophilic	nest-dwelling
exophilic	field- or forest-dwelling

host	an animal that harbors or nourishes a parasite
ixodid	a member of the family Ixodidae
meninges	the three membranes that envelop the brain and spinal cord
meningitis	inflammation of the meninges
meningoencephalitis	inflammation of the brain and spinal cord and their meninges
myelitis	inflammation of the spinal cord
questing	the specific host-seeking behavior of unfed, exophilic ticks
reservoir host	an animal that harbors pathogenic organisms and that serves as a source of infection
spirochete	corkscrew-shaped form of bacteria
vector	an organism, such as a tick, that transmits a pathogen to another organism
viremia	a medical condition where viruses enter the bloodstream and, hence, have access to the rest of the body
zoonotic disease	a disease affecting animals that can be transmitted to humans

1 Introduction

Ticks are arachnid ectoparasites that feed on the blood of terrestrial vertebrates. They occur worldwide and thrive in diverse habitats that range from deserts to tropical rain forests. Parasitism by ticks has been reported in a wide range of vertebrate taxa including mammals, birds, reptiles, and, although rarely, amphibians. Some tick species have been found to adversely affect animal and human health and are the cause of major economic losses (Steelman 1976, Jongejan & Uilenberg 2004, Anderson & Magnarelli 2008).

Ixodes ricinus (Linnaeus, 1758), also known as the sheep tick or castor bean tick, is the focus species of the present work. This species has a wide geographical distribution throughout Eurasia, ranging from 10°W (Ireland) to 48°E (Ural mountains, Russia) and from 30°N (Egypt) to 66°N (Sweden) (Randolph *et al.* 2002, Jaenson *et al.* 2012b), where it has been incriminated as the main vector of the causative agents of Lyme borreliosis and tick-borne encephalitis (Randolph 2001, Jin *et al.* 2012). Shifts in the distribution limit of ticks and tick-borne diseases in many European countries in the past two decades have spurred researchers to examine the role of the main factors influencing tick populations and the transmission of the pathogenic microorganisms they carry (e.g., Lindgren *et al.* 2000, Daniel *et al.* 2003, 2004, Jaenson *et al.* 2012b, Medlock *et al*. 2013). This study investigates the potential impact of forest conversion, defined here as the conversion of homogeneous coniferous forests into site-adapted mixed forests dominated by native broadleaved species, on *I. ricinus* abundance. Albeit an important facet of the current forest policy in Europe, forest conversion has received little attention in tick-related research.

In the following sections, we first describe *I. ricinus* as study species and its prominent role as a vector of diseases. Subsequently, we discuss a number of factors influencing ticks and tick-borne diseases and address the need to fill the gap in our knowledge regarding the influence of changes in forest management, forest conversion in particular, on *I. ricinus* tick populations. Finally, the objectives and outline of this thesis will be introduced.

1.1 Study species

1.1.1 Taxonomy

Ticks are classified in the class Arachnida, subclass Acari, order Parasitiformes, and suborder Ixodida. Guglielmone *et al.* (2010) recognized 896 species of ticks in three families that show significant differences in morphology and biology: Ixodidae (ixodid or hard ticks, 702 species), Argasidae (argasid or soft ticks, 193 species), and Nuttalliellidae (1 species). New tick species are still being discovered (Apanaskevich *et al.* 2011, Dantas-Torres *et al.* 2012b, Heath 2012, Venzal *et al.* 2012). The dominant family, the Ixodidae, comprises over 700 species in 14 genera: *Amblyomma*, *Anomalohimalaya*, *Bothriocroton*, *Cosmiomma*, *Cornupalpatum*, *Compluriscutula*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Margaropus*, *Nosomma*, *Rhipicentor*, and *Rhipicephalus* (Guglielmone *et al.* 2010). Although there are still considerable disagreements among taxonomists regarding the genus-level classifications, *Ixodes* is by far the largest genus of hard ticks with 243 species. The *Ixodes ricinus* species complex, hereafter *Ixodes* ticks, is a group of closely related ticks within the genus *Ixodes* (Keirans *et al.* 1999). Members of this species complex, including *I. ricinus* in Europe, *I. persulcatus* (Schulze, 1930) in Asia, *I. scapularis* (Say, 1821) in eastern North America, and *I. pacificus* (Cooley & Kohls, 1943) in western North America, have been identified as important vectors of several human pathogens (Parola & Raoult 2001, Nieto & Foley 2009, Dantas-Torres *et al.* 2012a). In fact, the causative agent of Lyme borreliosis is primarily transmitted by ticks of this species complex (Xu *et al.* 2003).

1.1.2 Morphology

The three active life stages of ticks are larva, nymph, and adult (male and female). The larvae of *I. ricinus* are smaller than 1 mm in size and have three pairs of legs. *Ixodes ricinus* has a single nymphal stage. The nymphs (1.2 to 1.5 mm) are larger than the larvae and have four pairs of legs. The adults also have four pairs of legs, but they are larger than the immature stages and have an apparent genital aperture. Adult females are slightly larger than males, ranging in length from 3.0 to 3.6 mm whereas males range in length from 2.4 to 2.8 mm (Fig. 1.1). The main morphological characteristics of *I. ricinus* are shown in Fig. 1.2.

The structure of *I. ricinus* is fused into two parts: the capitulum or gnathosoma, also called 'false head', and the idiosoma or 'body' (Hillyard 1996). The basal portion of the capitulum, the basis capituli, bears the mouthparts. All three life stages of *I. ricinus* have prominent mouthparts, clearly visible from above and below, which consist of a pair of movable, four-segmented pedipalps or palps, an immobile hypostome, and a pair of movable, two-segmented chelicerae that lie above the hypostome. The two chelicerae are scalpel-like structures that are used to puncture the epidermis of the host. The hypostome or feeding tube bears numerous denticles (recurved teeth) for anchoring the tick to the host's skin while feeding. The pedipalps carry numerous sensillae, which serve a chemosensory function. At rest, the pedipalps protect the upper surface of the hypostome and chelicerae. During feeding, they do not enter the wound but are pressed against the host's skin (Hillyard 1996, Anderson & Magnarelli 2008).

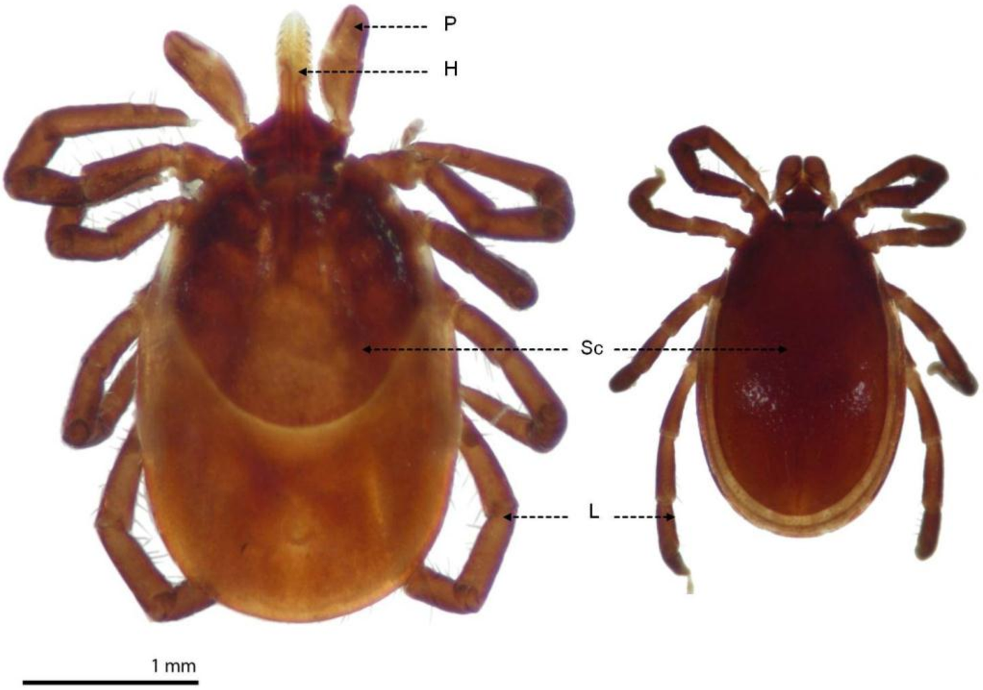


Fig. 1.1 Dorsal view of a female (*left*) and a male (*right*) adult of *Ixodes ricinus*. Note the difference in size between the two sexes. In females, the scutum covers only a part of the dorsal surface. In male ticks, the scutum is larger and completely covers the dorsal surface. H, hypostome; L, leg; P, pedipalp; Sc, scutum [Photograph: Maxime Madder].

The idiosoma contains most of the organs and bears the legs, the anal aperture, the genital aperture (in adults), and the paired spiracles (in nymphs and adults) (Hillyard 1996, Anderson & Magnarelli 2008). The spiracles regulate the gaseous exchange and limit water loss from the respiratory system into the surrounding environment (Pugh *et al*. 2008). Each leg comprises six segments: coxa, trochanter, femur, patella, tibia, and tarsus. The first segments (coxae) are often armed with internal and/or external spurs, which are important structures for species identification. Adult *I. ricinus* ticks, for instance, have a long internal spur on the coxae of the first pair of legs and a small external spur on the coxae of all four pairs of legs (Fig. 1.2). The furthest segment of each leg (tarsus) also bears an apotele or pretarsus, consisting of a pulvillus (a cushion-like structure) and a pair of claws that serves for attachment to a vertebrate host. *Ixodes ricinus* ticks do not have eyes. Instead, they have a unique sensory organ on the tarsi of the first pair of legs called Haller's organ. This structure is a complex of sensory pits and bristle-like sensillae that can detect various environmental stimuli such as changes in temperature, humidity, carbon dioxide, vibrations, ammonia, aromatic chemicals, and pheromones, which enables the tick to find a host (Klompen & Oliver 1993, Guerin *et al.* 2000, Leonovich 2004).

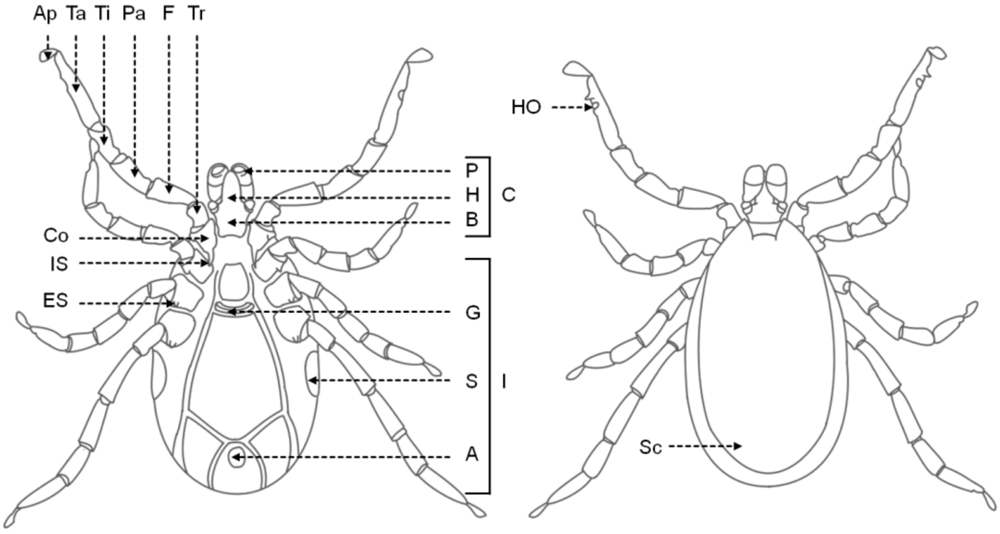


Fig. 1.2 Ventral (*left*) and dorsal (*right*) view of a male adult of *Ixodes ricinus*. A, anal aperture; Ap, apotele; B, basis capituli; C, capitulum; Co, coxa; ES, external spur; F, femur; G, genital aperture; H, hypostome; HO, Haller's organ; I, idiosoma; IS, internal spur; P, pedipalp; Pa, patella; S, spiracle; Sc, scutum; Ta, tarsus; Ti, tibia; Tr, trochanter.

Like all other members of the Ixodidae and unlike the Argasidae, *I. ricinus* has a hard sclerotized shield (scutum) on the upper surface of the idiosoma, which does not expand when the tick engorges (Hillyard 1996). In larvae, nymphs, and females, the scutum is confined to the anterior part of the idiosomal dorsum. In males, which only feed briefly and never gorge on blood, it covers the entire dorsal surface (Fig. 1.1 and Fig. 1.2). Thus, sexual dimorphism is evident in adult hard ticks. Also, the genital aperture in adults differs: in males, the aperture appears as a horizontal slit while in females it is a semicircular opening. The larvae and nymphs of *I. ricinus* show no clear sexual dimorphism and their genital aperture is closed and unapparent (Hillyard 1996, Anderson & Magnarelli 2008). However, it appears possible to distinguish between male and female nymphs according to their body dimensions (Dusbábek 1996).

1.1.3 Biology and ecology

Ixodes ricinus is a three-host tick species, which means each life stage (larva, nymph, adult female) feeds once on a separate vertebrate host and remains on its host only while feeding. Thus, each life stage seeks a host, attaches, takes a single blood meal, detaches after engorgement, and falls to the ground (Anderson & Magnarelli 2008). Ticks feed for several days, i.e., larvae 2–6 days, nymphs 3–8 days, and adult females 6–12 days (Hillyard 1996). The blood meal provides the necessary energy for off-host survival, for development of the larva and nymph to the next stage (interstadial development), and for reproduction in the adult stage.

Figure 1.3 illustrates the life cycle of *I. ricinus*. After hatching, the larvae seek a vertebrate host to obtain their first blood meal. A couple of days later, the blood-engorged larvae drop off their host to the ground to molt into nymphs. After having taken a blood meal on a second host, the nymphs detach from their host and develop into either male or female adults. The development and molting periods of larvae and nymphs can take several months. Mating typically occurs on a third host animal but can also occur in the vegetation (Gray 1987). Males locate potential mates by sensing female sex pheromones (Bouman *et al.* 1999). A male adult can fertilize several females but will die shortly afterwards. After mating, the female takes a final blood meal during which her body weight increases up to 100 times her pre-engorgement weight (Anderson & Magnarelli 2008). The engorged female then falls to the ground to lay a single large egg batch, and dies. The average number of eggs produced typically exceeds 1000 and is closely related to the size of the blood meal (Gray 1981, Jongejan 2001). The life cycle of *I. ricinus* is usually completed in three years, but it may vary from two to six years throughout its geographical range, depending on a complex interplay among temperature, humidity, vegetation type, photoperiod, and access to suitable hosts (Daniel *et al*. 1977, Gray 1998). In temperate environments, a life cycle of three years is considered typical because spring and summer temperatures are generally too low to allow progression through more than one life stage in a given year (Randolph *et al.* 2002).

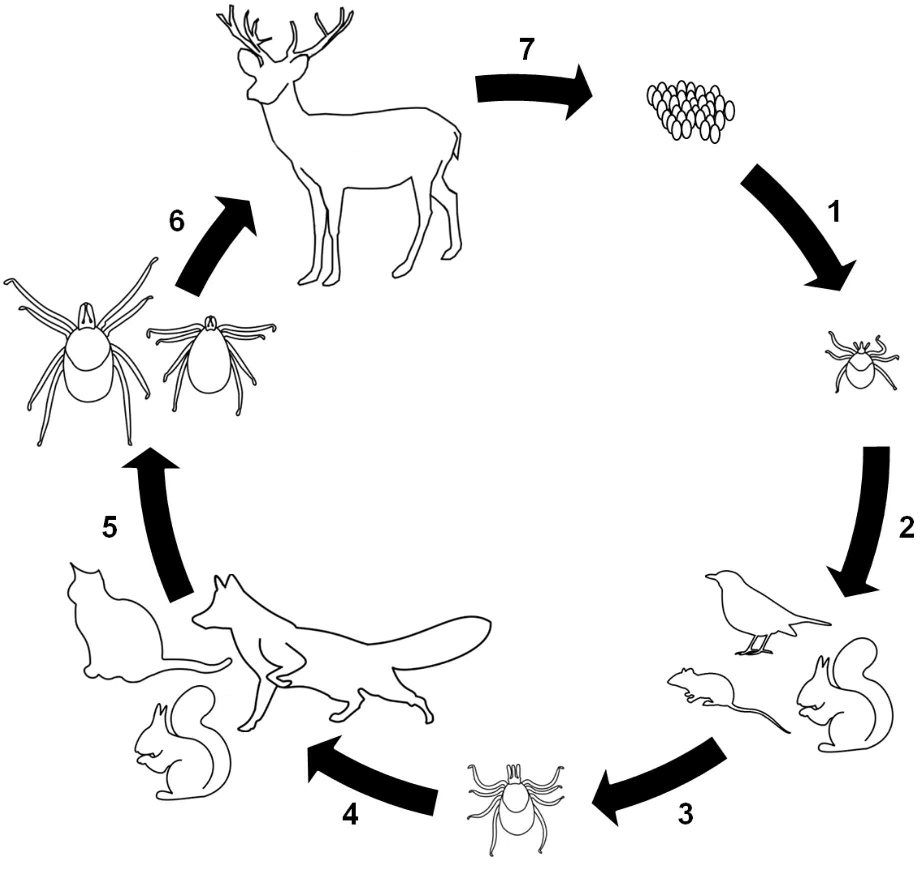


Fig. 1.3 Life cycle of *Ixodes ricinus* (typically 2–3 years). 1, eggs hatch into larvae; 2, larvae attach to and feed on a first host (typically birds and small mammals); 3, engorged larvae shelter and molt into nymphs; 4, nymphs attach to and feed on a second host; 5, engorged nymphs shelter and molt into adults; 6, female (*left*) and male (*right*) seek a third host (typically large mammals), the female then feeds on the host and mates with the male; 7, engorged female lays eggs (> 1000).

Depending on the type of host association, ticks can be divided into two groups. On the one hand, we have endophilic (nest-dwelling) specialist species that live in the nests or burrows of their hosts. Examples of endophilic species that occur in Belgium include, for instance, *Ixodes hexagonus* (Leach, 1815), *I. trianguliceps* (Birula, 1895), and *I. arboricola* (Schulze & Schlottke, 1929). *Ixodes hexagonus* is mainly associated with European hedgehog (*Erinaceus europaeus* Linnaeus, 1758), *I. trianguliceps* with small mammals (rodents), and *I. arboricola* with tree-hole nesting birds such as great tit (*Parus major* Linnaeus, 1758) (Randolph 1975, Heylen & Matthysen 2011, Pfäffle *et al*. 2011). Exophilic tick species, on the other hand, are not specialized to particular host species and occur in exposed habitats (e.g., forest, meadow) where some hunt their hosts and others use an ambush strategy (e.g., *I. ricinus*).

Ixodes ricinus is considered an extreme host generalist, parasitizing a large range of host species. This tick species has been recorded feeding on at least 237 different vertebrate species (Anderson & Magnarelli 1993). Larvae and nymphs feed on birds, reptiles, and small to large mammals, whereas females typically feed on medium-sized or large mammals. Although capable of feeding on many different vertebrate host species, *I. ricinus* appears to have hosts of predilection. Larvae often feed on rodent species, such as wood mouse (*Apodemus sylvaticus* Linnaeus, 1758), yellow-necked field mouse (*Apodemus flavicollis* Melchior, 1834), and bank vole (*Myodes glareolus* Schreber, 1780) (Matuschka *et al.* 1990, Tälleklint & Jaenson 1997), or on bird species such as common blackbird (*Turdus merula* Linnaeus, 1758), European robin (*Erithacus rubecula* Linnaeus, 1758), and pheasant (*Phasianus colchicus* Linnaeus, 1758) (Kurtenbach *et al.* 1998, Marsot *et al.* 2012). Roe deer (*Capreolus capreolus* Linnaeus, 1758) and other cervids are considered key hosts for adult ticks and play an important role in the ticks' population dynamics and dispersal (Chemini *et al.* 1997, Kiffner *et al.* 2010, Ruiz-Fons & Gilbert 2010). Tick populations are usually lower in the absence of deer (Gray *et al.* 1992), but livestock can also sustain populations of *I. ricinus* (Medlock *et al*. 2008)

Since *I. ricinus* ticks are intermittent parasites, they spend most of their life (> 90 %) off-host during which they alternate periods of host-seeking behavior with periods of resting and rehydrating in the litter layer. In order to find a host, *I. ricinus* ticks climb to the top of grass stems or other plants where they await passing hosts with their front legs — carrying Haller's organs — waving in the air. This host-seeking behavior, in which they exhibit an ambush strategy, is known as ‘questing’. When a suitable vertebrate host passes within reach, the ticks cling to the host animal upon contact. Since ticks are sensitive to desiccation during the off-host phase, they must find protection from high temperatures and low humidity. When the environmental conditions are unfavorable, they descend to seek shelter in the moist lower vegetation layers or litter layer where they restore their water balance by passive and active uptake of water (Lees 1946, Needham & Teel 1991). This behavioral pattern consisting of alternating questing and seeking shelter can repeat itself many times during the ticks’ life cycle.

1.1.4 Tick habitat

Ixodes ricinus is not only a host generalist but also a habitat generalist. The ticks can occur in diverse habitats, such as meadows, heathlands, and forests, as long as they have sufficient access to host animals and as long as they can retreat to a refuge to restore their water balance (Lees 1946). *Ixodes ricinus* habitat suitability is governed by a range of factors, such as climate, vegetation type, soil type, geology, and topography (Jensen *et al.* 2000, Medlock *et al.* 2008). Since *I. ricinus* ticks are susceptible to desiccation, they are mostly confined to habitats with a permanent layer of moist leaf litter such as forests, forest edges, and areas with shrubs and low vegetation (Gray 1998). Although *I. ricinus* populations can occur in high densities in open areas such as pastures, especially in regions with plentiful precipitation (Gray *et al.* 1995, Medlock *et al.* 2008), tick abundance is generally higher in forests because the forest canopy provides a climate buffering effect (Estrada-Peña 2001, Walker *et al.* 2001, Lindström & Jaenson 2003). Furthermore, forests provide forage and shelter for wildlife, including large mammals such as ungulates. In Europe, roe deer is by far the principal wild ungulate species in terms of abundance and distribution, and it is also the main host of adult *I. ricinus* ticks (Carpi *et al.* 2008, Kiffner *et al.* 2010). Since *Ixodes* ticks have only limited mobility and do not move horizontally over large distances, they rely on the movements of their hosts for dispersal (Carroll & Schmidtmann 1996, Madhav *et al.* 2004). Roe deer, which are important hosts of the egg-producing female adults, have a great dispersal capacity and are considered a key factor for the reproductive success and long-range dispersal of ticks (Scharlemann *et al.* 2008, Jaenson *et al.* 2012b). Forests dominated by oak (*Quercus* spp.) or common beech (*Fagus sylvatica* L.) that harbor a diverse fauna including larger mammals such as (roe) deer are generally considered the ideal habitat for *I. ricinus* to complete its life cycle (Gray 1998, Gray *et al.* 1998).

1.1.5 Tick phenology

In addition to knowing where ticks occur, it is also important to know how abundant they are and when they are active. This requires insight into the seasonal population dynamics of the different life stages of *I. ricinus*. Tick survival, development rates, and activity patterns are influenced by a number of abiotic factors, especially temperature and relative humidity (Perret *et al.* 2000, Randolph *et al.* 2002, Dobson *et al.* 2011). Both the development rate and questing activity of *I. ricinus* ticks are temperature-dependent. The development rate increases with increasing temperature up to 30°C (Randolph *et al.* 2002). The temperature threshold for onset of questing is set at a weekly averaged daily maximum temperature of 7°C for nymphs and adults, and 10°C for larvae (Perret *et al.* 2000, Randolph 2004). Consequently, host-seeking activity typically starts in spring and lasts until autumn when temperatures drop. During the winter months, the temperature is generally too low to allow interstadial development or questing activity, but questing can be observed in exceptionally mild winters (Dautel *et al.* 2008, Gassner *et al.* 2011). Extremely low temperatures can impair tick survival. Dautel and Knülle (1997) showed that winter-acclimatized, unfed nymphs and diapausing engorged larvae and nymphs had a lethal temperature LT50 (i.e., the estimated temperature lethal to 50 % of individuals after a 24 hour exposure) ranging from -14 to -19°C. They also found that the detrimental effects of cold are accumulative, and 50 % of the larvae and nymhs died after approximately one month when exposed to a constant temperature of -10.1°C. In summer, tick survival is mainly a function of relative humidity, as *I. ricinus* requires a relative humidity of at least 80 % at the base of the vegetation throughout the year (Kahl & Knülle 1988).

When the environmental conditions are unsuitable, ticks may enter a behavioral or developmental diapause, thereby increasing their chances of survival. Behavioral diapause is defined as a period of inactivity in unfed ticks, whereas developmental diapause is a physiological state in which a tick undergoes biochemical changes, particularly related to a slowing of metabolic processes (Belozerov 1982). In general, the seasonal activity pattern of *I. ricinus* can be described as either unimodal, with a single peak of activity, or bimodal with two peaks (one in spring and one in autumn) (Korenberg

2000). However, the seasonal activity patterns of ticks are subject to considerable variation and have been shown to vary from year to year, even at a given site (Tälleklint & Jaenson 1996, Perret *et al.* 2000, Randolph *et al.* 2002).

1.2 Ticks as vectors of diseases

Reports of ticks and tick-borne diseases are found throughout recorded history. The earliest reference was found in an Egyptian papyrus scroll (1550 BC) that referred to tick fever (Varma 1993). However, it was not until 1893 that ticks were suspected of transmitting diseases, when Smith and Kilbourne (1893) demonstrated that the cattle tick (*Boophilus annulatus* Say, 1821) was responsible for transmitting the protozoan *Babesia bigemina* causing Texas cattle fever. Since this discovery, a great deal of work has been carried out on the importance of ticks as ectoparasites and, more importantly, as vectors of diseases. Fortunately, about 90 % of all tick species prefer to feed on certain groups of wild animals, with some species being quite host specific, and do not pose a direct health risk to humans. The remaining 10 %, however, display a more opportunistic feeding behavior and also feed on humans and their domestic animals (Jongejan & Uilenberg 2004). Such tick species may not only cause direct damage through blood feeding, e.g., anemia, skin damage, reduced weight gain and milk production in livestock, and toxic conditions (paralysis, toxicosis, irritation, and allergy), but may also act as vectors of various viral, bacterial, rickettsial, helminth, and protozoan pathogens that cause debilitating diseases in humans and animals. Moreover, infection with tick-borne pathogenic microorganisms can be followed by immunosuppression, which may predispose animals to secondary infections (Woldehiwet 2008).

Ixodes ricinus is by far the most abundant tick species in Europe. The ticks feed indiscriminately on a wide range of vertebrate hosts, during which they can get infected by several pathogenic agents. Since *I. ricinus* ticks readily bite humans and their domestic animals, they are important vectors of a number of infectious diseases, such as Lyme borreliosis, tick-borne encephalitis, babesiosis, anaplasmosis, and tularemia (Parola & Raoult 2001, Beugnet & Marié 2009, Heyman *et al.* 2010). In Europe, tick-borne encephalitis and Lyme borreliosis are without doubt the two most commonly reported tick-borne diseases transmitted to humans (Randolph 2001, Piesman & Gern 2004, Rizzoli *et al.* 2011), and are briefly described below.

1.2.1 Tick-borne encephalitis

Tick-borne encephalitis is an infectious zoonotic disease that is caused by tick-borne encephalitis virus or TBEV (Flaviviridae: Flavivirus), which is medically the most important and widespread arbovirus transmitted by ticks in Eurasia. TBEV has been subdivided into three subtypes: the Western or European subtype, the (Ural-)Siberian subtype, and the Far Eastern subtype (Hubálek & Rudolf 2012). *Ixodes ricinus* is the main vector of the Western subtype, and *I. persulcatus* is the main vector of the Siberian and Far Eastern subtypes. The Western subtype is restricted to distinct foci within central, eastern, and northern Europe (Randolph 2001, Haglund & Günther 2003), where the total number of annually reported human cases of tick-borne encephalitis ranged between 1043 and 4513 in 1990–2009 (Süss 2011). Tick-borne encephalitis is not known to be endemic in Belgium, although TBEV antibodies have been detected in wild cervids in Belgium (Linden *et al. in press*).

Tick-borne encephalitis is associated with severe infection of the central nervous system and is the cause of mortality and long-term morbidity among people in many countries. The initial phase of the disease is usually associated with non-specific influenza-like symptoms that may or may not be followed by symptoms ranging from mild meningitis to severe meningo-encephalitis with or without myelitis. No specific treatment is known so far, but the disease can be successfully prevented by vaccination (Kunz 2003, Heinz *et al*. 2007). For a review on TBEV, its epidemiology and pathogenesis, the clinical manifestations, vaccination, and prevention, we refer to Haglund and Günther (2003) and Mansfield *et al*. (2009).

Uninfected ticks may acquire TBEV through systemic transmission, which occurs when they feed on the blood of a viremic host (i.e., the pathogenic agent is present systemically throughout the blood system of the host). Transovarial transmission, which is the transfer of the pathogen from an infected adult female to her offspring via the eggs, has also been shown to play a role in TBEV transmission (Danielová & Holubová 1991, Gustafson 1994). Yet, the persistence of TBEV in nature mainly depends on the non-systemic transmission of the virus from an infected *I. ricinus* nymph to many uninfected larvae co-feeding in time or space on the same rodent host (Labuda *et al.* 1993, Labuda & Randolph 1999). This transmission mechanism occurs via the host's skin and, thus, viremia is not needed. Infected ticks remain infected throughout their life cycle via transstadial transmission and may infect co-feeding ticks during a subsequent blood meal. Rodents, mainly of the genera *Apodemus* and *Myodes*, are the most competent hosts for both systemic and non-systemic transmission of TBEV (Süss 2003). A prerequisite for virus transmission by co-feeding is the synchronous host-seeking activity of larvae and nymphs. However, the peak period of questing by larval *I. ricinus* ticks typically occurs later in the year than for nymphs and adults as larvae require higher temperatures. Therefore, co-feeding will only occur under certain climatic conditions, which is one of the main reasons behind the focal distribution of tick-borne encephalitis (Randolph *et al.* 2000).

1.2.2 Lyme borreliosis

Lyme borreliosis, or Lyme disease, occurs throughout Europe with at least 85,000 human cases reported each year (Lindgren & Jaenson 2006). In Belgium, Lyme borreliosis is the most important disease transmitted by ticks, and more than thousand new cases of Lyme borreliosis occur annually (Fig. 1.4) (Ducoffre 2011). The risk of acquiring Lyme borreliosis is not similar across the country and is highest along a north-south axis, which is largely attributed to forest cover and roe deer abundance (Linard *et al.* 2007).

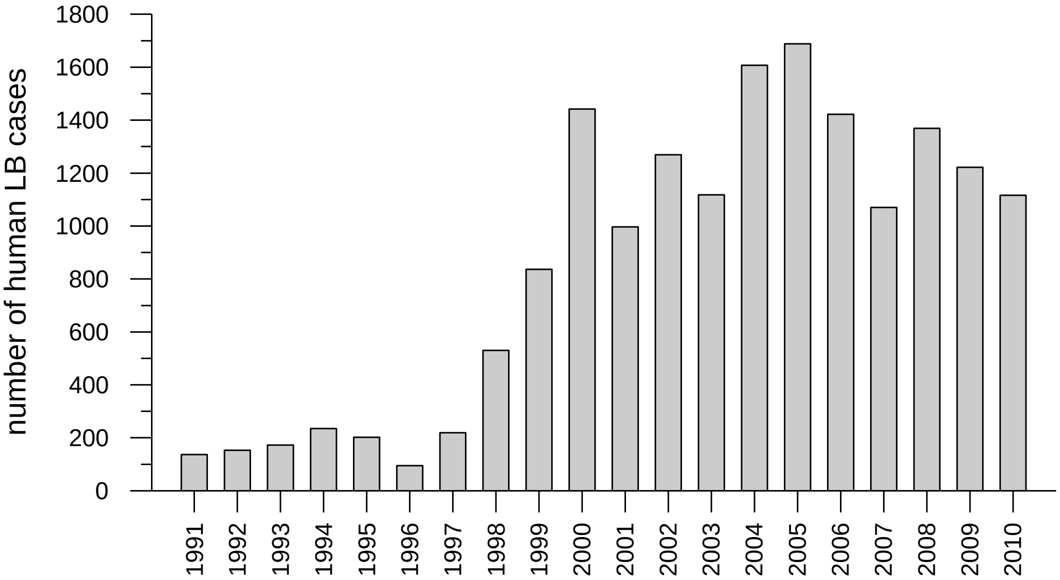


Fig. 1.4 Number of human Lyme borreliosis cases in Belgium in 1991–2010. Data source: Institute of Public Health.

Lyme borreliosis is a multisystemic illness caused by spirochetal bacteria of the *Borrelia burgdorferi* sensu lato (sl) genospecies complex, which now comprises up to eighteen genospecies worldwide (Stanek & Reiter 2011). Three of these genomic species, namely *B. garinii*, *B. afzelii*, and *B. burgdorferi* sensu stricto (ss) are known to be pathogenic to humans. All three genospecies occur in Europe, with *B. garinii* and *B. afzelii* being the most prevalent ones. Although these pathogenic genospecies may all cause influenza-like symptoms and erythema migrans — a red rash on the skin often with a central clearing — they are associated with different clinical manifestations in humans (Van Dam *et al.* 1993). *Borrelia garinii* is most often associated with neuroborreliosis, *B. afzelii* with a severe skin condition called acrodermatitis chronica atrophicans (ACA), and *B. burgdorferi* ss with neuroborreliosis and arthritis. Fortunately, Lyme borreliosis can be successfully treated with antibiotics. However, as the diagnosis of this infection is primarily clinical and the symptoms are largely non-specific, many Lyme cases go undiagnosed and thus untreated, and the number of cases annually reported is likely an underestimation of the true incidence. For an extensive description on the epidemiology, pathogenesis, clinical manifestations, and treatment of Lyme borreliosis, we refer to Stanek *et al.* (2011, 2012).

Borrelia spirochetes are primarily maintained in nature by systemic transmission between ticks belonging to the *Ixodes ricinus* species complex and terrestrial vertebrates that serve as reservoir hosts (Piesman & Gern 2004). Reservoir competence is the ability to maintain the pathogen internally and to act as a source of infection, i.e., transmit the pathogen to uninfected ticks. Larvae and nymphs get infected while feeding on an infected reservoir host and maintain the infection transstadially to the next stage. The transmission cycle is completed when an infected nymph or adult transmits the spirochetes to another reservoir host during feeding. Several mammalian and avian species are capable of transmitting *Borrelia burgdorferi* sl to ticks. Especially small and medium-sized rodents and birds have been incriminated as important reservoir hosts. In fact, the two most prevalent genospecies in Europe have been associated with particular reservoir hosts: *B. afzelii* has been associated with rodents (Hu *et al.* 1997, Hanincová *et al.* 2003a) and *B. garinii* with birds (Kurtenbach *et al.* 1998, Hanincová *et al.* 2003b, Comstedt *et al.* 2006, Dubska *et al.* 2009). Non-reservoir hosts, such as roe deer, do not support the circulation of the pathogenic agent as they cannot maintain the pathogenic organism and infect ticks. However, these hosts may contribute indirectly to transmission of *Borrelia* since they are important hosts for adult reproductive ticks in the typical woodland habitats. Furthermore, non-reservoir hosts may also play an important role in non-systemic transmission of *Borrelia* spirochetes between co-feeding ticks (Ogden *et al.* 1997, Pichon *et al.* 2000). Ticks may become infected when feeding very close to an infected tick on the same (uninfected) host or even from feeding close to a site where an infected tick has recently finished its blood meal (Tsao 2009). Transovarial transmission has been reported (Zhioua *et al.* 1994) but plays a negligible role in the transmission of *Borrelia* spirochetes (Richter *et al.* 2012).

1.3 Factors influencing ticks and tick-borne diseases

In Europe, a steady increase in the number of human cases of tick-borne infections such as Lyme borreliosis and tick-borne encephalitis has been observed since the 1980s (Randolph 2001). A similar increasing trend has been observed for Lyme borreliosis in the United States (Centers for Disease Control and Prevention 2008). This observed increase is to a great extent directly related to an enhanced awareness, recognition, and reporting of these infectious diseases. This is especially so in the case of Lyme borreliosis, from which the etiological agent has been discovered only for the past three decades (Burgdorfer *et al.* 1982, Barbour *et al.* 1983). However, it is now commonly recognized that the increase in human cases of both tick-borne infections reflects a true increase in incidence (World Health Organization 2004, Sood *et al.* 2011), which has raised political concern and has prompted researchers to gain a better understanding of the tick–host–pathogen interface and the major factors driving changes in disease risk. Essentially, the risk of contracting a tick-borne disease depends on the risk of being bitten by an infected tick, which in turn depends on the abundance of questing ticks, their infection rate, and the number of people that come into contact with ticks. The complex nature of tick-borne diseases thus entails the study of multiple interactions between the pathogenic agent, the tick vector, the vertebrate host, and human behavior to understand disease dynamics.

A particularly illustrative example is that of Sweden, where *I. ricinus* has increased in abundance and has expanded its northern range margin northward from approximately 61°N to 66°N in the past 30 years (Jaenson & Lindgren 2011, Jaenson *et al.* 2012b). This was linked to an exponential increase of the roe deer population in the 1980s and early 1990s, caused by an epizootic of scabies in the two most important predators of roe deer, namely the red fox (*Vulpes vulpes* Linnaeus, 1758) and the lynx (*Lynx lynx* Linnaeus, 1758). Furthermore, a series of milder winters during the early 1990s and a consequent prolonged growing season probably attributed to a better survival and increased abundance of roe deer, which would have been favorable for tick populations. The roe deer population level then gradually declined from the early 1990s due to an increase in fox and lynx numbers and deer hunting, until it was suddenly reduced due to the two harsh winters of 2009–2010. A sudden increase in tick-borne encephalitis incidence in 2011 was ascribed to high tick abundances together with this decline in roe deer numbers, which probably forced a substantial portion of the questing larvae and nymphs to feed on small rodents, which are competent transmission hosts for TBEV (Jaenson *et al.* 2012a). Additionally, unusually high spring temperatures provided suitable conditions for synchronous activity of larvae and nymphs. This resulted in a higher degree of co-feeding immature ticks on rodents, thus increasing the abundance of TBEV-infected ticks. Furthermore, the favorable weather conditions probably stimulated many people to spend more time outdoors and brought more people in contact with infected ticks, thereby increasing the probability of TBEV transmission to humans.

A phenomenon similar to that in Sweden was observed in central and eastern Europe, where the marked increase in the incidence of tick-borne encephalitis over the past two decades was attributed to a combination of changes in the socio-economic conditions and in the abiotic and biotic environment that occurred after the dissolution of the Sovjet Union. For instance, abandoned agricultural fields became suitable for rodents, wildlife hosts for ticks increased, and mushroom and berry harvesting increased due to unemployment, which led to an increase in the abundance of infected ticks and an increase in the exposure of humans to infected ticks (Šumilo *et al.* 2008).

1.3.1 Impact of forest management

As forest management affects directly the composition of structural species such as trees, shrubs, and herbs, it affects the vertical structure of forests, forms edge habitats, and affects the quantity and quality of available food for wildlife. As illustrated in the following paragraphs, significant changes in tree species composition, forest structure, or spatial configuration might greatly affect the tick–host–pathogen interactions and risk of human exposure to tick-borne infections.

Rizzoli *et al.* (2009) demonstrated that substantial changes in forest structure, serving as a proxy for small mammal host abundance, and roe deer abundance are most likely among the most crucial factors affecting the risk of tick-borne encephalitis emergence in Italy.

In the United States, researchers have postulated that masting (i.e., the periodic production of large seed crops) influences tick abundance and Lyme borreliosis risk through changes in the habitat selection of deer and changes in rodent abundance (Jones *et al.* 1998). However, their hypothesis of using acorn production as predictor of disease risk to humans has been strongly disputed by others (Randolph 1998), who stressed the importance of other wildlife hosts and human behavior in determining the risk of tick-borne infections. This is especially so in Europe, where more pathogenic genospecies of *B. burgdorferi* occur compared to the United States. Nonetheless, the promotion of mast-producing tree species by forest managers might affect the food availability for numerous animal species living in the forest, which could have a severe impact on the spatial and temporal variation in tick abundance and disease incidence.

In the last decade, changes in land use and land cover have also received increasing attention to explain shifts in tick abundance and infectious disease patterns (Estrada-Peña 2004, Halos *et al.* 2010). Deforestation of much of the northeastern United States during the 18th and 19th centuries resulted in the near total elimination of the white-tailed deer (*Odocoileus virginianus* Zimmerman, 1780), which is generally recognized to play a crucial role in the reproduction of the deer tick *I. scapularis*. Deforestation and the subsequent elimination of deer presumably destroyed conditions suitable for *I. scapularis* ticks and enzootic cycles of *B. burgdorferi* (Barbour & Fish 1993, Spielman 1994). The emergence of Lyme borreliosis as a public health problem at the end of the 20th century is attributable in part to the reforestation of the northeastern United States followed by the suburbanization of those woodlands. The abandonment of farms resulted in a change in the landscape through natural succession from open fields to deciduous forests, which resulted in an exponential rise in deer populations. As the forests and the deer returned, so did the deer tick. Subsequently, human development in those woodlands resulted in a higher number of forest patches interspersed with residential development, thus ensuring high contact rates and spirochete transmission between ticks and humans (Maupin *et al*. 1991, Frank *et al*. 1998). A similar situation occurred in Europe. Roe deer were virtually exterminated during the 1940s along with large tracts of forest. After World War II, changes in land use and land cover (e.g., reforestation, agricultural changes) and a behavioral adaptation in roe deer in relation to their tolerance of human disturbance resulted in a marked increase of roe deer populations throughout Europe (Cederlund *et al.* 1998), which has been ascribed a major role in the emergence and spread of *I. ricinus* ticks and Lyme borreliosis (Spielman 1994, Sood *et al.* 2011).

Moreover, forest fragmentation has been proposed to play an important role in defining local scale heterogeneity in tick abundance, infection prevalence in ticks, and disease incidence through influence on host diversity and abundance (Allan *et al.* 2003, Brownstein *et al.* 2005, Jackson *et al.* 2006). In the United States, the fragmented suburban landscape supports high densities of white-tailed deer as they favor edge habitat and benefit from the absence of predators (Barbour & Fish 1993, Frank *et al*. 1998). Forest fragmentation can also influence the abundance of small mammals that serve as hosts for the immature ticks and maintain the enzootic cycle of *Borrelia* spirochetes. The reduction in forest patch size in the northeastern United States has been proposed to be positively correlated with disease risk by increasing the densities of the white-footed mouse (*Peromyscus leucopus* Rafinesque, 1818), which is considered the main host for immature *I. scapularis* and the primary reservoir host for *B. burgdorferi*, and by decreasing the numbers of less-competent hosts (Allan *et al*. 2003).

1.4 Forest conversion needs

Europe experienced significant changes in landscape composition and configuration in the past centuries. At the end of the 18th century, following a period of destruction and overutilization of the woodlands, concerns about wood shortage triggered forest management in Europe to plant fast-growing coniferous species. Today, coniferous monocultures consisting mainly of Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) cover large areas in Europe and expand far beyond the limits of their natural ranges. Scots pine has a share of 31 % of the forest area in Europe, and Norway spruce has a share of 21 % (Spiecker *et al.* 2004).

In the past decades, however, the extensive use of coniferous monocultures has raised concerns regarding its potential impact on the ecological stability and flexibility of forests. Forests that consist of a mix of tree species tend to support higher biological diversity (Lust *et al.* 1998, Felton *et al.* 2010) and are more resistant against biotic and abiotic disturbances such as insect and wind damage (Farrell *et al*. 2000, Löf *et al*. 2004, Jactel & Brockerhoff 2007, Knoke *et al.* 2008). Also, an admixture of broadleaved tree species in coniferous stands has been suggested as a means to counteract soil acidification (Brandtberg *et al.* 2000). Furthermore, mixed forests are considered to have a higher amenity and recreation value compared to homogeneous forests (Bostedt & Mattsson 1995, Elsasser *et al.* 2010). Thus, the creation and restoration of natural woodland serving diverse socio-economic (e.g., recreation) as well as nature conservation purposes has become a major objective of sustainable forestry throughout Europe (Zerbe 2002). Conversion of Europe's homogeneous conifer plantations into (semi-)natural broadleaved forests is generally agreed upon by forest policy makers as an important component of multifunctional forest management (Olsthoorn *et al.* 1999, Stanturf & Madsen 2002, Zerbe 2002).

In Belgium too, fast-growing conifer species were planted to meet the demand for wood. This concerns approximately 172,400 ha of Norway spruce in Wallonia (southern Belgium) and 45,000 ha of pine trees in Flanders (northern Belgium) (Le Compte *et al*. 1999, Afdeling Bos en Groen 2001a). Of the total Flemish forest area (146,000 ha), homogeneous stands of Scots pine cover 22.7 % and stands of Corsican pine (*P. nigra* Arnold ssp. *laricio* (Poiret) Maire) cover 8 %. The Campine region, an ecoregion covering the northeast of Flanders, contains approximately 90 % of these stands. Most forests in this region were established in the 19th and first half of the 20th century, when wood was in high demand by the coal mining industry. Afforestation took place on marginal fields and former heathlands, which once formed an important component of the traditional agricultural system and covered vast areas of the Campine region. The main management system in these plantations was clear cut and reforestation with Scots pine or Corsican pine in a rotation period of 50–70 years. From the 1970s, the coal-mining industry declined and many forest owners lost their incentive for timber production activities. Today, the traditional management system based on even-aged, homogeneous pine stands is no longer appropriate in Flanders. Instead, special attention goes to the multifunctional character of forests, thus raising the need to convert the homogeneous pine stands to mixed, structure-rich stands with oak as one of the main constituents (Afdeling Bos en Groen 2001b). To achieve this, the Flemish Forest Decree of 1990, which applies to both public and private forests in Flanders, stresses the use of native deciduous tree and shrub species, the stimulation of spontaneous processes, and the development of a varied forest structure.

1.5 The Campine region as study area

The Campine region was chosen as study area for two main reasons. First, pine plantations currently dominate the forest area in the region, and the planned forest conversions will thus largely affect the forest landscape. Second, the region has a high incidence of Lyme borreliosis (Linard *et al.* 2007) and is intensely used for recreation. In addition, forest-based recreation in the Campine region is likely to increase in the future. Therefore, the Campine region is not only the Flemish region where the changes in forest composition and structure associated with forest conversion will be most pronounced, but it is also the region where forest conversion might have a great influence on tick populations and public health.

The following sections provide a general description of the climate, forest characteristics, and composition of the vertebrate host community in the forests in the Campine region. In addition, we discuss the main forests sites and forest stand types used in this thesis.

1.5.1 Climate

The climate is sub-atlantic. The mean annual precipitation amounts to 799 mm and is evenly distributed throughout the year, with mean monthly precipitation ranging from 53 mm in March to 79 mm in July. The mean annual temperature is 9.0°C, with minimum and maximum mean monthly temperatures of 1.4°C in January and 16.7°C in July (Royal Meteorological Institute of Belgium, http://www.kmi.be/, accessed on July 5, 2011).

1.5.2 Forest characteristics

Forest cover in the Campine region amounts to 16 % in the province of Antwerp and 21 % in Limburg. The region’s characteristic forests are pine plantations — mainly consisting of Scots pine and, to a lesser extent, Corsican pine — on nutrient-poor and acidic sandy soils. The coniferous stands are interspersed with deciduous stands of pedunculate oak (*Quercus robur* L.), red oak (*Q. rubra* L.), common beech (*Fagus sylvatica* L.), silver birch (*Betula pendula* Roth), and downy birch (*B. pubescens* Ehrh.) (Waterinckx & Roelandt 2001). The most common plant species in the shrub layer and among the seedlings are pedunculate oak, red oak, silver birch, rowan (*Sorbus aucuparia* L.), black cherry (*Prunus serotina* Ehrh.), and alder buckthorn (*Frangula alnus* Mill.). The ground vegetation consists of a mixture of grasses, ferns, dwarf shrubs, and herbs, mainly wavy hair grass (*Deschampsia flexuosa* (L.) Trin.), purple moor grass (*Molinia caerulea* (L.) Moench), broad buckler fern (*Dryopteris dilatata* (Hoffm.) A. Gray), narrow buckler fern (*Dryopteris carthusiana* (Vill.) H. P. Fuchs), bracken fern (*Pteridium aquilinum* (L.) Kuhn), blackberry (*Rubus fruticosus* agg.), bilberry (*Vaccinium myrtillus* L.), common heather (*Calluna vulgaris* (L.) Hull), and honeysuckle (*Lonicera periclymenum* L.) (Waterinckx & Roelandt 2001).

1.5.3 Vertebrate host community

Large and medium-sized mammals, which are present in the study area and are potential hosts for nymphal and adult ticks, include roe deer (*Capreolus capreolus* Linnaeus, 1758), red fox (*Vulpes vulpes* Linnaeus, 1758), European hare (*Lepus europaeus* Pallas, 1778), European rabbit (*Oryctolagus cuniculus* Linnaeus, 1758), red squirrel (*Sciurus vulgaris* Linnaeus, 1758), European hedgehog (*Erinaceus europaeus* Linnaeus, 1758), and mustelid species such as least weasel (*Mustela nivalis* Linnaeus, 1758), European pole cat (*Mustela putorius* Linnaeus, 1758), stoat (*Mustela erminea* Linnaeus, 1758), and stone marten (*Martes foina* Erxleben, 1777). Ground-foraging birds and small mammals, which are potential hosts for immature ticks, include European robin (*Erithacus rubecula* Linnaeus, 1758), common blackbird (*Turdus merula* Linnaeus, 1758), Eurasian jay (*Garrulus glandarius* Linnaeus, 1758), carrion crow (*Corvus corone* Linnaeus, 1758), magpie (*Pica pica* Linnaeus, 1758), wood pigeon (*Columba palumbus* Linnaeus, 1758), wood mouse (*Apodemus sylvaticus* Linnaeus, 1758), bank vole (*Myodes glareolus* Schreber, 1780), pygmy shrew (*Sorex minutus* Linnaeus, 1758), and common shrew (*Sorex araneus* Linnaeus, 1758) (Verkem *et al.* 2003). The European hedgehog and several species of mice, voles, shrews, and birds have been shown to be competent reservoirs of the Lyme borreliosis spirochetes and other pathogens (Gray *et al.* 1994, Gern *et al.* 1997, Kurtenbach *et al.* 1998, Hildebrandt *et al*. 2010).

1.5.4 Forest sites and forest stand types

Fieldwork was conducted in several forests in the provinces Antwerp and Limburg (Fig. 1.5), with most of the work carried out in two regions: site A, located near the border with the Netherlands in the municipality Postel, and site B, located approximately 30 km to the south. The fieldwork at site B was in fact carried out in two nearby forests, located in the municipalities Herselt and Tessenderlo and separated by 1–2 km of agricultural land and pasture. Both forests at site B are very similar in terms of topography, soil, vegetation composition, and historical land use and consist of a central core area of ancient forest (i.e., sites which have been continuously forested since 1775), surrounded by forest stands established in the 19th and first half of the 20th century.

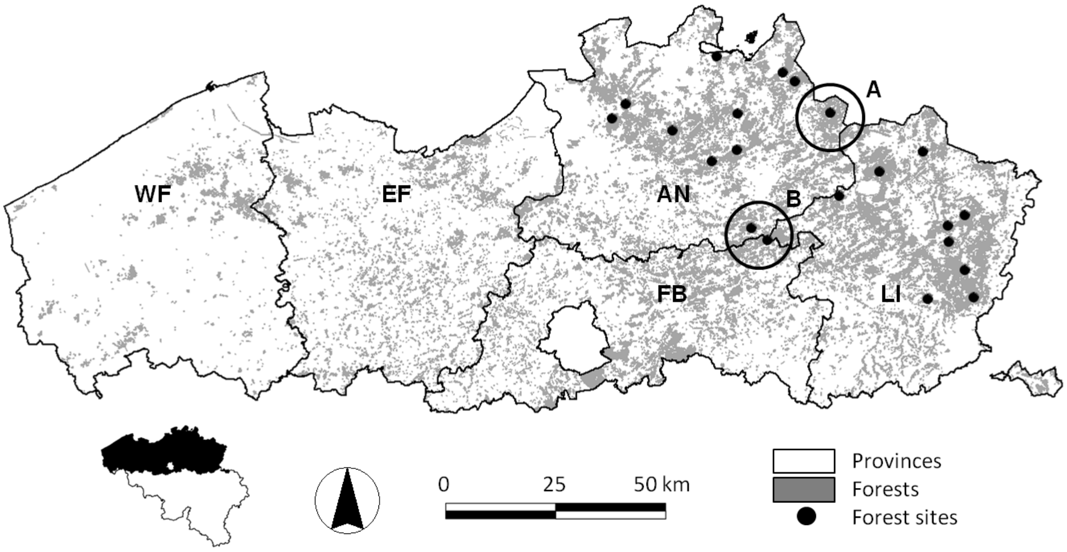


Fig. 1.5 Map of Flanders (northern Belgium) showing the provinces (AN, Antwerp; EF, East-Flanders; FB, Flemish Brabant; LI, Limburg; WF, West-Flanders), forest cover, and the location of the 21 forest sites where *Ixodes ricinus* ticks were sampled. The two main study sites A en B are depicted as two open circles.

Ticks were mostly sampled in four distinct forest stand types that differed in dominant tree species composition (coniferous *vs*. deciduous) and vertical structure (with or without a substantial shrub cover): forest stands composed of pine (either Scots pine or Corsican pine) or oak (mainly pedunculate oak), either structure poor or structure rich (Fig. 1.6).

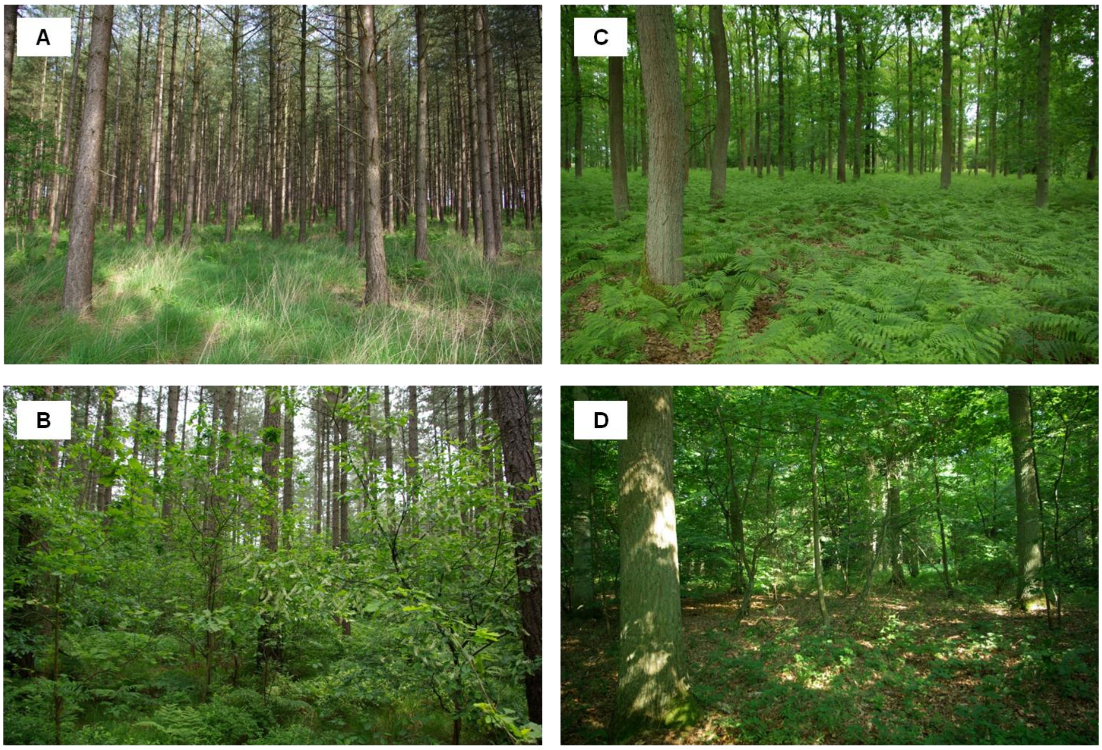


Fig. 1.6 The four main forest stand types that were sampled for *Ixodes ricinus* ticks in the present study. A, structure-poor pine stand; B, structure-rich pine stand; C, structure-poor oak stand; D, structure-rich oak stand [Photographs: A–D Wesley Tack].

1.6 Objectives and thesis outline

A necessary first step in understanding the epidemiology of tick-borne diseases is to determine the factors influencing the distribution and abundance of ticks. Forests are the main habitat for *I. ricinus*. By altering the characteristics of forests, e.g., tree species composition and vertical structure, the current close-to-nature management may have a considerable impact on the suitability of forests for tick populations and their main hosts, thereby influencing the dynamics of tick-borne infections (see section 1.3). As forest-based recreation is becoming increasingly important in many European countries, the role of ticks as vectors of zoonotic diseases may augment in the coming years due to an increase in human-tick contacts. This urges the need to better understand the regional as well as the local factors, e.g., forest management, that influence tick population dynamics. However, the effects of forest conversion on tick populations have scarcely been investigated. The main objective of this thesis is to evaluate the potential effect of forest conversion on tick abundance, with focus on the Campine region.

The specific objectives of the thesis were:

- (i) to compare the efficiency of two sampling methods for the sampling of questing ticks in different understory vegetation types,
- (ii) to assess the abundance of questing ticks in different forest stand types and relate observed differences to forest composition and vertical structure,
- (iii) to relate differences in tick abundance between forest sites to forest configuration,
- (iv) to relate fluctuations in the availability of food resources (acorn mast) to temporal variations in rodent and tick abundance.

The thesis is organized in four parts:

The first part (Chapter 2) deals with the comparison of two sampling methods for the sampling of questing *I. ricinus* ticks. Larvae, nymphs, and adults were sampled on 5 occasions in 2008. The two methods, the entire-blanket and strip-blanket, were evaluated for sampling of ticks in four different vegetation types.

The second part (Chapters 3 and 4) consists of two observational studies that aimed to determine the effect of forest composition, forest structure, and forest configuration on the abundance of larval, nymphal, and adult *I. ricinus*. Chapter 3 describes the results of a field survey carried out at the regional scale, during which a total of 176 forest stands at 21 forest sites (see Fig. 1.5) was sampled on one occasion in 2009. The effects of forest fragmentation on tick abundance were also addressed in this chapter. Chapter 4 describes the results of a longitudinal study, carried out in 25 forest stands at two forest sites (sites A en B in Fig. 1.5) in three successive years (2008–2010). For both observational studies, host-seeking ticks were sampled in the four distinct forest stand types shown in Fig. 1.6.

The third (Chapter 5) and fourth part (Chapter 6) of our study follow an experimental approach. Both studies were carried out in the same forest site (site B in Fig. 1.5). In Chapter 5 we present the adverse effects of a shrub-removal experiment on the abundance of larvae, nymphs, and adults. This study was carried out in 6 pine stands in 2008–2010 (12 sampling occasions in total). Chapter 6 describes the effect of a seed-addition experiment and a mast year on the abundance of small rodents, and the association between these rodents and both attached and questing immature ticks. This study was carried out in 6 pine stands and 6 oak stands. Questing ticks were sampled on 16 sampling occasions (2008–2011) and rodents were sampled three times (September 2008, 2009, and 2010).

The final chapter (Chapter 7) summarizes the main findings of the observational studies and experiments, leading to general recommendations for forest management.

[Photograph Gert Arijs]

2 The evaluation of two sampling methods in different understory vegetation types

After: Tack W, Madder M, De Frenne P, Vanhellemont M, Gruwez R, Verheyen K (2011) The effects of the sampling method and vegetation type on the estimated abundance of *Ixodes ricinus* ticks in forests. Experimental and Applied Acarology 54, 285–292

2.1 Abstract

Estimating the spatial and temporal variation in the abundance of disease-transmitting ticks is of great economic and medical importance. Entire-blanket dragging is the most widely used method to sample exophilic host-seeking ixodid ticks. However, this technique is not equally efficient in different vegetation types. The height and structure of the understory vegetation will not only determine the degree of contact between the blanket and host-seeking ticks, but will also determine the rate of dislodgement. The purpose of this study was to determine whether the alternative strip-blanket is more efficient in sampling ticks than the standard entire-blanket. Sampling was carried out in four understory vegetation types that differed in height and structure (bracken fern, bilberry, purple moor grass, and short grass) on five sampling occasions between April and September 2008. A total of 8068 *Ixodes ricinus* (Linnaeus, 1758) ticks was collected. The highest numbers of ticks were collected along the forest trails where the dominant vegetation consisted of short grasses. The lowest numbers of ticks were collected in sites dominated by bracken fern, where the understory greatly hampered sampling efforts. Surprisingly, in each vegetation type, significantly more nymphs and adults were collected using the entire-blanket. Conversely, the strip-blanket was more efficient in collecting larvae, especially in dense and tall vegetation.

2.2 Introduction

Accurate estimates of tick abundance are essential in order to evaluate the risk of exposure to ticks during recreational and work-related outdoor activities. Drag sampling is the most commonly used method to estimate the abundance of host-seeking exophilic ticks because of its convenience and inexpensiveness. The technique involves dragging a blanket over the vegetation for a fixed time or distance and counting the number of ticks found clinging to it (Gray & Lohan 1982, Ginsberg & Ewing 1989, Falco & Fish 1992). However, since drag sampling requires contact between the host-seeking ticks and the blanket, the vertical distribution of ticks in the vegetation and the physical properties of the vegetation will influence sampling efficiency. Questing height, i.e., the height above the soil surface at which ticks wait for passing hosts, is species and life stage dependent, which is probably related to differences in desiccation tolerance (Needham & Teel 1991, Randolph & Storey 1999) and the size of their preferred hosts (Mejlon & Jaenson 1997). Larvae of *I. ricinus* typically quest for small mammals or birds in the leaf litter or near the base of the vegetation, nymphs readily ascend vegetation in search of medium-sized and large mammals, and adults usually climb even higher in search of large mammals (Tälleklint & Jaenson 1994, Mejlon & Jaenson 1997). Because larvae tend to quest lower in the vegetation than nymphs and adults, sampling difficulties can be expected in certain vegetation types. In dense vegetation, the blanket might not penetrate deep enough to reach the larvae, resulting in an underestimation of larval tick abundance (Gray 1985). Furthermore, adults, and to a lesser extent nymphs, are much larger than larvae and are more prone to dislodgement from the blanket. Rough vegetation surfaces especially will cause more ticks to get brushed off (Milne 1943, Li & Dunley 1998).

To date, we are aware of only one study that compared the entire-blanket and the strip-blanket, the latter being a slightly modified version of the commonly used entire-blanket, for collecting *I. ricinus* ticks. Gray and Lohan (1982) found the strip-blanket to be more efficient for capturing *I. ricinus* nymphs on a range of vegetation types (heather, bog, bracken, and short grass), which was attributed to the strips rotating considerably during dragging and protecting nymphs to some extent from being dislodged.

In the present study, the efficiency of the strip-blanket and the entire-blanket was assessed for collecting larval, nymphal, and adult *I. ricinus* ticks in four vegetation types, differing in height and structure, in a predominantly coniferous forest. We hypothesized that the strip-blanket would be superior compared to the entire-blanket in collecting nymphs and adults, confirming the findings of Gray and Lohan (1982). Furthermore, we expected the strip-blanket to be a more efficient sampling method than the entire-blanket for collecting larvae, especially in dense vegetation, because the strip-blanket allows for a better penetration of the vegetation and is expected to result in a higher degree of contact.

2.3 Materials and methods

2.3.1 Study area

Fieldwork was conducted at Het Wik (50°58’N, 5°25’ E), a 110 ha nature reserve situated in the Campine region (see section 1.5). Tick surveillance since 1999 has shown *I. ricinus* to be consistently present in this nature reserve for the past decade (unpublished results of Cox 1999, Hoeyberghs 2000, and Tormans 2008).

We sampled ticks in four vegetation types less than 1 km apart, which were selected based on the dominant vegetation: (a) European larch (*Larix decidua* Mill.) stands with a dense, homogeneous understory of bracken fern (*Pteridium aquilinum* (L.) Kuhn); (b) Scots pine (*Pinus sylvestris* L.) stands dominated by bilberry (*Vaccinium myrtillus* L.); (c) small forest gaps (< 1 ha) dominated by purple moor grass (*Molinia caerulea* (L.) Moench); and (d) forest trails with predominantly short grasses (generally 30–50 cm tall), such as wavy hair grass (*Deschampsia flexuosa* (L.) Trin.) and velvet grass (*Holcus lanatus* L.). Bracken is a perennial fern with triangular-shaped, stiff, upright fronds. New fronds emerge in early May, grow rapidly until August (approximately 180 cm tall), and start to wither in September. Bilberry, an ericaceous dwarf shrub with rigid stems, reaches a height of 30–50 cm and purple moor grass, a tussock-forming perennial, reaches a height of approximately 90–120 cm.

Numerous potential mammalian and avian hosts (or signs of their presence, such as tracks, burrows, or feces) were observed on several occasions during sampling, including roe deer (*Capreolus capreolus* Linnaeus, 1758), red fox (*Vulpes vulpes* Linnaeus, 1758), red squirrel (*Sciurus vulgaris* Linnaeus, 1758), European hare (*Lepus europaeus* Pallas, 1778), European hedgehog (*Erinaceus europaeus* Linnaeus, 1758), wood mouse (*Apodemus sylvaticus* Linnaeus, 1758), common blackbird (*Turdus merula* Linnaeus, 1758), carrion crow (*Corvus corone* Linnaeus, 1758), magpie (*Pica pica* Linnaeus, 1758), wood pigeon (*Columba palumbus* Linnaeus, 1758), and European robin (*Erithacus rubecula* Linnaeus, 1758).

2.3.2 Tick sampling

Ticks were collected from the vegetation by drag sampling. Two blanket types were used: the standard entire-blanket and the strip-blanket. Both blankets had the same contact surface (1 m × 1 m), but the latter was cut into ten strips, each 1 m long and 10 cm wide, to ease movement through the vegetation. The fabric (white flannel) was attached with Velcro tape to a wooden dowel and weighted at the opposite end using lead curtain weights (ten weights, each of them weighing 35 g) to aid contact between the blanket and the vegetation.

Ticks were sampled during five visits to the study area between April and September 2008, between 07:00 am and 07:00 pm. Sampling was performed on dry and non-windy days to minimize the potential impact of precipitation and wind on the efficiency of the blankets. On each sampling occasion and in each vegetation type, nine 1 min drags (each extending a distance of *ca*. 30 m) were performed with each blanket. In total, 360 drags were performed (5 sampling occasions × 4 vegetation types × 2 blanket types × 9 drags). The two blankets were used side by side simultaneously and were exchanged between the two operators throughout the day to avoid operator bias. Before each pair of drags (one with each blanket), air temperature and relative humidity were recorded at the soil surface and at a height of 125 cm above the soil surface with a portable digital temp/RH meter (DM509, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). To avoid time of day and changing meteorological conditions as a source of bias, the four vegetation types were alternately sampled throughout the day. After each drag, ticks adhering to the blankets were removed in the field using forceps and stored in 70 % ethanol. Ticks were counted and identified morphologically to species and developmental stage using a stereo-microscope and the identification keys of Hillyard (1996). Tick abundance was expressed as the number of ticks collected per drag.

2.3.3 Statistical analysis

As our abundance data showed a non-normal distribution and common transformation procedures failed, the assumptions for parametric methods could not be met. Instead, the generalized estimating equation approach of Liang and Zeger (1986) was applied for each life stage (larva, nymph, and adult) separately. Each individual drag sample was used as a separate case. A negative binomial distribution and a log link function was applied to assess the influence of sampling occasion, vegetation type, and blanket type on relative tick abundance, taking repeated sampling of the same dragging sites into account. All two-way interactions were included in the analysis. Post-hoc comparisons were done using the sequential Sidak multiple comparison test. The Spearman rank order correlation was applied to determine the correlation between the numbers of ticks gathered and the air temperature and relative humidity measured during tick collection. All statistical tests were performed with SPSS 15.0 (SPSS 2006).

2.4 Results

During tick sampling, the ambient air temperature ranged from 3.5 to 31.4°C (mean = 17.4°C, SD = 6.1°C) and the relative humidity ranged from 32 to 94 % (mean = 66 %, SD = 16 %). The temperature and relative humidity at the soil surface ranged from 3.2 to 32.0°C (mean = 17.6°C, SD = 5.9°C) and from 45 to 98 % (mean = 83 %, SD = 10 %), respectively. For the recorded values of temperature and relative humidity on each sampling occasion, we refer to Table 2.1.

Table 2.1 The minimum, maximum, and mean (SD, standard deviation) temperature and relative humidity measured at the soil surface and at a height of 125 cm above the soil surface on five sampling occasions during tick sampling.

	Temperature (°C)			Relative humidity (%)		
	min	max	mean (SD)	min	max	mean (SD)
At the soil surface						
April	3.2	17.6	12.3 (3.2)	72	97	85 (7)
May	9.7	21.0	14.8 (2.7)	77	96	87 (6)
June	15.5	26.8	20.9 (3.5)	77	95	86 (5)
July	13.4	32.0	25.2 (4.6)	45	92	69 (12)
September	7.4	20.7	14.6 (3.5)	78	98	89 (5)
At a height of 125 cm						
April	3.5	15.8	12.1 (2.8)	51	94	72 (13)
May	9.1	18.7	14.0 (2.8)	54	94	71 (13)
June	14.9	26.2	20.8 (3.4)	58	89	70 (10)
July	13.5	31.4	25.5 (4.7)	32	83	48 (16)
September	7.5	20.2	14.8 (3.5)	49	93	66 (13)

A total of 8068 *I. ricinus* was collected, of which 5370 were larvae, 1920 were nymphs, and 778 were adults (422 males and 356 females). The abundance of questing ticks was significantly affected by time and vegetation type, with a significant interaction between both factors (Table 2.2 and Fig. 2.1). Larvae were found in significantly lower numbers in spring (April and May) compared to the warmer summer months (June–September), which resulted in a positive correlation between larval abundance and air temperature ($r = 0.598$, $p < 0.001$, $n = 180$) and in a negative correlation with relative humidity ($r = -0.304$, $p < 0.001$, $n = 180$). The larval abundance was significantly highest along the grass-dominated forest trails (64 % of total larval catch) and lowest in the bracken-dominated larch stands (only 6 %), but no significant difference was found between the forest gaps with purple moor grass (14 %) and the bilberry-dominated pine stands (16 %). Nymphs and adults were present throughout the study period with a significantly higher abundance of nymphs in May and a significantly lower abundance of both nymphs and adults in September. Neither the nymphal nor the adult abundance was significantly correlated with temperature or humidity. The abundance of nymphs and adults was significantly lowest in the bracken-dominated larch stands, which was especially noticeable in June and July when the bracken understory was very tall (> 150 cm) and dense. Furthermore, the abundance of nymphs was significantly higher along the grass-dominated trails compared to the bracken sites and the forest gaps dominated by purple moor grass.

Table 2.2 The effects of sampling occasion (time), vegetation type, and blanket type on the abundance of *Ixodes ricinus* larvae, nymphs, and adults. Larval data from April were excluded from the analysis because of the low number of ticks caught (only 0.3 % of the total larval catch). Bold: $p < 0.05$.

	Larvae		Nymphs		Adults	
	df	p	df	p	df	p
Within-subjects						
Time	3	**< 0.001**	4	**< 0.001**	4	**< 0.001**
Time × Vegetation type	9	**<0.001**	12	**< 0.001**	12	**< 0.001**
Time × Blanket type	3	0.534	4	0.393	4	0.142
Between-subjects						
Vegetation	3	**< 0.001**	3	**< 0.001**	3	0.133
Blanket type	1	**0.019**	1	**0.012**	1	**0.002**
Vegetation type × Blanket type	3	**0.019**	3	0.550	3	0.619

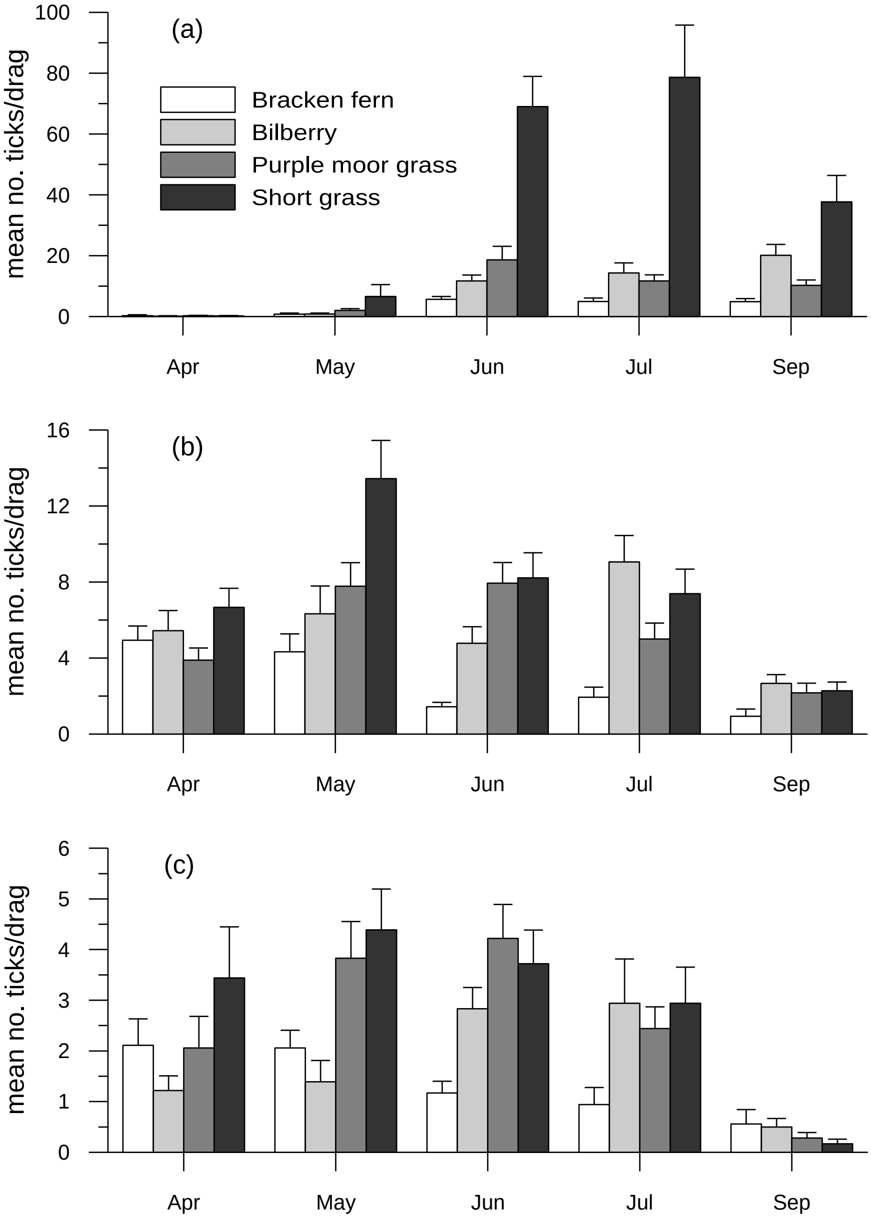


Fig. 2.1 Abundance of (a) larval, (b) nymphal, and (c) adult *Ixodes ricinus* ticks collected in four vegetation types. The results from the strip-blanket and entire-blanket were pooled. Bars represent the mean number of ticks collected per drag (n = 18), error bars indicate the standard error of the mean. Note the difference in values on the y-axis.

Additionally, a significant effect of blanket type on the abundance of all life stages and a significant vegetation × blanket type interaction term for the larvae was discernible (Table 2.2 and Fig. 2.2). For both nymphs and adults, the entire-blanket appeared to be more efficient than the strip-blanket in each vegetation type. Overall, the entire-blanket sampled 12 % more nymphs and 14 % more adult ticks than the strip-blanket. The strip-blanket, on the other hand, was more efficient for collecting larvae. This was especially apparent in the bracken-dominated larch stands. Although the total number of larvae collected was low compared to the other vegetation types, twice as many larvae were collected with the strip-blanket.

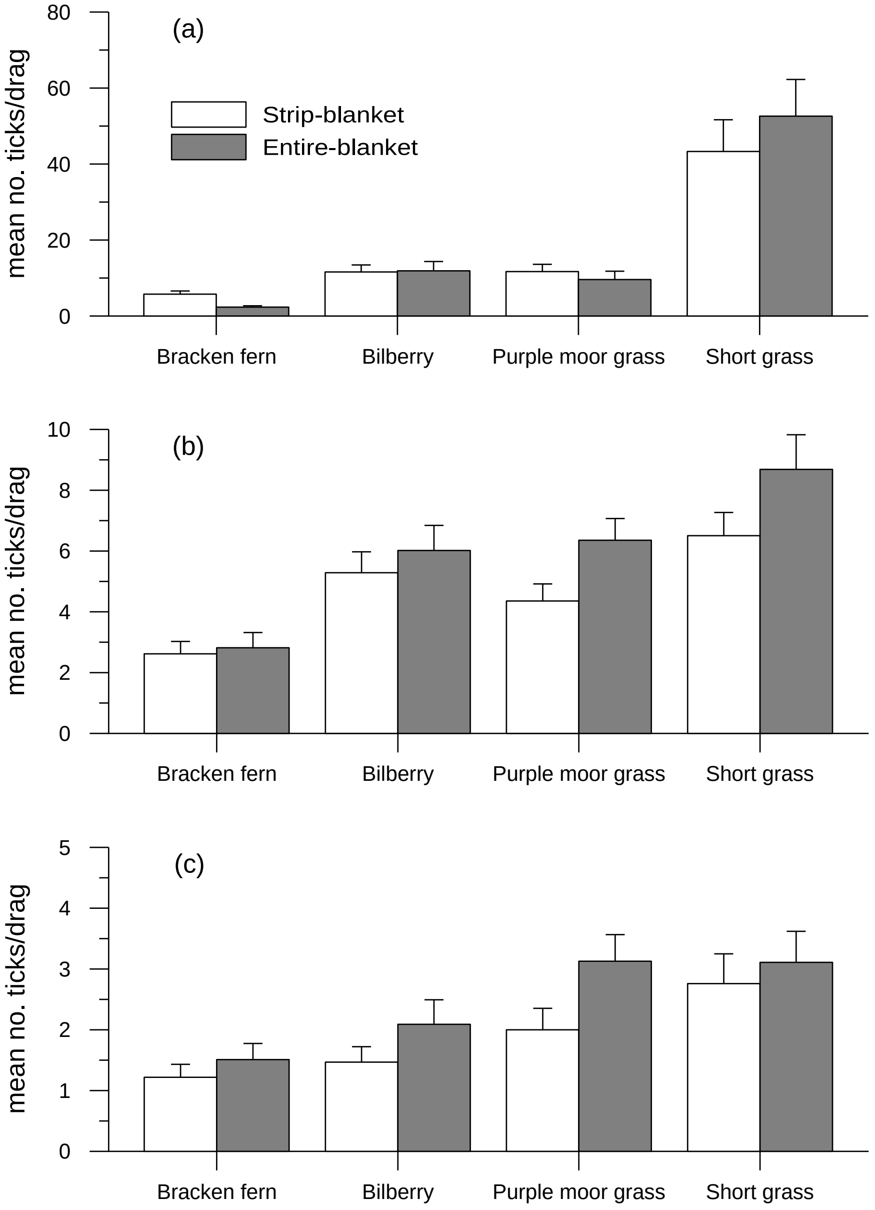


Fig. 2.2 Comparison of the efficiency of the strip-blanket and the entire-blanket for sampling (a) larval, (b) nymphal, and (c) adult *Ixodes ricinus* ticks in four vegetation types. The results from the different sampling occasions were pooled. Bars represent the mean number of ticks collected per drag (n = 45), error bars indicate the standard error of the mean. Note the difference in values on the y-axis.

2.5 Discussion

Entire-blanket sampling is an established method for tick collection (Milne 1943, Falco & Fish 1992). However, adult ticks are easily dislodged from the blanket through contact with the vegetation during dragging (Milne 1943, Li & Dunley 1998) and larvae are often missed in dense vegetation because the blanket inadequately contacts the lowest parts of the vegetation and the litter layer (Gray 1985). In the present study, the strip-blanket was proposed as an alternative for the conventional entire-blanket to overcome these problems.

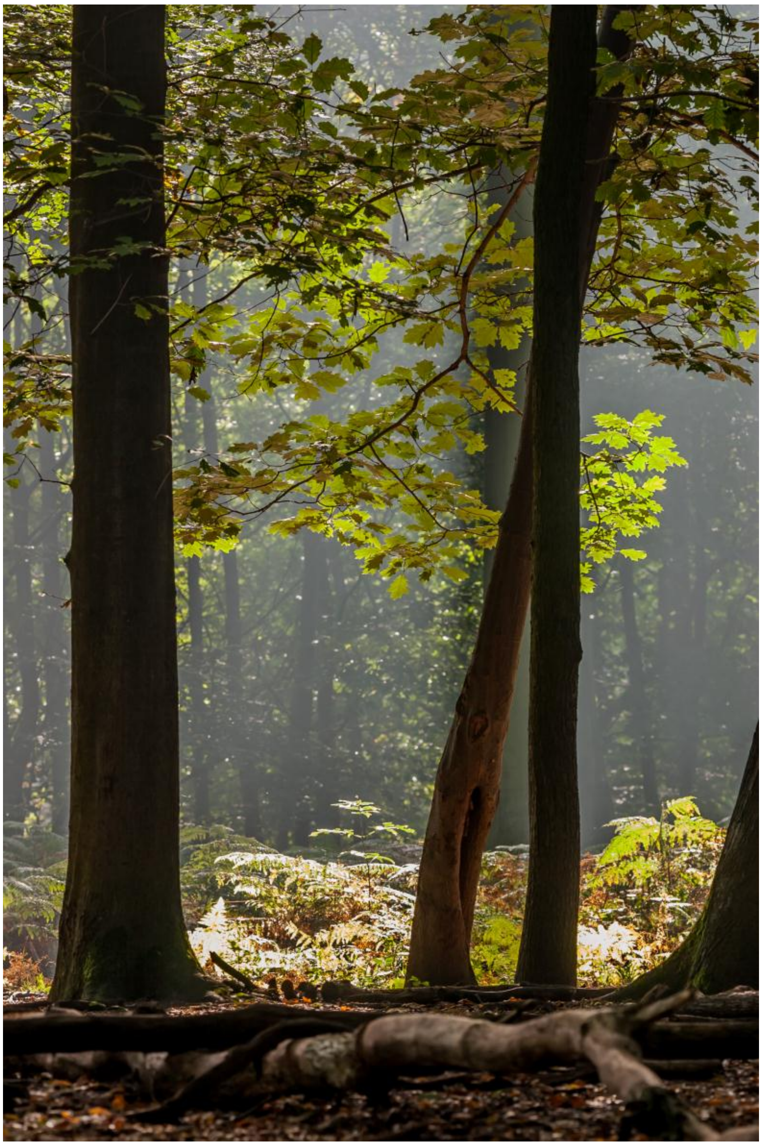
Surprisingly, we showed that significantly more nymphs and adults were collected with the entire-blanket instead of the strip-blanket. Rotation of the strips did not seem to protect ticks from being dislodged at all, as was suggested by Gray and Lohan (1982). On the contrary, the strips often got stuck in the vegetation, which caused undesirable abrupt movements of the blanket and hence, very probably, lead to a higher drop-off rate. Tick sampling was particularly difficult in the forest stands with a dense bracken fern understory. In spring, when the new fronds had just emerged and started to grow, tick abundance differed little between vegetation types. In summer, however, the abundant bracken foliage strongly interfered with tick sampling and ticks were easily knocked off of the blanket, resulting in significantly lower numbers of nymphs and adults.

In line with our expectations, the strip-blanket was more efficient for sampling larvae in dense vegetation. The bracken understory made it very difficult to reach the questing larvae with the entire-blanket and the strip-blanket, which resulted in overall low larval abundance compared to the other vegetation types. However, the strip-blanket did make it easier to move through the ferns and picked up significantly more larvae than the entire-blanket. In the other vegetation types, where the vegetation was less dense, the strip-blanket did not seem to offer an advantage over the entire-blanket.

The lower abundance of larvae, nymphs, and adults in the bracken-dominated larch stands was clearly caused by the height and the structure of the understory, which impeded tick sampling. The higher abundance of larvae and nymphs along the forest trails can be explained partly by the fact that the short grasses did not obstruct the blankets from making close contact with questing ticks and did not cause the blankets to get stuck, but may also be down to other factors, such as differences in roe deer habitat selection. Because adult *I. ricinus* ticks predominantly feed on wild cervids such as roe deer, the distribution of roe deer will determine the location where engorged females drop off and lay eggs (Ruiz-Fons & Gilbert 2010). Roe deer are probably highly attracted to the palatable grass and herbaceous cover along the trails, resulting in a higher import of eggs.

2.6 Conclusion

In summary, our results show that the strip-blanket did not appear to provide an advantage over the standard entire-blanket for sampling nymphal and adult ixodid ticks. However, the alternative strip-blanket method may have its utility in collecting larval ticks, especially in dense vegetation where the penetration capacity of the entire-blanket is limited. Moreover, it is important to note that the vegetation structure strongly influences the efficiency of drag sampling, which must be taken into consideration when tick densities between different sites and vegetation types are to be compared.



[Photograph Gert Arijs]

3 Local habitat and landscape affect *Ixodes ricinus* tick populations

After: Tack W, Madder M, Baeten L, Vanhellemont M, Gruwez R, Verheyen K (2012) Local habitat and landscape affect *Ixodes ricinus* tick abundances in forests on poor, sandy soils. Forest Ecology and Management 265, 30–36

3.1 Abstract

Conversion of homogeneous pine forests to structure-rich deciduous or mixed forests might create favorable habitats for the tick *Ixodes ricinus* (Linnaeus, 1758), which is an important vector of numerous pathogenic microorganisms causing disease in animals and humans. Considering the threat to human health, it is important to know which factors regulate tick abundance. We studied the influence of local habitat and landscape variables on the abundance of host-seeking larval, nymphal, and adult *I. ricinus* ticks, which were collected at 176 locations in 21 forests in northern Belgium. *Borrelia*-infected nymphs occurred throughout the study area, which means that this region represents an area of risk for contracting Lyme borreliosis. At the forest stand level, the main tree species and the shrub cover significantly affected the abundance of all life stages of *I. ricinus*. Tick abundance was higher in oak stands compared to pine stands, and increased with increasing shrub cover. Additionally, at the landscape level, a positive effect on the abundance of nymphs and adults was found for forest edge length but not for forest cover. These patterns may be explained by the habitat preferences of the ticks' main hosts. Our results indicate that forest conversion might create suitable habitats for ticks, which highlights the need for intensive information campaigns and effective tick control measures.

3.2 Introduction

Since the prevention of tick attacks is considered the most effective measure against tick-borne infections, it is important to know where and why ticks are abundant. Adequate vegetation cover, providing protection against adverse environmental conditions, and access to vertebrate hosts for feeding and reproduction are essential for the survival and development of *Ixodes ricinus* (Linnaeus, 1758). As a result, the abundance of ticks may vary strongly between different habitat types (Gray *et al.* 1998, Jaenson *et al.* 2009, Gassner *et al.* 2011). Logically, several studies have focused attention on habitat types as indicators of risk of human exposure to ticks. The abundance of *I. ricinus* is generally higher in forests compared to open habitats such as pastures and moorland, because forests provide a cooler, more humid microclimate as well as forage and shelter for a diverse mix of host species (Walker *et al.* 2001, Lindström & Jaenson 2003, Ruiz-Fons & Gilbert 2010). Within forest variation in tick abundance may be important as well. Deciduous forests and mixed forests dominated by deciduous trees — especially oak (*Quercus* spp.) forests with a diverse fauna, including cervids such as roe deer (*Capreolus capreolus* Linnaeus, 1758) — are generally considered the ideal habitat for *I. ricinus*.

Moreover, the spatial configuration and connectivity of landscape features influence spatial variations in the occurrence of ticks and tick-borne diseases (Ostfeld *et al.* 2005). In northern Spain, for instance, Estrada-Peña (2003) demonstrated that habitat patches with high tick abundance form part of a network of corridors linking patches of high tick abundance. Patches where the climate and vegetation were highly suitable, but where ticks were absent or present at low abundance, were located far from this main network. These results suggest that the distribution of ticks in a zone is highly affected not only by abiotic variables such as temperature and relative humidity but also by host movements, which are influenced by landscape configuration. In the United States, Allan *et al.* (2003) reported a higher density of questing nymphs of *Ixodes scapularis* (Say, 1821) and higher nymphal infection prevalence with *Borrelia* spirochetes in smaller than in larger forest fragments, which was attributed to elevated population densities of the white-footed mouse (*Peromyscus leucopus* Rafinesque, 1818). This generalist species thrives in fragmented landscapes and serves as the main host of *I. scapularis* larvae. In a subsequent study, however, a lower prevalence of immature ticks on white-footed mice was observed in smaller than in larger fragments (Wilder & Meikle 2004), which contradicts the results of Allan *et al*. (2003). This demonstrates the fact that effects of forest fragmentation on ticks and their host animals are still not well understood.

In the present study, various forest sites throughout the Campine region in northern Belgium were surveyed for the abundance of *I. ricinus* ticks. Collected ticks of the nymphal stage, which is probably the predominant life stage responsible for pathogen transmission (Rizzoli *et al.* 2011), were examined for *Borrelia burgdorferi* spirochetes. Although the Campine region is a region of high endemicity for Lyme borreliosis (Linard *et al.* 2007), until now, no regional-scale survey of *I. ricinus* ticks has been carried out in this part of the country. We selected forest stands varying in dominant tree species (pine or oak), shrub cover, and herb cover to determine the importance of those factors. Landscape-level fragmentation variables were also included to control for potential differences among forest complexes, e.g., related to host dispersal. We specifically addressed the following questions: (i) is the abundance of host-seeking larval, nymphal, and adult *I. ricinus* ticks higher in oak stands compared to pine stands, (ii) what is the influence of shrub and herb cover on tick abundance, and (iii) what is the influence of landscape variables (forest cover and forest edge length) on tick abundance?

3.3 Materials and methods

3.3.1 Study area

The study was carried out at 21 forest sites in the Campine region in northeastern Belgium (see Fig. 1.5 in section 1.5). At each of the 21 forest sites, 5–15 forest stands (176 forest stands in total) were selected for tick sampling. Our general aim was to select pine- and oak-dominated stands at each forest site, both with and without a substantial shrub layer. Only forest stands on poor sandy soils where the relative contribution of *Pinus* spp. or *Quercus* spp. to the total cover of the tree layer was greater than or equal to 70 % were selected for our study. Care was taken to select forest stands where the herbaceous layer was dominated by wavy hair grass (*Deschampsia flexuosa* (L.) Trin.), purple moor grass (*Molinia caerulea* (L.) Moench), broad buckler fern (*Dryopteris dilatata* (Hoffm.) A. Gray), narrow buckler fern (*Dryopteris carthusiana* (Vill.) H. P. Fuchs), or bilberry (*Vaccinium myrtillus* L.) to maintain a comparable blanket contact when sampling for ticks. Forest stands with a dense bracken fern (*Pteridium aquilinum*

(L.) Kuhn) understory were avoided because this vegetation can seriously hamper tick sampling, especially during summer when the bracken foliage is abundant (Chapter 2).

A total of 176 forest stands was sampled, 109 of which were pine stands and 67 were oak stands. Of the selected forest stands, 37 % had less than 10 % shrub cover (35 pine and 30 oak stands), 30 % had a shrub cover of 10–50 % (35 pine and 17 oak stands), and 33 % had more than 50 % shrub cover (39 pine and 20 oak stands). Each forest stand was sampled once between July 4 and September 8, 2009. This time period was chosen because it corresponds to the period during which all life stages, including larvae, are found questing in the study area (Chapter 2). We specifically wanted to include larvae in our study, as their presence in a given location is a good indication of the presence of large maintenance hosts (e.g., roe deer), which implies that the ticks are part of an established population. Furthermore, the weather conditions were favorable during the two-month sampling period. The minimum daily temperature ranged from 9.9 to 19.7°C (mean = 14.2°C, SD = 2.3°C), the maximum daily temperature ranged from 16.7 to 33.4°C (mean = 23.7°C, SD = 3.5°C), and the daily precipitation ranged from 0 to 16.8 mm (mean = 1.8 mm, SD = 3.5 mm) (Klein Tank *et al.* 2002).

3.3.2 Local habitat and landscape characterization

In each forest stand, the percentage cover of the tree layer (> 7 m), shrub layer (1–7 m), and herb layer (< 1 m) was estimated visually. The following local habitat measurements were used for the analysis: main tree species (pine or oak), shrub cover (in %), and herb cover (in %). The digital forest cover map (Afdeling Bos en Groen 2001a) and the GIS software package Arcview 3.1 (ESRI 2004) were used to calculate landscape variables within a circular buffer created at the centre of each forest site. A buffer radius of 3 km was chosen, which defined a buffer at each site large enough to contain all forest stands visited for tick sampling at that particular site and small enough to minimize the degree of overlap between the buffers. Landscape variables were also calculated using buffer sizes of 1 and 2 km. These buffer sizes were selected based on the results of Kie *et al.* (2002) and Anderson *et al.* (2005). They found that correlation between certain landscape variables and the size of home range and movements of ungulates such as mule deer (*Odocoileus hemionus*) and red deer (*Cervus elaphus* Linnaeus, 1758) occurred at large spatial scales (i.e., radii of 2–3 km) but not at smaller spatial scales (i.e., radii ≤ 1 km). We calculated the following landscape variables: the percentage of forest cover, the number and mean size (ha) of forest patches, and the total forest edge length (km). We defined forest patches as areas of continuous tree canopy, with a minimum area of 0.5 ha, a minimum width of 25 m, and a minimum canopy cover of 20 %. Forest edges were defined as the boundary between forest and any other land cover type, mainly agricultural fields, meadows, grasslands, heathlands, roads, and residential areas.

3.3.3 Tick sampling

Tick sampling consisted of dragging a white flannel blanket (1 m × 1 m) over the herbaceous vegetation and litter. An entire-blanket was used as described in section 2.3.2. Sampling was always performed on dry and non-windy days (< 2 Beaufort) during day time (between 10:00 am and 05:00 pm) when the vegetation was dry. The forest stands were sampled in random order to eliminate time of day and changing meteorological conditions as a source of bias. Four 1 min blanket drags were established at random in each stand. Each drag covered about 25 m of distance. After each drag, larvae, nymphs, and adults were removed from the blanket using forceps and stored in vials containing 70 % ethanol for later identification and counting in the laboratory. The sampled ticks were identified morphologically using a stereo-microscope and the identification keys of Hillyard (1996).

3.3.4 Identification of *Borrelia* infections

In total, five hundred nymphs were used for further molecular analysis of the presence of *B. burgdorferi* sl spirochetes. We did not identify the *Borrelia* genospecies. Instead, only screening up to species level was performed to get an idea of the overall infection prevalence. The analysis was carried out on five pools (five nymphs per pool) for each of twenty forest sites. One of the 21 forest sites was excluded due to the low numbers of nymphs collected.

DNA was extracted using the method of Boom *et al.* (1990). This method is based on the lysing and nuclease-inactivating properties of proteinase K together with the nucleic acid-binding properties of silica particles. A standard PCR amplification was performed in 25 µl reaction mixtures containing 5 µl of the extracted DNA, 1.65 mM $MgCl_2$, 0.2 mM of all four dNTPs, 10 pM of two primers (BorrSLospAF/BorrSLospAR) (Demaerschalck *et al.* 1995), 1 UTaq polymerase enzyme (Promega), and 1 µl Yellow SubTM (GENEO Bioproducts, Hamburg, Germany). After a hot start of 10 s at 84°C, an initiation of 4 min at 92°C was performed, then followed by a 40 cycles denaturation-hybridisation-elongation step (30 s at 92°C, 45 s at 58°C, and 60 s at 72°C). The PCR ended with an extension step of 10 min at 72°C. Five µl of each reaction mixture was mixed with 2 µl of loading buffer and loaded onto 2 % agarose gels (Sigma) to be examined for the presence of DNA fragments. A 1.5 kb DNA ladder (MBI Fermentas, Lithuania) was loaded on every gel. The samples were run for 20 min at 100 V, stained in ethidium bromide for 30 min, washed under running tap water, and photographed under UV illumination.

3.3.5 Statistical analysis

Tick abundance was expressed as the total number of ticks collected per 100 m^2. Each life stage (larva, nymph, and adult) was analyzed separately. Because of the hierarchical nature of the survey (176 forest stands clustered by 21 forest sites), the data were analyzed using mixed effects models with forest site as a random effect term and local habitat variables (stand level) and landscape variables (site level) as fixed effects. Adult abundances were modeled with a generalized linear mixed model with a Poisson error distribution and a log link function using the *lmer* function of the *lme4* library (Bates *et al.* 2011) in R 2.13.0 (R Development Core Team 2011). Larval and nymphal abundances were first $\log_{10}(n + 1)$ transformed to approach normality, which was verified using the Kolmogorov-Smirnov test, and subsequently modeled with linear mixed models using the *lme* function of the *nlme* library in R (Pinheiro *et al.* 2011). Full factorial models were first simplified before estimating parameter values and their significance. That is, the fixed effects of local habitat and landscape variables were removed one by one from the model, and their chi-square statistic and significance level were estimated using maximum likelihood.

3.4 Results

A total of 25,204 *I. ricinus* ticks was collected, of which 21,502 were larvae, 3456 were nymphs, and 246 were adults (138 males and 108 females). All life stages of *I. ricinus* were found at each of the 21 forest sites sampled (except for adults in the municipality Neerpelt), with a mean abundance ± standard error (SE) of 122.2 ± 14.6 larvae, 19.6 ± 1.8 nymphs, and 1.4 ± 0.1 adults per 100 m^2. Table 3.1 shows the results at each forest site. *Borrelia*-positive nymphs were found at 17 out of the 20 forest sites examined. Overall, 38 out of a total of 100 pools tested positive for the presence of *B. burgdorferi* sl. The estimated mean nymphal infection prevalence was calculated to be 9.1 % with a 95 % confidence interval of 6.6 to 12.2 %.

Table 3.1 Mean (standard error) abundance of *Ixodes ricinus* larvae, nymphs, and adults at each of 21 forest sites. Tick abundance was expressed as the total number of ticks per 100 m². Lat, latitude; Lon, longitude.

Lat	Lon	Municipality	Larvae	Nymphs	Adults
51°18’27”	4°31’49”	Brasschaat	41.2 (15.4)	13.8 (3.5)	1.2 (1.0)
51°16’42”	4°29’09”	Brasschaat	159.7 (54.5)	12.7 (6.6)	2.0 (1.4)
51°15’17”	4°40’45”	Zoersel	29.5 (10.8)	23.3 (7.5)	2.2 (1.4)
51°24’09”	4°49’21”	Hoogstraten	43.0 (14.6)	16.8 (5.7)	1.2 (0.7)
51°22’13”	5°02’02”	Ravels	383.5 (153.9)	27.5 (11.9)	1.2 (0.3)
51°21’03”	5°04’24”	Arendonk	215.4 (78.9)	21.6 (9.3)	1.8 (0.7)
51°17’12”	4°53’14”	Lille/Vosselaar	91.2 (24.7)	23.4 (7.5)	1.8 (0.6)
51°12’53”	4°53’02”	Kasterlee	83.3 (61.4)	10.2 (3.9)	0.7 (0.5)
51°11’35”	4°48’14”	Herentals	106.7 (37.5)	16.0 (4.6)	2.7 (1.0)
51°03’27”	4°55’42”	Herselt	160.9 (27.0)	20.6 (3.6)	1.7 (0.4)
51°17’17”	5°11’09”	Mol	242.7 (62.4)	31.0 (4.7)	3.1 (0.4)
51°01’59”	4°58’47”	Tessenderlo	99.2 (23.5)	28.2 (5.8)	2.2 (0.7)
51°07’14”	5°12’36”	Ham/Leopoldsburg	40.0 (23.5)	5.6 (2.1)	0.3 (0.2)
51°10’04”	5°20’23”	Hechtel-Eksel	77.8 (44.0)	6.0 (1.6)	0.2 (0.1)
51°12’29”	5°28’44”	Neerpelt	24.3 (18.3)	1.8 (1.0)	-
51°04’42”	5°36’41”	Meeuwen-Gruitrode	99.0 (39.7)	43.9 (22.5)	2.1 (1.5)
51°03’30”	5°33’21”	Opglabbeek	105.5 (56.0)	17.5 (6.8)	0.3 (0.2)
51°01’31”	5°33’28”	Opglabbeek	75.1 (35.5)	12.6 (4.5)	0.4 (0.3)
50°58’08”	5°36’27”	Maasmechelen	108.0 (44.1)	12.7 (6.4)	0.2 (0.2)
50°54’46”	5°38’06”	Lanaken	76.9 (66.2)	26.2 (13.1)	0.9 (0.4)
50°54’38”	5°29’13”	Bilzen	20.1 (8.1)	11.6 (2.7)	1.1 (0.3)

The landscape variables were strongly correlated with each other (Table 3.2). Mean forest patch size was positively correlated with forest cover ($r = 0.892$, $p < 0.001$) and negatively correlated with number of forest patches ($r = -0.701$, $p < 0.001$), while forest edge length was positively correlated with number of forest patches ($r = 0.589$, $p = 0.005$). Forest cover and forest edge length were not linearly correlated, but we did find a significant quadratic relationship ($r = 0.372$, $p = 0.015$). To avoid problems of multicollinearity, only forest cover and forest edge length were retained for further analysis. Forest cover in the 3-km buffer ranged from 16.5 to 66.2 % with a mean ± SE of

35.1 ± 2.8 %, and total forest edge length ranged from 77.7 to 190.3 km with a mean ± SE of 130.8 ± 6.0 km. The mean percentage of coniferous and deciduous forest cover in the 3-km buffers was 71 and 29 %, respectively.

Table 3.2 Pearson's linear correlation coefficients indicating the correlation between four landscape variables (n = 21). Significant effects in bold, (*)0.05 < p < 0.1, **p < 0.01, ***p < 0.001.

	No. forest patches	Forest edge length	Forest cover
Mean forest patch size	**-0.701*** **	-0.190	**0.892*** **
Number of forest patches		**0.589** **	-0.417(*)
Forest edge length			0.218

The linear mixed effects models revealed significant effects of both local habitat and landscape variables on tick abundance (Table 3.3). At the forest stand level, the results indicated a highly significant effect of tree species and shrub cover for all life stages, whereas the herb layer cover and the interaction terms were not related to tick abundance. Tick abundance was significantly higher in the oak stands compared to the pine stands (Fig. 3.1). The mean tick abundance ± SE in the pine stands was 77.7 ± 10.1 larvae, 13.0 ± 1.3 nymphs, and 0.9 ± 0.1 adults per 100 m^2. In the oak stands, these values were higher: 194.5 ± 32.8 larvae, 30.5 ± 4.0 nymphs, and 2.2 ± 0.3 adults per 100 m^2. Thus, larvae were 2.5 times more abundant, and both nymphs and adults were 2.4 times more abundant in oak stands.

In addition, a significant positive effect of shrub cover was found for all life stages (Fig. 3.1). Larvae, nymphs, and adults were respectively 1.7, 2.0, and 1.9 times more abundant in forest stands with high shrub cover than in stands with low shrub cover. Consequently, oak stands with high shrub cover yielded 6–7 times more ticks than homogeneous pine stands with little or no shrubs.

Furthermore, at the landscape level, a significant positive effect of total forest edge length was found for nymphs and adults and a marginally positive effect was found for larvae (Table 3.3). Forest cover had no effect on tick abundance. The same relationships between tick abundance and forest edge length and cover were found when the analysis was performed using buffers with a radius of 1 and 2 km.

Table 3.3 Effect of local habitat and landscape variables on the abundance of *Ixodes ricinus* larvae, nymphs, and adults (n = 176). A positive effect for Main Tree Species means higher tick abundance in oak stands compared to pine stands. Bold: $p < 0.05$.

	Larvae		Nymphs		Adults	
	χ^2	p	χ^2	p	χ^2	p
Local habitat variables						
Main tree species	16.66	< **0.001**	29.08	< **0.001**	39.57	< **0.001**
Shrub cover	17.81	< **0.001**	25.09	< **0.001**	23.04	< **0.001**
Herb cover	0.00	0.994	1.82	0.178	0.05	0.823
Main tree × Shrub cover	0.24	0.622	0.45	0.503	0.64	0.424
Main tree × Herb cover	0.27	0.600	0.14	0.707	0.48	0.490
Shrub × Herb cover	0.38	0.540	0.24	0.626	0.22	0.640
Landscape variables						
Forest edge length	3.76	0.053	9.35	**0.002**	8.26	**0.004**
Forest cover	0.09	0.766	0.17	0.679	2.43	0.119
Forest edge × cover	0.00	0.984	0.97	0.325	1.69	0.194

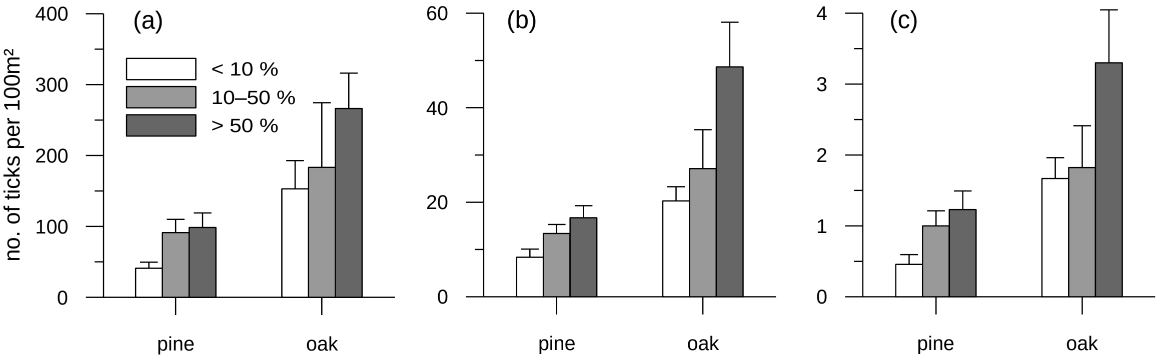


Fig. 3.1 The effect of shrub cover on the abundance of (a) larval, (b) nymphal, and (c) adult *Ixodes ricinus* ticks in pine and oak-dominated stands. For graphic reasons, the shrub cover estimates were grouped into three classes: low (< 10 %), intermediate (10–50 %), and high shrub cover (> 50 %). Bars represent the mean number of ticks collected per 100 m² and error bars indicate the standard error of the mean. Note the difference in values on the y-axis.

3.5 Discussion

We conducted a large-scale survey of *I. ricinus* ticks in forests on poor sandy soils — the first tick survey in northern Belgium as far as we know — to investigate the effects of local habitat and landscape variables on questing tick abundance and to determine the nymphal infection prevalence with *Borrelia* spirochetes. *Borrelia*-infected nymphs were found throughout the study area. Spirochetes were absent in three forest sites, but this was probably due to the small sample size (25 nymphs per forest site) and not because of a true absence. The overall mean infection prevalence in host-seeking nymphs was 9.1 %, which is consistent with the mean infection prevalence in Europe (10.1 %) (Rauter & Hartung 2005). We wish to emphasize the fact that we did not identify the *Borrelia* genospecies, which could be considered a shortcoming of our study. However, *B. afzelii* and *B. garinii* are the most common *Borrelia* species in Europe (Rauter & Hartung 2005) and are both known to be responsible for causing Lyme borreliosis in humans. The genospecies *B. valaisiana* may also be very common, especially in western Europe. Although its pathogenicity is still uncertain (Stanek & Reiter 2011), a number of studies have suggested that this genospecies may also be pathogenic for humans (Rijpkema *et al.* 1997, Ryffel *et al.* 1999).

Although each forest stand was sampled only once and we could not take into account the temporal variation in tick abundance, our study does provide important information about the habitat preferences of *I. ricinus* ticks and the influence of landscape structure on tick abundances in summer, when most people visit forests for recreation. Our results confirm the importance of forest type and forest edge length for the distribution and abundance of *I. ricinus* ticks. Consistent with previous studies (Estrada-Peña 2001, Lindström & Jaenson 2003), we found (i) a higher tick abundance in oak stands compared to pine stands, (ii) a positive effect of shrub cover on tick abundance, and (iii) a higher abundance of ticks in fragmented forest sites with a high amount of forest edge habitat, which is most likely related to more optimal environmental conditions and a higher density and activity of the ticks' main hosts. Many prior studies have discussed the importance of deer in maintaining tick populations. Gray *et al.* (1992) and Pichon *et al.* (1999), for instance, have stressed the importance of red deer (*Cervus elaphus* Linnaeus, 1758) and roe deer for adult *I. ricinus* in Europe. Duffy *et al.* (1994) and Stafford *et al.* (2003) focused on white-tailed deer (*Odocoileus virginianus* Zimmerman, 1780) as key host for adult *I. scapularis* (Say, 1821) in the United States.

Roe deer are the most common large mammals in the study area and, thus, are probably the most important hosts for adult ticks. They most likely play a pivotal role in the observed differences in tick abundance, as deer habitat use largely determines the location where engorged female ticks drop off and lay eggs (Gray *et al.* 1992, Ruiz-Fons & Gilbert 2010). From spring to autumn — the period of *I. ricinus* activity — the structure-rich oak stands in the study area are probably more attractive to roe deer than the homogeneous pine stands, as they provide both high-quality forage and shelter. Although roe deer are considered generalist herbivores, they are highly selective feeders, preferentially feeding on deciduous browse, fruits, and seeds (Tixier & Duncan 1996). Especially in spring and summer, when female roe deer invest heavily in the care for their offspring (Andersen *et al.* 1998), coniferous browse is shunned whereas easily digestible forage rich in soluble carbohydrates (e.g., forbs and deciduous browse) is preferred (Tixier *et al.* 1997, Cornelis *et al.* 1999). Oak browse is considered highly palatable and acorns can make up a large part of the roe deer diet when they are sufficiently available (e.g., up to 89 % in a mast year, Maillard & Picard 1987).

Roe deer also require appropriate habitat for birthing and fawn rearing during spring and summer, with enough cover and concealment to hide fawns against potential predators and to protect them from adverse influences of microclimate (Tufto *et al.* 1996, Linnell *et al.* 1999, Van Moorter *et al.* 2009). The positive effect of forest edge length on tick abundance can be explained by the fact that forest edges are high-quality habitats for roe deer as forest edges provide a good interspersion of cover and forage. For example, previous studies reported higher roe deer population densities (Wahlström & Kjellander 1995), smaller home range sizes (Saïd & Servanty 2005), and higher utilization rates by roe deer (Tufto *et al.* 1996) in edge habitats.

Similarly, habitat selection by several small mammal species, which serve as hosts for immature ticks, is strongly influenced by factors related with forage and cover. Bank vole (*Myodes glareolus* Schreber, 1780) and wood mouse (*Apodemus sylvaticus* Linnaeus, 1758) in particular have been identified by several authors as key hosts for larval ticks (Tälleklint & Jaenson 1997, Gray *et al.* 1999, Estrada-Peña *et al.* 2005). Bank vole, a typical forest-dwelling rodent, prefers deciduous plant species to conifers (Hjältén *et al.* 2004) and prefers forests with well-developed lower vegetation (Ecke *et al.* 2002, Miklós & Žiak 2002). Wood mouse, on the other hand, is a habitat generalist that also uses open habitats (Tattersall *et al.* 2001) and thrives in situations where forests meet open land (Geuse *et al.* 1985, Tellería *et al.* 1991, García *et al.* 1998). In fact, in the study by Boyard *et al.* (2008), this rodent species was more abundant in the forest-pasture ecotone than inside forests or pastures in a bocage landscape and was highlighted as the major means of transfer of *I. ricinus* larvae from woodland to pasture. Besides being important hosts for immature ticks, rodents are also important reservoir hosts for *B. burgdorferi* sl (Kurtenbach *et al.* 1998). Therefore, factors affecting rodent population dynamics could also determine the fraction of tick meals taken from them, which could influence the infection prevalence of ticks. Halos *et al.* (2010) found that *B. burgdorferi* prevalence was favored by forest fragmentation in woodlands while in pastures, it was favored by shrubby vegetation at the perimeter of the pastures, probably because large amounts of edges or shrubs increase the abundance of small vertebrates as reservoir hosts. In the United States, a similar association between populations of competent rodent reservoirs for Lyme borreliosis and ecotones was described (LoGiudice *et al.* 2003).

3.6 Conclusion

In summary, our results show that the forests in the Campine region (northern Belgium) are a potential risk area for acquiring Lyme borreliosis and that tick abundance was significantly affected by both local habitat and landscape variables, which we postulate is largely caused by the habitat associations of the ticks' main hosts. The abundances of all life stages of *I. ricinus* were influenced by dominant tree species and shrub cover. Ticks were more abundant in oak stands compared to pine stands, and they were more abundant in forest stands with a high shrub cover. At the landscape level, the abundance of nymphs and adults was positively affected by forest edge length. These results indicate that forest management may strongly influence the suitability of habitats for ticks and, consequently, influence the epidemiology of tick-borne diseases, such as Lyme borreliosis.

[Photograph Sandy Adriaenssens]

4 Tick abundance depends on tree species composition and shrub cover

After: Tack W, Madder M, Baeten L, De Frenne P, Verheyen K (2012) The abundance of *Ixodes ricinus* ticks depends on tree species composition and shrub cover. Parasitology 139, 1273–1281

4.1 Abstract

Woodlands are the main habitat for *Ixodes ricinus* (Linnaeus, 1758) ticks. Assessing to what extent tick abundance and infection with *Borrelia* spirochetes are affected by forest composition and structure is a prerequisite for effective prevention of Lyme borreliosis. We selected a total of 25 pine and oak stands, both with and without an abundant shrub layer, in northern Belgium and estimated tick abundance between April and October 2008–2010. Additionally, the presence of deer beds was used as an indicator of roe deer (*Capreolus capreolus* Linnaeus, 1758) habitat use. *Borrelia* infections in questing nymphs were determined by polymerase chain reactions. We found that the abundance of host-seeking larvae, nymphs, and adults was higher in oak stands compared to pine stands. Furthermore, the abundance of the three life stages was higher in structure-rich compared to structure-poor forest stands. The same relationship with dominant tree species and shrub cover was observed for the deer beds. Our results suggest that the observed differences in tick abundance are greatly attributed to differences in habitat use by the ticks' main hosts. Whereas tick abundance was markedly higher in the structure-rich oak stands compared to the structure-poor pine stands, the *Borrelia burgdorgferi* infection rates in nymphs did not differ significantly.

4.2 Introduction

The conversion of monospecific coniferous forests into mixed, structure-rich forests dominated by native broadleaved species — a major objective of sustainable multipurpose forest management in many European countries — will cause large-scale landscape changes that might influence the suitability of forests for ticks and their vertebrate hosts, which might influence the epidemiology of tick-borne diseases (i.e., forest conversion creating an ecosystem dysfunction). Yet, there have been relatively few studies addressing the variation in tick abundance and infection prevalence between forest types. In Chapter 3, we quantitatively showed that the abundance of *Ixodes ricinus* (Linnaeus, 1758) is higher in oak stands compared to pine stands and increases with increasing shrub cover. Yet, so far, little is known on the longer-term temporal variation in tick abundance and on the effects of forest composition and structure on the resulting *Borrelia* infection rate.

Here, we selected a total of 25 pine (*Pinus* spp.) and oak (*Quercus* spp.) stands, both with and without a substantial shrub layer, and sampled *I. ricinus* tick populations between April and October in three successive years in northern Belgium to describe the spatiotemporal variation in the abundance of larvae, nymphs, and adults and to relate this variation to forest composition and structure. Additionally, habitat use by roe deer (*Capreolus capreolus* Linnaeus, 1758) was determined by counting the number of deer beds. *Borrelia burgdorferi* sl spirochete infections in tick nymphs, the life stage predominantly responsible for pathogen transmission, were determined by polymerase chain reactions to assess the potential impact of different forest characteristics on the infection prevalence.

4.3 Materials and methods

4.3.1 Study area

The study was carried out at two sites in the Campine ecoregion in northeastern Belgium (site A and B, see Fig. 1.5 in section 1.5). At both sites, six pine stands and six oak stands were selected with half of the stands having little or no shrub layer (< 15 % shrub layer cover in the 1–7 m height class) and the other half having a well-developed shrub layer (> 50 % cover). An additional oak stand was selected with low shrub cover at site B.

Thus, ticks were sampled in 25 forest stands (12 in site A and 13 in site B) and in four distinct forest stand types: pine stands and oak stands, both with and without a substantial shrub layer (see Fig. 1.6 in section 1.5).

The relative contribution of *Pinus* spp. (*P. sylvestris* L. or *P. nigra* Arnold ssp. *laricio* (Poiret) Maire) or *Quercus* spp. (mainly *Q. robur* L.) to the total estimated canopy cover of the tree layer (> 7 m) was greater than or equal to 80 % in each pine and oak stand, respectively. In each forest stand, the percentage cover of the shrub layer (1–7 m) and herb layer (< 1 m) was estimated visually. Shrub cover estimates were very comparable between pine and oak stands at both forest sites. The structure-rich oak stands had an average shrub cover of 66.7 % at site A and 70.0 % at site B, and the pine stands had an average shrub cover of 70.0 % at site A and 58.3 % at site B. The shrub layer mainly consisted of alder buckthorn (*Frangula alnus* Mill.), black cherry (*Prunus serotina* Ehrh.), and rowan (*Sorbus aucuparia* L.) in the pine stands and alder buckthorn, pedunculate oak (*Quercus robur* L.), and sycamore (*Acer pseudoplatanus* L.) in the oak stands. The herbaceous layer was dominated either by wavy hair grass (*Deschampsia flexuosa* (L.) Trin.), purple moor grass (*Molinia caerulea* (L.) Moench), broad buckler fern (*Dryopteris dilatata* (Hoffm.) A. Gray), narrow buckler fern (*Dryopteris carthusiana* (Vill.) H. P. Fuchs), or bilberry (*Vaccinium myrtillus* L.), which are representative vegetation types for the Campine region.

4.3.2 Tick sampling

Tick sampling was carried out between April and October in 2008, 2009, and 2010 for a total of eleven occasions at site A and twelve occasions at site B (12 stands × 11 occasions + 13 stands × 12 occasions = 288). In each forest stand and at each sampling occasion, we performed six 1 min blanket drags (each extending a distance of *ca.* 25 m) at random. After each two drags, we recorded the air temperature and relative humidity at the soil surface and at a height of 125 cm above the soil surface. Temperature and humidity were measured using a portable digital temperature and relative air humidity meter (DM509, Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). The four forest stand types were sampled in random order on each sampling day. For a more detailed description on tick sampling and identification, we refer to section 3.3.3.

Additionally, the number of fecal pellet groups and beds of roe deer were counted at each sampling occasion, along the same transects used for tick sampling. Pellet-group counting is a widely used method for assessing habitat use by deer. In our study, however, the number of pellet groups counted was too small (only 21 pellet groups in total) for proper analysis. Instead, we have used the number of deer beds in each forest stand type to examine differences in habitat selection for bedding sites (Smith *et al.* 1986, Bíró *et al.* 2006). Deer beds were easily detectable in the sandy soil of the study area and were distinguished as oval depressions in the soil or as flattened areas of vegetation, often accompanied by other signs of roe deer (e.g., hoof prints, hair).

4.3.3 Identification of *Borrelia* infections

Twenty pooled samples per forest site per year (20 samples × 2 sites × 3 years = 120), with each sample consisting of five nymphs (600 nymphs in total), were used for further molecular analyses for the presence of *B. burgdorferi* sl spirochetes. We did not identify the *Borrelia* genospecies. Instead, only screening up to species level was performed to get an idea of the overall nymphal infection prevalence. For each site and each year, ten samples consisted of nymphs collected in pine stands with low shrub cover while the other ten samples were collected in oak stands with high shrub cover. Potential differences in infection prevalence are most likely to occur between these two contrasting forest stand types. DNA was extracted using the method of Boom *et al.* (1990), as described in section 3.3.4.

4.3.4 Statistical analysis

Questing tick abundance, expressed as the number of ticks collected per 100 m^2, was first $\log_{10}(n+1)$ transformed to approach normality, which was verified using the Kolmogorov-Smirnov test. Subsequently, log-transformed tick abundances were modeled with linear mixed models using the *lmer*-function of the *lme4*-library (Bates *et al.* 2011) in R 2.13.0 (R Development Core Team 2011). Data for each life stage (larva, nymph, and adult) were analyzed separately. Models included tree species (pine *vs*. oak), shrub cover (in %), herb cover (in %), year, and all their two-way interactions as fixed effects and forest stand (nested within site (A or B)) and sampling occasion as non-nested random effect terms. To analyze the effects of tree species, shrub cover, year, and all their two-way interactions on the presence of roe deer (scored as 1 or 0 depending on whether deer beds were (1) or were not (0) encountered in the forest stand while dragging), we applied a generalized linear mixed model (GLMM) with a similar random-effects structure as above, but with a binomial error distribution and logit link function. Analysis of nymphal infection with *B. burgdorferi* sl (pooled samples of nymphs infected (1) or not (0)) were also performed with a GLMM with binomial error distribution and logit link function. This model included forest type (pine stands with low shrub cover *vs*. oak stands with high shrub cover), year, and their interaction term as fixed effects and forest stand (nested within forest site) as random effect term.

We always compared all possible models (i.e., build by each combination of the fixed effects terms) using Akaike's Information Criterion, adjusted for sample size (AIC_C) (Hurvich & Tsai 1989). The ΔAIC_C of a model was then calculated as the difference in AIC_C value for that model and the model with the lowest AIC_C value (best fit to the data). Models with $\Delta AIC_C \leq 4$ were considered equivalent (Bolker 2008). To determine the relative importance of the explanatory variables, we used the sum of Akaike weights of the set of all top models ($\Delta AIC_C \leq 4$) in which the variable appeared (Burnham & Anderson 2002). The Akaike weight reflects the weight of evidence in support of a particular model relative to the entire model set, and varies from 0 (no support) to 1 (complete support). Finally, the parameter values of the model with the lowest AIC_C value were estimated with restricted maximum likelihood estimation.

4.4 Results

During tick collection, the temperature ranged from 6.5 to 30.2°C (mean = 18.9°C, SD = 5.2°C) at the soil surface and from 7.1 to 31.7°C (mean = 20.2°C, SD = 5.5°C) at a height of 125 cm above the soil surface (Table 4.1). The relative humidity at the soil surface and at a height of 125 cm ranged from 46 to 99 % (mean = 85 %, SD = 10 %) and from 28 to 93 % (mean = 59 %, SD = 14 %), respectively. For the recorded values of temperature and relative humidity on each sampling occasion, we refer to Table 4.1.

A total of 110,770 *I. ricinus* ticks was collected, of which 89,017 were larvae, 18,685 were nymphs, and 3068 were adults (1634 males and 1434 females). The mean ± standard error of the number of ticks collected per 100 m^2 was 206.1 ± 20.8 larvae (range 0–4263), 43.3 ± 2.1 nymphs (range 1–215), and 7.1 ± 0.4 adults (range 0–44). On each sampling occasion, all three life stages were active and ticks were found questing in all 25 forest stands studied. In May 2009, a very high number of larvae was collected along a single transect in one of the oak stands with high shrub cover, which resulted in a peak in larval activity in May (Fig. 4.1a). This high variance in larval abundance was not unexpected and reflects the limited dispersal capability of larvae after emergence from the egg mass, consisting of up to 2000 eggs (Jongejan 2001). By considering this single transect as outlier, questing larvae showed a summer peak (August) each year.

Table 4.1 The minimum, maximum, and mean (SD, standard deviation) temperature and relative humidity measured at the soil surface and at a height of 125 cm above the soil surface on each sampling occasions (except May 2009).

	Temperature (°C)			Relative humidity (%)		
	min	max	mean (SD)	min	max	mean (SD)
At the soil surface						
May 2008	17.1	27.7	23.7 (3.6)	46	72	60 (9)
Aug 2008	15.3	27.0	20.0 (2.8)	76	94	86 (5)
Oct 2008	7.6	18.1	13.6 (2.9)	85	99	92 (4)
Apr 2009	6.5	16.9	13.4 (2.8)	85	95	90 (3)
Jul 2009	18.1	30.2	23.4 (3.1)	52	92	79 (11)
Aug 2009	18.0	24.4	20.7 (1.9)	80	95	88 (4)
Oct 2009	8.3	15.4	10.8 (2.4)	86	96	92 (3)
May 2010	11.8	25.8	17.9 (3.6)	70	93	83 (5)
Jul 2010	21.3	30.1	25.3 (2.6)	51	86	72 (9)
Aug 2010	18.0	25.9	20.6 (2.1)	82	95	89 (4)
Oct 2010	10.1	22.5	16.6 (4.4)	87	97	91 (2)
At a height of 125 cm						
May 2008	16.9	27.2	23.3 (3.4)	28	57	42 (11)
Aug 2008	15.5	28.1	20.8 (3.0)	52	84	66 (9)
Oct 2008	7.1	20.7	14.3 (3.6)	55	93	76 (12)
Apr 2009	9.5	17.3	14.5 (2.5)	62	81	69 (5)
Jul 2009	20.2	30.7	25.2 (2.9)	35	73	54 (10)
Aug 2009	18.6	29.3	23.4 (2.7)	34	78	54 (13)
Oct 2009	9.7	17.7	12.2 (2.8)	47	63	56 (6)
May 2010	11.1	27.7	19.6 (4.4)	31	79	47 (13)
Jul 2010	22.2	31.7	26.7 (2.8)	29	56	42 (7)
Aug 2010	17.7	27.5	21.9 (2.5)	46	70	59 (7)
Oct 2010	9.9	25.7	17.7 (5.3)	60	84	70 (7)

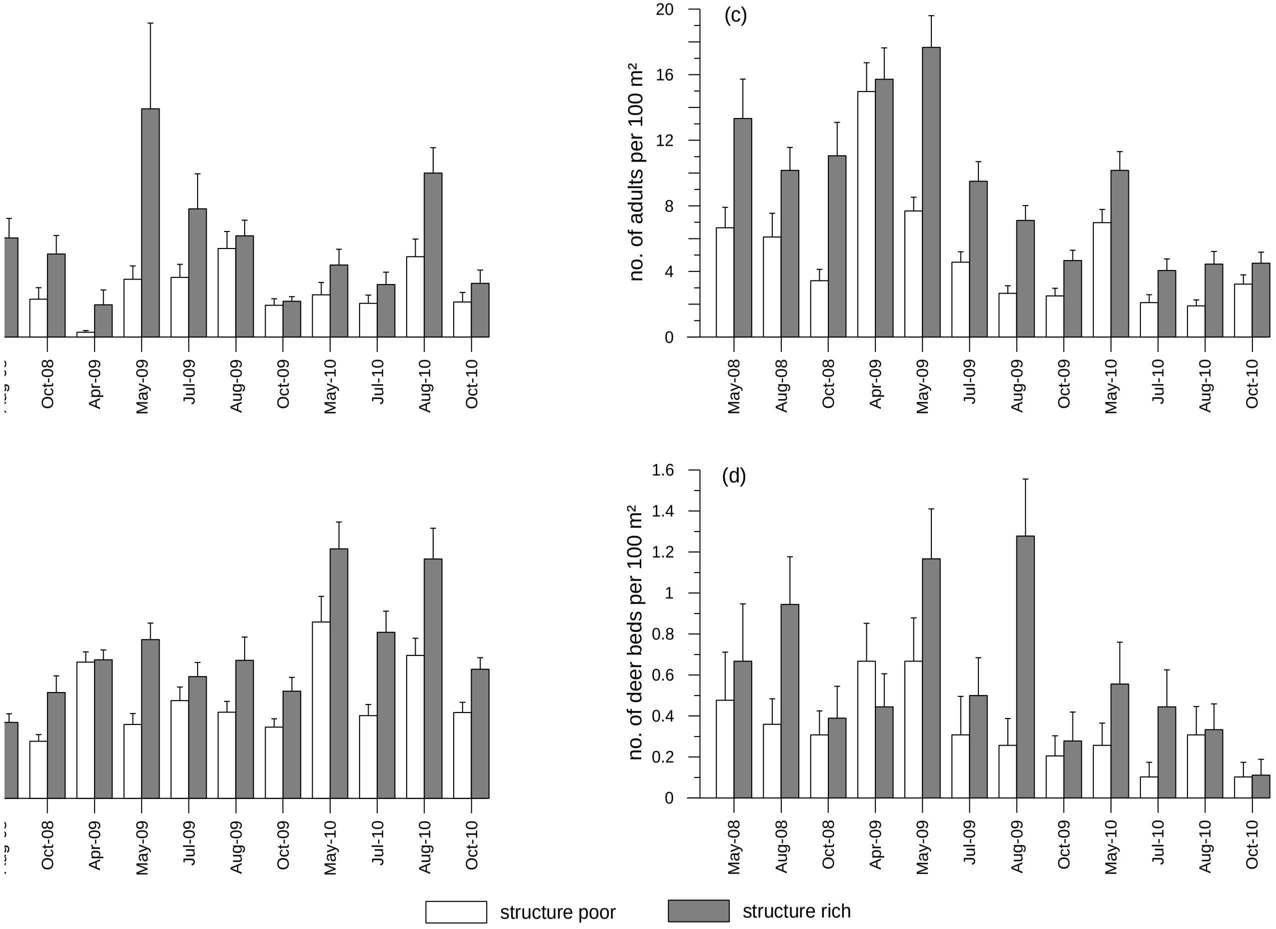


Fig. 4.1 Mean number of *Ixodes ricinus* larvae, nymphs, and adults (a–c) and mean number of deer beds (d) in pine and oak stands between May and October in three successive years. The results from the two forest sites were pooled. Error bars denote the standard error of the mean. Note the difference in values on the y-axis.

Nymphs were active throughout the study period without displaying a clear peak (Fig. 4.1b). Adult tick abundance peaked in spring (April–May) each year and steadily declined in summer (Fig. 4.1c). Our data were not suited to study seasonal variation in tick abundance, but our results are in line with those of Gassner *et al.* (2011), who examined the temporal dynamics of *I. ricinus* in a neighboring country, the Netherlands.

For both larvae and adults, the best model explaining the variation in tick abundance included tree species and shrub cover as explanatory variables (Table 4.2). For adults, a second closely competing model also included a tree species by year interaction term. The best model for nymphs included tree species, shrub cover, and year, whereas the second best model included only tree species and year (Table 4.2).

Table 4.2 Model selection statistics for the analyses of effects of tree species (T), shrub layer cover (S), and year (Y) on the abundance of *Ixodes ricinus* larvae, nymphs, and adults and on the presence of deer beds. ΔAIC_C: the difference in values of the corrected Akaike Information Criterion (AIC_C) between a model and the best model having the lowest AIC_C value; *w*: Akaike weight, indicating relative support for the model.

Response	Model	df	ΔAIC_C	*w*
Larvae	T + S	7	-	0.630
	T + S + Y	9	2.31	0.198
	T	6	2.60	0.172
Nymphs	T + S + Y	9	-	0.684
	T + Y	8	1.54	0.316
Adults	T + S	7	-	0.490
	T × Y + S	11	1.17	0.273
	T + S + Y	9	2.75	0.124
	T	6	2.94	0.113
Deer beds	T + S + Y	8	-	0.311
	T + S	6	0.42	0.253
	T × S + Y	9	2.04	0.112
	T × S	7	2.43	0.092
	T × Y + S	10	3.00	0.070
	T + Y	7	3.13	0.065
	T	5	3.55	0.053
	T + S × Y	10	3.89	0.044

Herb cover did not appear in any of the top models. Dominant tree species, on the other hand, was present in all top models of each life stage and was therefore the variable with the highest relative importance in explaining the abundance of larvae, nymphs, and adult ticks (Table 4.3). The temporal fluctuations in tick abundance were very similar in oak and pine stands, but the mean abundance was consistently higher in the oak stands (Fig. 4.1a–c; Table 4.4). Larvae, nymphs, and adults were on average 3.3, 1.6, and 1.5 times more abundant in the oak stands.

Shrub cover was also a variable of high relative importance (Table 4.3) and had a positive effect on tick abundance (Table 4.4). On each sampling occasion, the mean number of ticks collected was higher in forest stands with high shrub cover compared to stands with low shrub cover. Overall, the number of larvae, nymphs, and adults was 2.1, 1.5, and 1.8 times higher in forest stands with high shrub cover (> 50 % cover) (Fig. 4.2a–c). Hence, mean tick abundance was lowest in pine stands with low shrub cover (43.3 ± 8.2 larvae, 20.7 ± 2.0 nymphs, and 3.8 ± 0.5 adults per 100 m^2) and highest in oak stands with high shrub cover (418.9 ± 72.2 larvae, 61.6 ± 4.9 nymphs, and 11.1 ± 1.0 adults per 100 m^2) (Fig. 4.2a–c).

Table 4.3 Relative importance of each explanatory variable, calculated across all top models ($\Delta AIC_C \leq 4$, see Table 4.2) in which the variable appeared.

Variable	Larvae	Nymphs	Adults	Deer beds
Tree species	1.000	1.000	1.000	1.000
Shrub cover	0.828	0.684	0.887	0.882
Year	0.198	1.000	0.397	0.602
Tree species × Shrub cover	0.000	0.000	0.000	0.204
Tree species × Year	0.000	0.000	0.273	0.070
Shrub cover × Year	0.000	0.000	0.000	0.044

A very similar pattern was observed regarding the number of deer beds we encountered during tick sampling (Fig. 4.1d and 4.2d). The best model explaining the presence of deer beds included tree species, shrub cover, and year (Table 4.2), with the first two being the variables with the highest relative importance (Table 4.3). The probability of encountering deer beds was significantly higher in oak stands (n = 288, p = 0.006) and in forest stands with high shrub cover (n = 288, p = 0.015) (Table 4.4). The mean number of deer beds was 1.6 times higher in forest stands with high shrub cover and twice as high in oak stands, which resulted in four times as many deer beds in oak stands with high shrub cover compared to pine stands with low shrub cover.

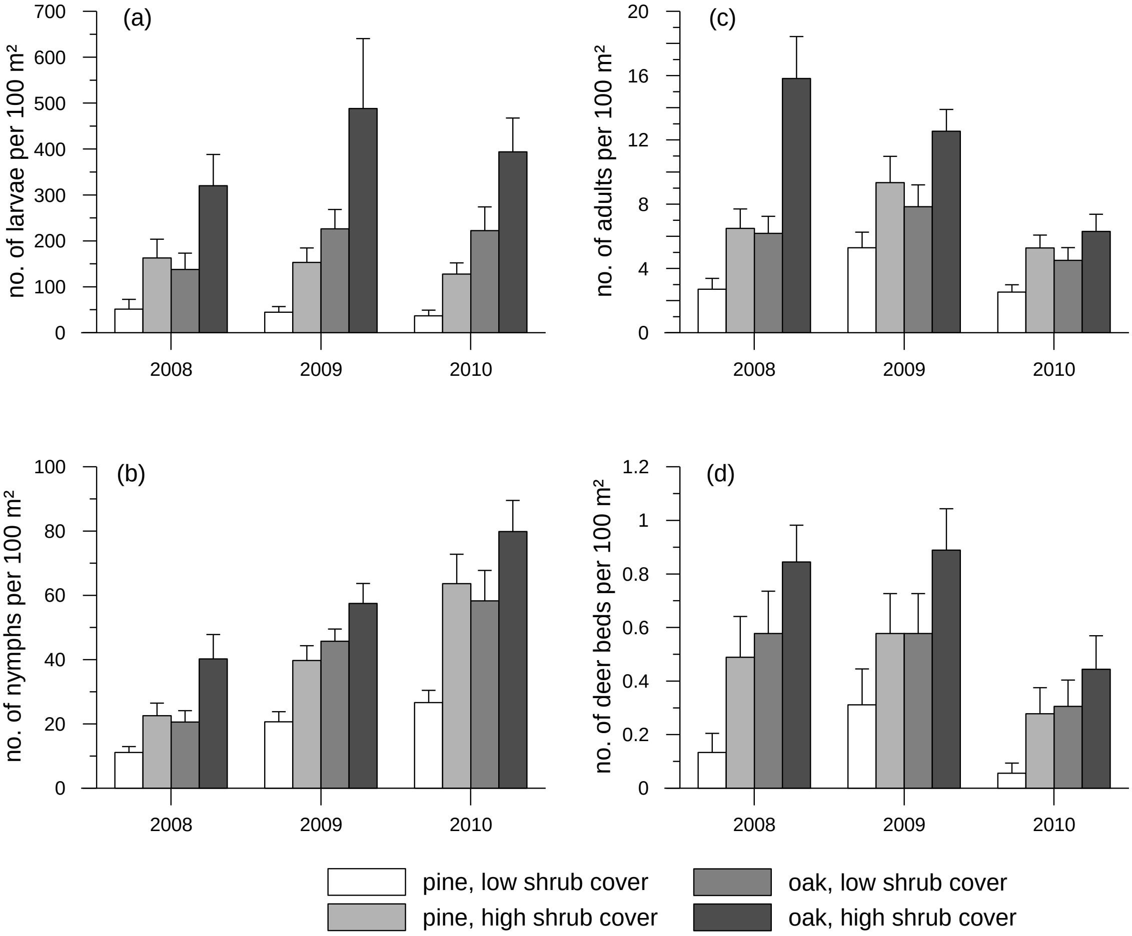


Fig. 4.2 The effects of tree species and shrub layer cover on the number of *Ixodes ricinus* larvae, nymphs, and adults (a–c) and on the number of deer beds (d) in three successive years. Shrub cover estimates were grouped into two classes: low (< 15 %) and high (> 50 %) cover. The results from the two forest sites were pooled. Error bars denote the standard error of the mean. Note the difference in values on the y-axis.

Table 4.4 Parameter estimates (PE) of the best model (see Table 4.2) for the abundance of *Ixodes ricinus* larvae, nymphs, and adults and for the presence of deer beds. A positive effect for tree species means a higher tick abundance or deer presence in oak stands compared to pine stands. A positive effect for the year 2009 or 2010 means a higher tick abundance or deer presence in that year compared to 2008.

	Larvae		Nymphs		Adults		Deer beds	
	PE	t	PE	t	PE	t	PE	Z
Intercept	2.93	8.87	2.33	12.17	1.34	7.27	-0.87	-1.47
Tree species	1.43	5.51	0.60	4.87	0.39	3.59	1.19	2.76
Shrub cover	0.02	4.29	0.01	4.38	0.01	4.73	0.02	2.43
Year 2009			0.59	2.91			-0.59	-0.99
Year 2010			0.87	4.14			-1.45	-2.29

Borrelia-positive nymphs were found each year at sites A en B. The average infection rate with *B. burgdorferi* sl was 8.3 % (95 % confidence interval: 4.8–13.2 %) in 2008, 11.3 % (7.0–16.9 %) in 2009, and 6.2 % (3.4–10.7 %) in 2010. A similar infection rate was observed at both sites in the first two years of our study, but in 2010, the infection rate at site A (1.0 %) was considerably lower compared to site B (12.9 %). No significant difference in infection rate was observed between the structure-poor pine stands and the structure-rich oak stands (n = 120, p = 0.850). The average infection rate was 8.3 % (5.4–12.2 %) in the former and 8.7 % (5.7–12.8 %) in the latter.

4.5 Discussion

Our results show that tree species composition and vertical structure are important variables in explaining tick abundance in forests. The abundance of all three life stages was higher in oak stands compared to pine stands, and was higher in structure-rich compared to structure-poor stands. Interestingly, this pattern was observed at both forest sites and on almost every sampling occasion. So, although some annual and seasonal fluctuations in tick numbers occurred, the mean tick abundance was always lowest in the structure-poor pine stands and almost always highest in the structure-rich oak stands. On average, the abundance of larvae, nymphs, and adults was 9.7, 3.0 and 2.9 times higher in the structure-rich oak stands than in the structure-poor pine stands, while intermediate abundances were recorded in the structure-poor oak stands and the structure-rich pine stands.

The observed differences in tick abundance between the forest stand types must not necessarily depend directly on differences in tree species composition or structure, but may rather be caused by differences in activity of host animals. Our observations from deer bed counts indicate that roe deer were more often present in oak stands and in stands with high shrub cover, most likely because of the availability of high-quality forage and shelter. The importance of deer in maintaining tick populations has been stressed in several European studies (Gray *et al.* 1992, Pichon *et al.* 1999, Ruiz-Fons & Gilbert 2010). Being the most common large mammals in the study area, roe deer probably play an important role in the reproduction of *I. ricinus* and, therefore, their habitat use largely determines the location where engorged female ticks drop off and lay eggs. The immature stages (larvae and nymphs) may also feed on roe deer, but they generally feed on small to medium-sized mammals and birds. Rodents, such as bank vole (*Myodes glareolus* Schreber, 1780) and wood mouse (*Apodemus sylvaticus* Linnaeus, 1758), have been identified by several authors as key hosts for larval ticks (Tälleklint & Jaenson 1997, Humair *et al.* 1999, Estrada-Peña *et al.* 2005). These rodent species, together with other mammal species such as the red fox (*Vulpes vulpes* Linnaeus, 1758) and European hedgehog (*Erinaceus europaeus* Linnaeus, 1758), are common in the study area and provide immature ticks the opportunity to obtain a blood meal and develop into the next life stage, which explains the relatively high nymphal and adult abundances in our study.

Besides being important hosts for immature ticks, small mammals and birds are also important reservoir hosts for *Borrelia* spirochetes. *Borrelia afzelii* has been associated with mice, voles, and red squirrels (*Sciurus vulgaris* Linnaeus, 1758), *B. burgdorferi* sensu stricto with red squirrels, and *B. garinii* and *B. valaisiana* mainly with birds (Humair & Gern 1998, Kurtenbach *et al.* 1998, Humair *et al.* 1999, Hanincová *et al.* 2003a, b). The different genospecies tend to cause distinct clinical manifestations affecting different systems (Van Dam *et al.* 1993) and, thus, the vertebrate host composition will determine not only the density of *Borrelia* infected ticks but also the relative risk of different clinical forms of Lyme borreliosis. We did not identify the *Borrelia* genospecies, which can be considered a shortcoming of our study. However, *B. afzelii* and *B. garinii*, both known to be pathogenic to humans, are the two most common

Borrelia species in Belgium, the Netherlands, and northern France (Rauter & Hartung 2005), which suggests that most larvae feed on small rodents and birds in this region. A study carried out in the Netherlands (Gassner *et al.* 2008) showed a significantly higher nymphal abundance and *Borrelia* infection rate in oak plots than in pine plots, which was ascribed to differences in rodent densities. In our study, however, the nymphal infection rate with *Borrelia* varied substantially for the different forest sites and years, but no significant effect was found for forest type (structure-rich oak *vs*. structure-poor pine). Yet, as the absolute number of ticks was considerably higher in the oak stands and in the stands with an abundant shrub layer, the chance of getting a tick bite while outdoors is influenced by forest type.

4.6 Conclusion

Our results indicate that structure-rich deciduous forests can support higher tick population levels than structure-poor coniferous forests. However, whereas tick abundance was highly affected by tree species and shrub cover, the overall *Borrelia* infection rates in ticks were similar in the two contrasting forest stand types. On the other hand, it is important to note that monospecific pine stands cover most of the area in both study sites, while oak stands, especially those with an abundant shrub layer, are relatively scarce. Large-scale forest conversion programs could change the composition and abundance of wildlife communities to the extent that the relative proportion of reservoir-competent and incompetent hosts changes, thereby influencing not only tick abundance but the infection prevalence in ticks as well. In the past decade, increasing attention has been paid to the role of biodiversity in mediating infection levels and disease, termed the dilution effect (Ostfeld & LoGiudice 2003). The current study underlines the importance of considering spatial heterogeneity in forest habitat quality when studying tick populations. However, further studies will be required in order to fully understand the effects of forest conversion on Lyme borreliosis risk.

[Photograph Wesley Tack]

5 Shrub clearing adversely affects tick abundance

After: Tack W, Madder M, Baeten L, Vanhellemont M, Verheyen K (2013) Shrub clearing adversely affects the abundance of *Ixodes ricinus* ticks. Experimental and Applied Acarology, *in press*

5.1 Abstract

In order to get a better understanding of the importance of vertical forest structure as a component of *Ixodes ricinus* (Linnaeus, 1758) tick habitat, an experiment was set up in a coniferous forest on sandy soils in northern Belgium. Ticks were sampled in six control and six treatment plots on various sampling occasions in 2008–2010. In the course of the study period, a moderate thinning was carried out in all plots and shrub clearing was performed in the treatment plots. Thinning had no effect on tick abundance, while shrub clearing had an adverse affect on the abundance of all three life stages (larva, nymph, adult) up to two years post-clearing. Our findings are especially relevant in the light of the ongoing efforts to improve vertical forest structure in Belgium and many other parts of Europe, which might create suitable habitats for ticks and change the epidemiology of tick-borne diseases. Also, our results indicate that shrub clearing could be applied as a tick control measure in recreational areas where there is a high degree of human-tick contact.

5.2 Introduction

The conversion of coniferous forests into mixed deciduous forests might improve the living conditions for *Ixodes ricinus* (Linnaeus, 1758) ticks (i.e., a more suitable microclimate) and for their main hosts (e.g., increased food resources and shelter). Well-structured, deciduous forests, particularly those containing oak (*Quercus* spp.) and common beech (*Fagus sylvatica* L.), have been shown to provide excellent conditions for the survival and development of *I. ricinus* (Gray 1998, Gray *et al.* 1998, Chapter 3, Chapter 4). Recent observational studies in western Europe showed that both tick abundance and disease dynamics are influenced by the tree species composition and the vertical stratification of forests, which was mainly attributed to the habitat preferences of the ticks' main hosts such as roe deer and rodents (Rizzoli *et al.* 2009, Chapter 4). Experiments manipulating the structural attributes of forests may further increase our understanding of how the changes in forest management may influence tick abundances. Information on the effects of habitat modification, especially the removal of leaf litter, herbaceous vegetation, and shrubs, is available for North America (Wilson 1986, Schulze *et al.* 1995), but inadequate research has been conducted on the effects on *I. ricinus* ticks in European forests.

Here we present the results of a shrub-removal experiment in a coniferous forest on sandy soils in northern Belgium. We compared the relative tick abundance of all three *I. ricinus* life stages in shrub-cleared plots and adjacent controls before and one and two years after the treatment. We hypothesize that the establishment of a shrub layer as a component of the conversion of coniferous forest increases the incidence of ticks.

5.3 Materials and methods

5.3.1 Study area and experimental setup

The study was conducted in one of the two forests at site B, situated in the municipality Herselt in the Campine region (see Fig. 1.5 in section 1.5). Six mature, homogeneous stands of Corsican pine (*Pinus nigra* Arnold subsp. laricio (Poiret) Maire) with a well-developed shrub layer (> 50 % shrub layer cover) were chosen for our study. These forest stands were at least 450 m apart and located within a radius of 1.5 km of each other. The shrub layer mainly consisted of black cherry (*Prunus serotina* Ehrh.), alder buckthorn

(*Frangula alnus* Mill.), juneberry (*Amelanchier lamarckii* F. G. Schroed.), and rowan (*Sorbus aucuparia* L.). The herbaceous vegetation was dominated by either wavy hair grass (*Deschampsia flexuosa* (L.) Trin.), purple moor grass (*Molinia caerulea* (L.) Moench), broad buckler fern (*Dryopteris dilatata* (Hoffm.) A. Gray), or bilberry (*Vaccinium myrtillus* L.). In each forest stand, two 65 m × 65 m square plots (one control and one treatment plot) were established for tick sampling. In the winter of 2008–2009, all shrubs were removed in the six treatment plots. As an integral part of the forest management, a moderate thinning of canopy trees was carried out in the winter of 2008–2009 in three of these stands. The other three stands were thinned in 2009–2010. Special care was taken to minimize damage to the shrub layer in the control plots. Logging slash was removed by hand from all plots.

5.3.2 Tick sampling

In each plot, questing *I. ricinus* ticks (larvae, nymphs, and adults) were drag-sampled in 2008 (May, August, and October), 2009 (April, May, July, August, and October), and 2010 (May, July, August, and October): 12 sampling occasions in total. Six 1 min blanket drags were performed at random in each plot at each sampling occasion. For a more detailed description on tick sampling and identification see section 3.3.3.

5.3.3 Temperature measurements

Temperature was recorded at a height of 10 cm (T_{10}) and 125 cm (T_{125}) above ground level (at intervals of 15 min) inside the control and adjacent treatment plots in four forest stands, of which two stands were thinned in 2008 and two were thinned in 2009, using Decagon EHT Humidity/Temp sensors connected to Decagon Em50 dataloggers (Decagon Devices Inc., Pullman, WA, USA). Due to sensor failure and data logger malfunctioning, we only have temperature data from February 5 to June 30, 2009.

5.3.4 Statistical analysis

Questing tick abundance was expressed as the number of ticks collected per 100 m² ground surface. For each forest stand and each sampling occasion, we computed an effect size as the natural log of a response ratio, i.e., tick abundance in the treatment plot divided by tick abundance in the control plot (Hedges *et al.* 1999).

Since tick abundance was sometimes zero, an adapted effect size was calculated as

$$\mathrm{E} = \ln\left[\frac{\mathrm{A_{TREATMENT}}+1}{\mathrm{A_{CONTROL}}+1}\right] \qquad (5.1)$$

with E the adapted effect size and A the abundance of larvae, nymphs, or adults in the treatment or control plots. Effect sizes were then analyzed with linear mixed models with year of sampling (2008, 2009, and 2010), year of thinning (2008 *vs*. 2009), and their interaction as fixed effects and with forest stand identity as a random effects term to account for the repeated measurements in the same stands. Each life stage (larva, nymph, adult) was analyzed separately. Temperature data were analyzed with ANOVA. The statistical analyses were conducted in R 2.13.0 (R Development Core Team 2011).

5.4 Results

In February–June 2009, T_{10} was significantly lower in the treatment plots than in the controls ($F = 33.1$, $p < 0.001$), but no significant difference was found for T125 ($p > 0.9$). The overall mean T_{10} was 0.39°C lower in the treatment plots (Table 5.1). The difference in T_{10} between treatment and control plots decreased steadily from -0.67°C in February to -0.26°C in June and was mainly caused by lower night-time temperatures in the treatment plots.

In total, 35,356 *I. ricinus* ticks were collected, of which 27,344 were larvae, 7018 were nymphs, and 994 were adults (530 males and 464 females). All three life stages were active on each sampling occasion. The mean ± standard error of the number of ticks collected per 100 m² ground surface was 126.6 ± 10.9 larvae (range 0–720), 32.5 ± 2.4 nymphs (range 2–184), and 4.6 ± 0.3 adults (range 0–20). Figure 5.1 depicts mean tick abundances in the control and treatment plots in the three consecutive sampling years. In 2008, before shrub clearing and thinning, the abundances of ticks were similar in the control and treatment plots. In 2009 and 2010, however, tick abundances in the control plots were higher than in the treatment plots, suggesting a negative effect of shrub clearing on ticks. Compared to the controls, the overall numbers of larvae, nymphs, and adults in the treatment plots were respectively 1.5, 2.2, and 2.3 times lower in 2009 and 1.4, 3.2, and 2.5 times lower in 2010. This finding was statistically confirmed by the linear mixed effects models, which revealed significant effects of sampling year on effect sizes (Table 5.2), but not for year of thinning ($p > 0.2$) or their interaction term ($p > 0.07$).

Table 5.1 Overall and monthly mean air temperature at a height of 125 cm (T_{125}, °C) and 10 cm (T_{10}, °C) above ground level in the control plots between February 5 and June 30, 2009 (*left*). For the treatment plots, overall and monthly mean differences in T_{125} and T_{10} versus the control plots are shown (*right*), with positive numbers denoting a higher temperature in the treatment plots.

	Control		Treatment	
Month	T_{125}	T_{10}	T_{125}	T_{10}
February	4.22	4.33	-0.13	-0.67
March	6.81	6.81	-0.07	-0.49
April	12.62	12.50	0.04	-0.32
May	14.35	14.27	0.07	-0.26
June	15.01	14.95	0.05	-0.26
Overall	**10.71**	**10.67**	**-0.01**	**-0.39**

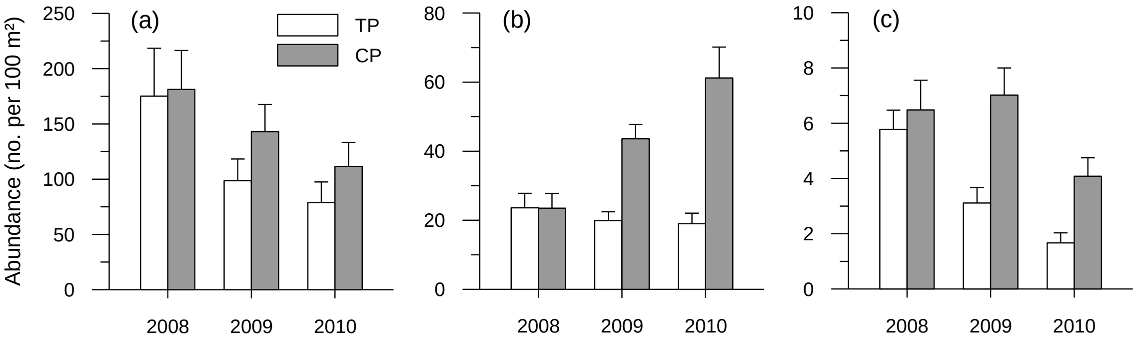


Fig. 5.1 Abundances of questing (a) larval, (b) nymphal, and (c) adult *Ixodes ricinus* ticks (mean ± SE) in the control plots (CP) and treatment plots (TP) in 2008–2010. Note the difference in values on the y-axis.

The effect sizes of larvae, nymphs, and adults were clustered around zero on each sampling occasion in 2008, while negative values — depicting a lower abundance of ticks in the treatment plots — were observed on most occasions in 2009 and 2010 (Fig 5.2). The overall mean ± standard error of the effect size for larvae, nymphs, and adults was respectively -0.50 ± 0.15, -0.88 ± 0.11, and -0.63 ± 0.11 in 2009 and -0.94 ± 0.37, -1.13 ± 0.12, and -0.63 ± 0.11 in 2010.

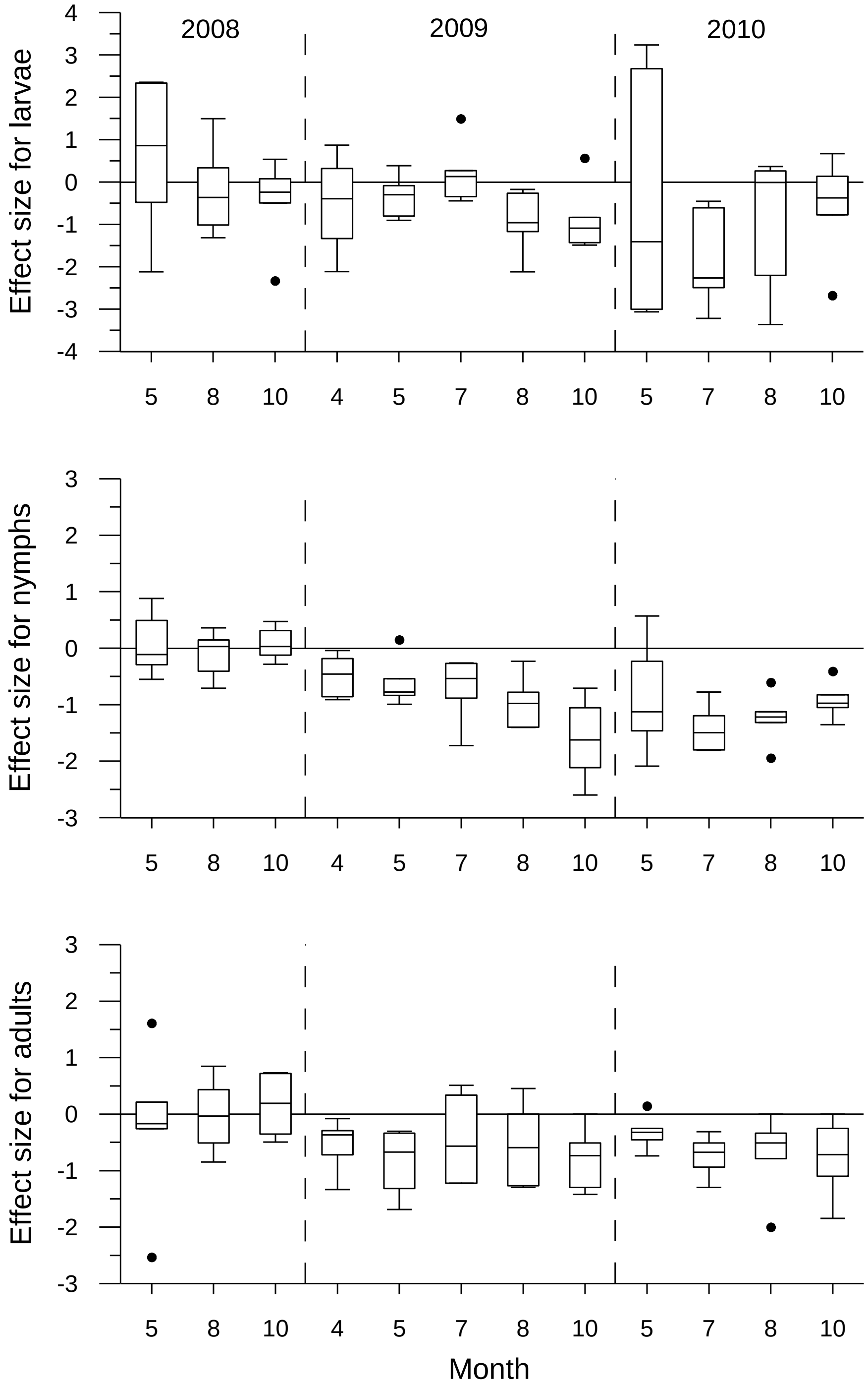


Fig. 5.2 Effect sizes (mean ± SE) of shrub clearing in winter 2008 on the abundance of *Ixodes ricinus* larvae, nymphs, and adults on each of the sampling occasions in 2008–2010. The effect size is calculated with Eq. 5.1.

Table 5.2 Outcome of the general linear mixed model with effect size as the log ratio of tick abundance in treatment versus control plots for *Ixodes ricinus* larvae, nymphs, and adults as response variables (n = 72 for each life stage) and with year as explanatory variable. A negative effect for year (2009 or 2010) means a smaller effect size compared to 2008. Bold: $p < 0.05$.

	Larvae		Nymphs		Adults	
	t	p	*t*	p	*t*	p
Intercept	-0.03	0.973	0.04	0.970	-0.22	0.824
Year 2009	-1.23	0.224	-5.21	**<0.001**	-3.09	**0.003**
Year 2010	-2.31	**0.024**	-6.39	**<0.001**	-2.95	**0.004**

5.5 Discussion

In Chapter 4, we found that tick abundance was lower in homogeneous Corsican pine plots with low (< 15 %) shrub cover compared to pine plots with high (> 50 %) shrub cover. The present study was carried out to experimentally verify the importance of a well-developed shrub layer on *I. ricinus* tick populations, which is especially relevant to policy makers considering the ongoing efforts to improve vertical forest structure. The present experimental study and the observational study of Chapter 4 were carried out in the same forest area and in plots with similar canopy characteristics. In both chapters, sampling was carried out on the same sampling occasions under similar weather conditions using the same tick sampling methodology.

In 2008, when all control and treatment plots had a shrub cover > 50 %, tick abundance estimates in these particular plots were at the same level, as indicated by effect sizes clustered around zero (Fig. 5.2). Tick abundances in these control and treatment plots were, however, higher compared to the pine plots with low shrub cover of Chapter 4: mean tick abundance in the control and treatment plots combined was 2.3, 3.3, and 2.7 times higher for larvae, nymphs, and adults, respectively. In 2009 and 2010, tick abundance remained relatively high in the controls while it decreased in the cleared treatment plots to a level equivalent to the structure-poor pine plots of Chapter 4, suggesting a negative effect of the removal of the shrub layer on tick populations. Indeed, statistical analysis showed that shrub clearing in winter resulted in a significant suppression of all three life stages of *I. ricinus* in the two following years. This could be related to a deterioration of local microclimatic conditions due to shrub clearing. Several studies have shown that the spatial and temporal variation in tick abundance is strongly associated with temperature, relative humidity, and vegetation type (e.g., Ruiz-Fons *et al.* 2012). In temperate regions, *I. ricinus* ticks have highly seasonal population dynamics as the winter temperatures are too low to allow interstadial development and questing activity. A temperature threshold of 7°C has been proposed as a prerequisite for tick activity in spring (Perret *et al.* 2000). Ticks are also sensitive to desiccation and require a relative humidity exceeding 80 % at the base of the vegetation throughout the year (Kahl & Knülle 1988). Shrub clearing is likely to modify the microclimatic conditions, which could be detrimental to ticks. We found significant lower T_{10} values in the treatment plots. However, the temperature difference between control and treatment plots was highest in the winter months, when ticks were not actively questing. Considering the fact that ticks are very cold-hardy and can survive 24-hour exposure to temperatures below -14°C (Dautel & Knülle 1997), the observed temperature difference was probably too small to significantly affect tick survival. We think a more plausible explanation for our results is that shrub clearing has affected local host density. For instance, bank vole, the predominant rodent species in the study area (Chapter 6), is a key host for immature ticks and has been shown to prefer forests with high tree and shrub cover (Hille & Mortelliti 2010). The shrub clearing might have reduced bank vole abundances, thus influencing the feeding success of immature ticks and reducing tick abundance in the treatment plots.

5.6 Conclusion

The results of this study indicate that the presence of a well-developed shrub layer indeed has a beneficial effect on tick populations. This means that forest management may strongly influence the suitability of habitats for ticks. Our findings indicate that shrub clearing can locally suppress tick populations and, thus, the probability of getting a tick bite up to two years post-clearing. Therefore, shrub clearing is a potentially important component of an integrated tick control strategy, especially in areas used for recreational activities such as campsites, picnic areas, and paths, which opens a promising avenue for further investigation.

[Photograph Gert Arijs]

6 The influence of acorn mast on rodents and their tick parasites

After: Tack W, Heyman P, Cochez C, Baeten L, Bonte D, De Blander E, Madder M, Vanhellemont M, Verheyen K (2012) The influence of acorn mast on rodents and their tick parasites.Ticks and Tick Borne Diseases, *submitted*

6.1 Abstract

The production of large seed crops could be a primary determinant of variation in tick abundance and disease incidence by influencing the population dynamics of rodents, as rodents are important feeding hosts for immature ticks and reservoirs for many human pathogens. We studied (i) the numerical response of wood mouse (*Apodemus sylvaticus* Linnaeus, 1758) and bank vole (*Myodes glareolus* Schreber, 1780) after a seed-addition experiment and a mast year and (ii) the association between these rodent species and both attached and questing ticks. Rodents were live-trapped, attached ticks were collected from captured rodents and questing ticks were collected by drag sampling. The rodents mainly carried larval and nymphal *Ixodes ricinus* (Linnaeus, 1758) and secondarily *I. trianguliceps* (Birula, 1895). Wood mice harbored greater numbers of larvae and nymphs than bank voles, and males carried a higher larval burden than females. The abundance of both rodent species increased after the seed-addition experiment and the mast year, although the effect was significant only for bank voles. The abundance of questing *I. ricinus* nymphs depended on the abundance of both rodent species and of larvae in the prior year. These lagged responses appeared to be more important than local vegetation composition and structure. Thus, rodent abundance and the abundance of questing *I. ricinus* nymphs showed a lagged response to acorn mast and might as such impact disease dynamics. Data on seed production will allow better understanding of spatial and temporal variation in tick abundance, and better planning of prevention and control programs. Finally, forest management may strongly influence the epidemiology of numerous tick-borne diseases by favoring certain mast-producing tree species.

6.2 Introduction

Over the last decades, the emergence and spread of new infectious disease agents has become a prominent topic on both the medical and political agendas. Up to 75 % of emerging human diseases have been identified as zoonotic in origin (Taylor *et al.* 2001), with rodents being among the most important sources of pathogens threatening human health and well-being (Meerburg *et al.* 2009). The hantaviruses (genus Hantavirus, family Bunyaviridae) and arenaviruses (family Arenaviridae), for instance, are strongly associated with murid rodents. These haemorrhagic fever viruses, which can be transmitted from rodents to humans via direct contact, are globally widespread and are the cause of considerable human morbidity and mortality (Mills 1999). Especially tick-borne diseases for which rodents serve as reservoir hosts, such as Lyme borreliosis and tick-borne encephalitis (see section 1.2), have received much attention of the media and the scientific community in recent years. Other tick-borne pathogens for which rodents serve as reservoirs include, e.g. the bacterium *Anaplasma phagocytophilum* and *Babesia* protozoa that cause human granulocytic anaplasmosis and babesiosis, respectively (Meerburg & Kijlstra 2009).

Because rodents play an important role in the spread of numerous human pathogens, variation in disease incidence may be related to rodent population dynamics. Previous studies have suggested that mast seeding — the intermittent and synchronous production of large seed numbers — may be a useful predictor of increased risk of diseases such as hantavirus infection (Tersago *et al.* 2009) and Lyme borreliosis (Ostfeld *et al.* 2001) through changes in rodent abundance. Host-seeking *Ixodes scapularis* (Say, 1821) nymphs and the abundance of *Borrelia*-infected nymphs were correlated with rodent abundance in the prior year and with acorn production two years earlier in US forests (Ostfeld *et al.* 2001). In the autumn of a mast year, deer spend more time in oak forests feeding on acorns and import attached adult ticks into these forests, which results in a higher abundance of larvae the following summer (Jones *et al.* 1998). Furthermore, the production of large acorn numbers results in a higher abundance of rodents the following summer, which are not only important feeding hosts of larvae but also important reservoirs for *Borrelia* spirochetes. The concurrence of the increased larval and rodent abundance appears to increase the abundance of infected nymphs in the next year (Ostfeld *et al.* 2001). Similar results were described in a Russian study by Korenberg *et al.* (2002). They found that the density of host-seeking *Ixodes persulcatus* (Schulze, 1930) nymphs and their infection prevalence with *B. burgdorferi* are positively correlated with rodent abundance during the previous year.

Whether similar trophic links exist in western Europe, where *Ixodes ricinus* (Linnaeus, 1758) is the most common and widespread tick vector of zoonotic diseases, remains unclear. A better understanding of these trophic relations is, however, a prerequisite for interpreting and predicting the effects of our changing environment on disease incidence. In many European countries, the forest policy aims at converting monospecific coniferous forests into deciduous forests dominated by native species such as oak. Yet, oak stands have been shown to support higher tick population levels than pine stands, which is thought to be related to the habitat preferences of the main tick hosts (Chapter 3 and Chapter 4). Furthermore, climate change is expected to cause altered seed production dynamics (Verheyen *et al.* 2010) and significant changes in tree species richness and forest community types (Iverson & Prasad 2001). The expected changes in forest composition, structure, and seed production may subsequently affect the spatial and temporal patterns in population densities of seed-consuming rodents and their tick parasites, which might cause significant changes in infectious disease levels. However, currently, we do not sufficiently understand the complex relationships between food resources, rodent hosts, pathogens, and ticks to be able to accurately predict changes in the distribution and incidence of infectious diseases.

This chapter reports on a three-year study of the relationship between rodents and their food resources (acorns), and between rodents and tick populations. The aim of our study was to determine whether the occurrence of a mast event can result in a significant increase in abundance of questing *I. ricinus* nymphs (the life stage predominantly responsible for pathogen transmission to humans) through an increase in rodent abundance. We examined (i) the population responses of two rodent species, wood mouse (*Apodemus sylvaticus* Linnaeus, 1758) and bank vole (*Myodes glareolus* Schreber, 1780) after a seed-addition experiment (in a very poor seed year) and after a very good seed year and (ii) the relationship between the abundances of immature *I. ricinus* ticks and their rodent hosts. We also took blood samples of wood mouse and bank vole to evaluate the presence of hantaviruses and *A. phagocytophilum* in local rodent populations.

6.3 Materials and methods

6.3.1 Study area

The study was conducted in the two forests at site B, situated in the Campine region (see Fig. 1.5 in section 1.5). Six stands dominated by Corsican pine (*P. nigra* Arnold ssp. *laricio* (Poiret) Maire) and six stands dominated by pedunculate oak (*Quercus robur* L.) were selected for our study. The percentage cover of the tree layer (height > 7 m), shrub layer (1–7 m), and herb layer (< 1 m) was estimated visually in each stand. The relative contribution of *P. nigra* or *Q. robur* to the total estimated tree canopy cover was greater than or equal to 70 % in each pine and oak stand, respectively. The shrub layer mainly consisted of black cherry (*Prunus serotina* Ehrh.), alder buckthorn (*Frangula alnus* Mill.), and juneberry (*Amelanchier lamarckii* F. G. Schroed.) in the pine stands and pedunculate oak, sweet chestnut (*Castanea sativa* Mill.), and rhododendron (*Rhododendron ponticum* L.) in the oak stands. The herbaceous layer was dominated either by wavy hair grass (*Deschampsia flexuosa* (L.) Trin.), purple moor grass (*Molinia caerulea* (L.) Moench), broad buckler fern (*Dryopteris dilatata* (Hoffm.) A. Gray), or bilberry (*Vaccinium myrtillus* L.). In each forest stand, a 49 m × 49 m square plot was established for sampling ticks and small mammals. These plots were at least 75 m apart. In 2008, we took advantage of the low acorn production to carry out an acorn-addition experiment in half of the pine and half of the oak plots, while the remaining six pine and oak plots served as control plots. Approximately 200 kg of acorns of *Q. robur* were distributed in the autumn of 2008 in each of the experimental plots (*ca.* 800 kg.ha^{-1}). In a good mast year, seed production can amount to over 600 kg.ha^{-1} (Den Ouden *et al.* 2005). The year 2009 was a good mast year, which gave us the opportunity to study the effect of subsequent natural mast seeding on rodent abundance.

6.3.2 Sampling of questing ticks

Questing ticks were drag-sampled in 2008 (May, August, and October), 2009 (April, May, July, August, and October), 2010 (May, July, August, and October), and 2011 (May, June, August, and September): 16 sampling occasions in total. Six 1 min blanket drags (each extending a distance of *ca.* 25 m) were performed at random in each plot at each sampling occasion. At each sampling occasion, all plots were sampled within a period of 5–10 days and in random order to avoid time of day and changing meteorological conditions as a source of bias. All three life stages were sampled, except in 2011 when only nymphs and adults were collected due to time constraints. For a more detailed description on tick sampling and identification, see section 3.3.3.

6.3.3 Sampling of small mammals and attached ticks

Trapping sessions were performed in each plot in September 2008 (before acorn addition), September 2009 (the year after the acorn addition), and September 2010 (the year after the mast event). Each trapping session consisted of a prebaiting period of two days followed by four consecutive nights of trapping, during which the traps were checked two or three times per night. Forty-nine live traps were placed in a 7 × 7 grid with 7 m between the traps (Trip-Trap live traps, Procter Brothers Ltd, Pantglas Industrial Estate, Bedwas, Caerphilly, UK). Thus, small mammals were captured in a 49 m × 49 m plot. The original plastic nest box of each trap was replaced by a larger wooden nest box (6 cm × 7 cm × 18 cm) to reduce stress in captured animals (Fig. 6.1). Hay was supplied as nesting material, and a mixture of peanut butter and muesli was used as bait. Captured animals were identified to species and released at the point of capture immediately after being marked by fur clipping.

The mark-recapture data were used to estimate rodent abundance in each plot as described by Schnabel (1938), which is a modified Lincoln-Peterson estimator that accounts for multiple trapping occasions (Seber 1982). The abundance of rodents was calculated as

$$\mathrm{N} = \frac{\sum_{\mathrm{t}}[\mathrm{C_t} \times \mathrm{M_t}]}{\sum_{\mathrm{t}} \mathrm{R_t}} \qquad (6.1)$$

with N the estimated population, C_t the number of individuals caught in the sample, M_t the number of marked individuals in the population just before sample t, and R_t the number of recaptures in the sample. When densities were too low to calculate rodent abundance we used the number of captured individuals instead.

After the final nighttime trapping session in 2010, the live traps in all 12 plots were baited and checked the next morning. A total of 123 mature, non-pregnant rodents was selected for further study: 30 wood mice (6 females and 24 males) and 93 bank voles (24 females and 69 males). The animals were euthanized with an overdose of isoflurane, and blood samples were taken from the heart. Subsequently, the animals were examined for ticks (Fig. 6.1). Attached ticks were removed from the head, ears, and neck (typical attachment sites of ticks), counted and identified to species level and developmental stage. The abundance of attached ticks was expressed as the number of ticks attached per individual host animal.

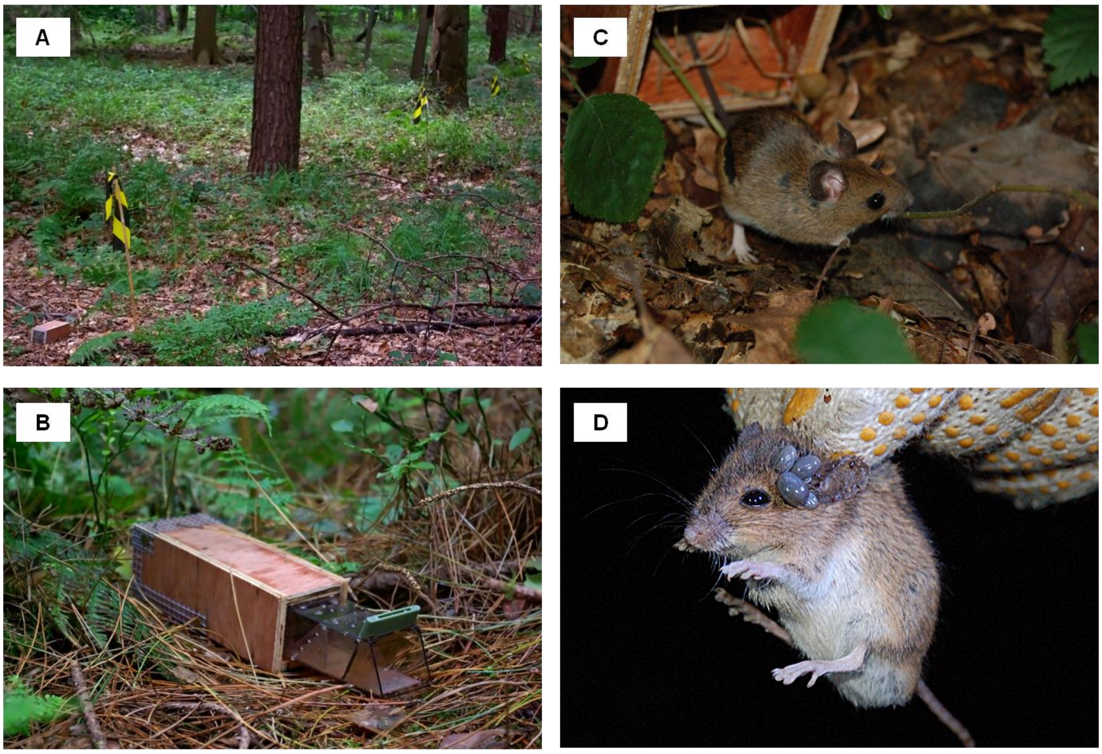


Fig. 6.1 Trapping of small mammals. A, 49 traps were placed in a 7 × 7 grid (with 7 m between the traps) and marked with bamboo sticks; B, Trip-Trap live trap with a wooden nest box; C, animals were marked by fur clipping; D, attached ticks were removed from the head, ears, and neck of the animals [Photographs: A–B Wesley Tack; C–D Diemer Vercayie].

6.3.4 Detection of pathogens

Whole blood from the 123 rodents was captured onto filter paper cards (Whatman FTA cards, Whatman International Ltd, Maidstone, Kent, UK) and stored at -20°C until testing. The blood was then eluted from the filter paper using 500 µl of phosphate-buffered saline solution (PBS). The eluates were analyzed with a commercial immunoglobulin G (IgG) PUUV and Hantaan (HTNV) ELISA test adapted to rodents with a goat anti-mouse IgG peroxidase-labeled conjugate (Sigma, St. Louis, MO, USA) and with a commercial *A. phagocytophilum* IgG immunofluorescence assay (IFA) (Focus Diagnostics, Cypress, CA, USA) with a goat anti-mouse IgG FITC-labeled conjugate (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). Both assays were applied following the manufacturer's instructions.

6.3.5 Statistical analysis

All statistical analyses were conducted in SPSS 15.0 (SPSS 2006) and R 2.13.0 (R Development Core Team 2011). Only the data on wood mouse and bank vole were analyzed because they were the two most abundant rodent species in the study area and because they have been identified by several authors as key hosts for larval ticks (Tälleklint & Jaenson 1997, Kurtenbach *et al.* 1998). Furthermore, unlike the insectivorous shrews, wood mouse and bank vole were expected to benefit directly from a surplus of acorns as they depend on tree seeds for a substantial part of their diet, especially in autumn (Watts 1968).

To test for the effect of the acorn-addition experiment on the abundances of wood mouse, bank vole, or their sum (Rodents = wood mice + bank voles), we applied linear mixed models with treatment (experimental *vs*. control plots), tree species (oak *vs*. pine), year (2008 *vs*. 2009), and all two-way interaction terms as fixed effects. Plot identity was included as a random effects term to account for the repeated measurements on the same permanent plots. To test the effect of the natural mast event on the abundance of wood mouse, bank vole, or their sum (Rodents), we only used the data from the control plots as they were not influenced by the acorn addition treatment in 2008. We applied linear mixed models with tree species (oak *vs*. pine), year (2009 *vs*. 2010), and their interaction as fixed effects and with plot identity as a random effects term.

Questing tick abundance was expressed as the number of ticks collected per 100 m² ground surface. The abundances of questing nymphs in the different sampling years were averaged to obtain a single estimate per plot per year. Subsequently, nymphal abundance was modelled with generalized linear mixed models (GLMM) with a Poisson error distribution and a logit link function. Plot identity was included as a random effects term. First, we determined whether to use the current or prior year's abundances of wood mouse and bank vole as explanatory variables. This was tested for 2009 and 2010, the years for which we had data on both the current and prior year's abundances of rodents. We used the Akaike Information Criterion, adjusted for sample size (AIC_C) (Hurvich & Tsai 1989), to select the model with the highest probability of matching our data (smallest AIC_C). Next, we constructed models based on the entire dataset, which included main effects of tree species (oak *vs*. pine), shrub cover (in %), herb cover (in %), the abundances of wood mouse and bank vole in the previous year, and the log-transformed abundances of larvae in the previous year. The latter variable was included because the abundance of nymphs is expected to depend on the larval abundance in the preceding year and the survival of the larval cohort to the nymphal stage (Randolph *et al.* 2002). Again, AIC_C was used to compare all possible competing models.

The data on the infestation of rodents with larval and nymphal *I. ricinus* were analyzed with generalized linear models (GLM) with a negative binomial error distribution and a logit link function. These models included rodent species (wood mouse *vs*. bank vole), animal sex, tree species, shrub cover, herb cover, and the interactions between species and the other factors as fixed effects.

6.4 Results

6.4.1 Rodent responses to acorn addition and mast

We captured a total of 1437 individual small mammals of five species (Table 6.1). Overall, bank vole represented 50.7 % of all the captured specimens, wood mouse 37.8 %, pygmy shrew 8.6 %, common shrew 2.6 %, and field vole (*Microtus agrestis* Linnaeus, 1758) 0.3 %. The rodent species wood mouse and bank vole were the most abundant species in each of the 12 plots in each year. The percentage of rodents (numbers of wood mouse and bank vole combined) in the individual plots ranged from 61 to 100 % (mean = 82 %; SD = 14 %) in 2008, from 67 to 100 % (mean = 88 %; SD = 10 %) in 2009, and from 83 to 100 % (mean = 93 %; SD = 5 %) in 2010. Although both rodent species were present in all plots, bank vole occurred especially in areas with a high herbaceous or shrub cover whereas wood mouse also occurred in more exposed areas with little or no cover.

In 2009, one year after the acorn-addition experiment, wood mouse increased in abundance in the control plots (+18.3 %) and in the experimental plots (+121.6 %), whereas bank vole declined in the control plots (-11.2 %) and increased in the experimental plots (+134.9 %) (Fig. 6.2). Consequently, total rodent abundance remained relatively constant in the control plots (+2.7 %) and increased in the experimental plots (+129.5 %). The acorn addition only significantly affected the abundance of bank vole and total rodent abundance, as indicated by a significant interaction between treatment and year (bank vole: $t = 2.8$, 10 df, $p = 0.019$ and Rodents: $t = 3.2$, 10 df, $p = 0.009$). No significant treatment × year interaction was found for wood mouse ($t = 1.3$, 10 df, $p = 0.237$). Tree species and the other interaction terms were not significant ($p > 0.1$).

Table 6.1 Total number and percentage of individual small mammals caught, by species and year

	2008		2009		2010		Total	
Species	No.	%	No.	%	No.	%	No.	%
Rodentia	**252**	**79.0**	**331**	**87.6**	**694**	**93.9**	**1277**	**88.9**
Apodemus sylvaticus	113	35.4	145	38.4	285	38.6	543	37.8
Myodes glareolus	134	42.0	186	49.2	409	55.3	729	50.7
Microtus agrestis	5	1.6	0	0.0	0	0.0	5	0.3
Insectivora	**68**	**21.3**	**47**	**12.4**	**45**	**6.1**	**160**	**11.1**
Sorex minutus	53	16.6	31	8.2	39	5.3	123	8.6
Sorex araneus/coronatus	15	4.7	16	4.2	6	0.8	37	2.6

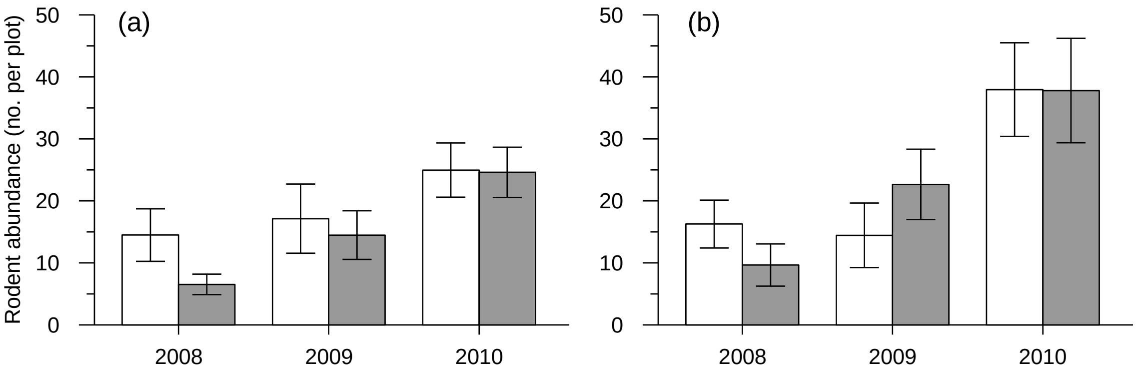


Fig. 6.2 Abundances of (a) wood mouse (*Apodemus sylvaticus*) and (b) bank vole (*Myodes glareolus*) (mean ± SE) in the control (open bars) and experimental plots (filled bars) in 2008–2010. The results from the pine and oak plots were pooled.

In 2010, i.e., the year following the mast event, the abundance of bank vole in the control plots was significantly higher compared to the previous year ($t = 4.7$, 5 df, $p = 0.005$), but this was not the case for wood mouse ($t = 1.1$, 5 df, $p = 0.316$) (Fig. 6.2). Total rodent abundance increased significantly between 2009 and 2010 ($t = 3.7$, 5 df, $p = 0.015$). Overall, wood mouse increased by 45.7 %, bank vole by 162.7 %, and total rodent abundance by 99.2 %. This increase in abundance was observed not only in the oak plots, but in the pine plots as well. No significant effect of tree species and year × tree species interaction term was retrieved ($p > 0.1$).

6.4.2 Questing ticks

A total of 77,079 questing *I. ricinus* ticks was collected: 60,504 larvae in 2008–2010, and 14,519 nymphs and 2056 adults (1124 males and 932 females) in 2008–2011. All three life stages were active on each sampling occasion. The mean ± standard error of the number of ticks collected per 100 m^2 ground surface was 280.2 ± 34.6 larvae (range 0–4263), 50.4 ± 2.7 nymphs (range 1–196), and 7.2 ± 0.5 adults (range 0–48).

The abundance of questing nymphs was better predicted by the prior year's abundances of wood mouse (A_{t-1}) and bank vole (M_{t-1}) as main effects compared to their current year's abundances ($A_t + M_t$). The next best model (with $\Delta AIC_C = 2.12$) included M_t instead of M_{t-1} as a main effect together with A_{t-1}. Whereas M_{t-1} and M_t were significantly correlated ($n = 24$, $r = 0.597$, $p = 0.002$), no significant correlation was detected between A_{t-1} and A_t ($n = 24$, $r = 0.234$, $p = 0.271$). The final model, and all other models with $\Delta AIC_C \leq 4$, included A_{t-1}, M_{t-1}, and L_{t-1} (Table 6.2); these variables had a higher relative importance than the habitat variables: tree species, shrub cover, and herb cover. The abundance of questing nymphs was higher with higher abundances of wood mouse, bank vole, and questing larvae in the previous year (Fig. 6.3). Moreover, the abundances of both larvae and nymphs were higher in the oak plots than in the pine plots (Fig. 6.4) and increased with increasing shrub cover, but these habitat variables were not retained in the final model.

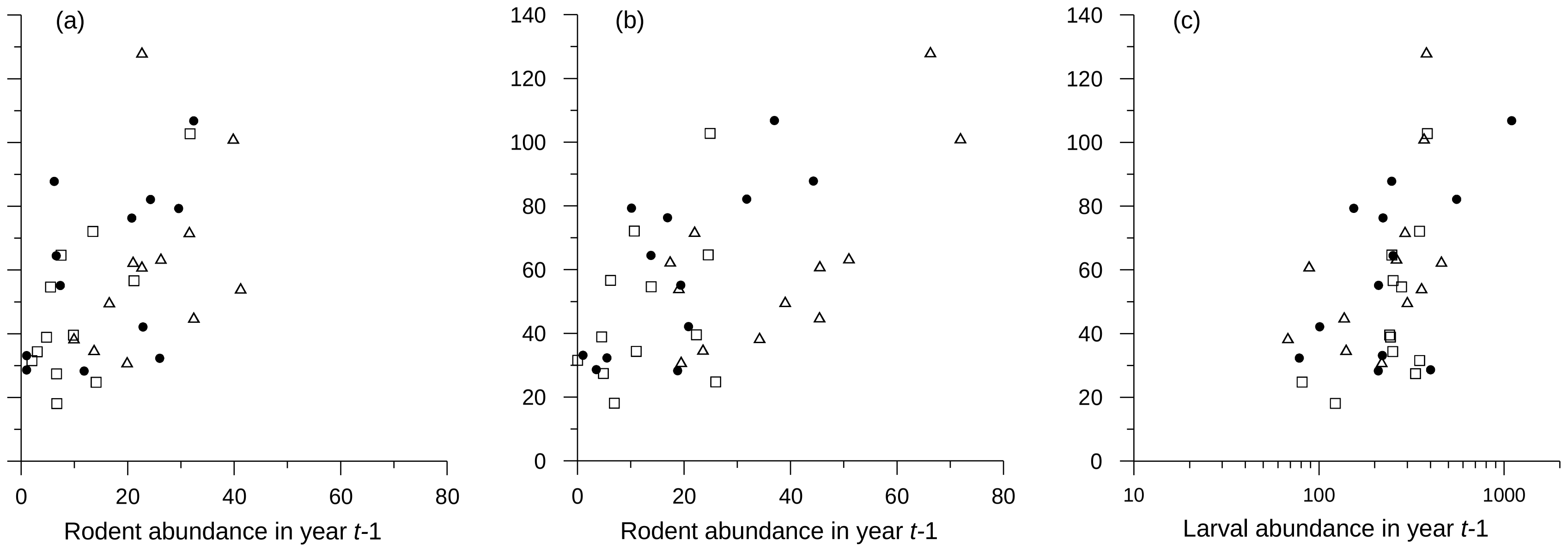


Fig. 6.3 The relationship between the abundance of questing *Ixodes ricinus* nymphs in year *t* and the abundance of (a) wood mouse (*Apodemus sylvaticus*), (b) bank vole (*Myodes glareolus*), and (c) *I. ricinus* larvae in year *t*-1 for *t* = 2009 (open square), 2010 (filled circle), and 2011 (open triangle).

Table 6.2 Outcome of the generalized linear mixed models with the abundance of questing *Ixodes ricinus* nymphs in year *t* (2009, 2010, and 2011) as response variable and with tree species (T), shrub cover (S), herb cover (H), and the abundance of *I. ricinus* larvae (= L), wood mouse (*Apodemus sylvaticus*) (= A), and bank vole (*Myodes glareolus*) (= M) in year *t*-1 as explanatory variables. Each combination of the individual explanatory variables that proved to be equivalent in terms of explanatory power (i.e., $\Delta AIC_C \leq 4$) is reported. *w*: Akaike weight, indicating relative support for the model. See also Fig. 6.3 and Fig. 6.4.

Variables		df	ΔAIC_C	*w*
$A_{t-1} + M_{t-1} + L_{t-1}$		5	-	0.559
$A_{t-1} + M_{t-1} + L_{t-1} + T$		6	2.40	0.168
$A_{t-1} + M_{t-1} + L_{t-1} + S$		6	2.76	0.141
$A_{t-1} + M_{t-1} + L_{t-1} + H$		6	2.89	0.132
Variables	Value	SE	*z*-value	*P*
Intercept	2.408	0.347	6.939	< **0.001**
A_{t-1}	0.009	0.003	3.430	< **0.001**
M_{t-1}	0.006	0.002	3.802	< **0.001**
L_{t-1}	0.229	0.062	3.688	< **0.001**

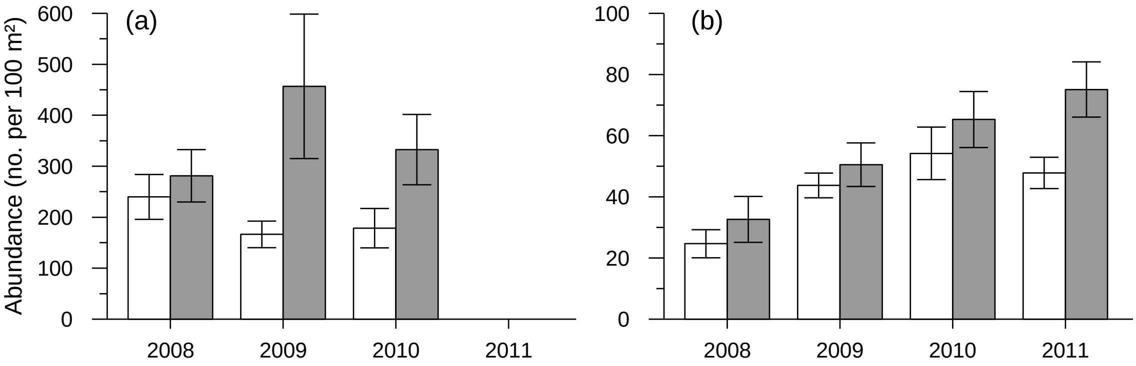


Fig. 6.4 Abundances of questing (a) larval and (b) nymphal *Ixodes ricinus* ticks (mean ± SE) in the pine (open bars) and oak plots (filled bars) in 2008–2011. Note the difference in values on the y-axis.

6.4.3 Tick burden on rodents

A total of 1566 ticks was removed from rodents: 1541 *I. ricinus* (1486 larvae and 55 nymphs) and 25 *I. trianguliceps* (Birula, 1895) (20 larvae, 2 nymphs, and 3 females). *Ixodes ricinus* was found in all plots, whereas *I. trianguliceps* was found in 7 out of the

12 plots. Data on *I. trianguliceps* were excluded from further analysis, since only a few ticks were collected. Furthermore, *I. trianguliceps* is a nest-dwelling tick that is restricted almost exclusively to small mammals and, thus, poses less of a threat to human health than *I. ricinus*. All wood mice and 79.6 % of the bank voles carried *I. ricinus* ticks. The mean ± standard error of the number of immature ticks per rodent host was 30.3 ± 4.7 larvae (range 3–115) and 1.6 ± 0.5 nymphs (range 0–11) for wood mouse and 6.2 ± 1.1 larvae (range 0–69) and 0.06 ± 0.03 nymphs (range 0–2) for bank vole. The overall ratio of larval to nymphal *I. ricinus* was 19:1 for wood mouse and 96:1 for bank vole. The generalized linear model revealed a significant effect of rodent species ($\chi^2 = 39.9$, 1 df, $p < 0.001$) and sex ($\chi^2 = 11.3$, 1 df, $p < 0.001$) on the larval *I. ricinus* burden: wood mouse harbored greater numbers of larvae than bank vole, and males carried more larvae than females (Fig. 6.5a). No significant effect was found for tree species, shrub cover, herb cover or the two-way interactions ($p > 0.08$). Similar results were obtained for the nymphal *I. ricinus* burden (Fig. 6.5b), but the GLM showed a significant effect for rodent species only ($\chi^2 = 35.8$, 1 df, $p < 0.001$).

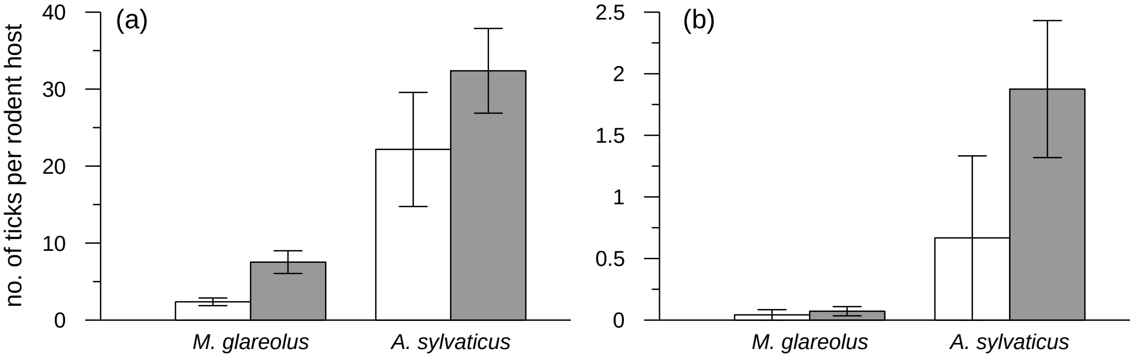


Fig. 6.5 Number of attached (a) larval and (b) nymphal *Ixodes ricinus* ticks (mean ± SE) on female (open bars) and male (filled bars) bank vole (*Myodes glareolus*) and wood mouse (*Apodemus sylvaticus*). Note the difference in values on the y-axis.

6.4.4 Detection of pathogens

In 2010, 123 rodent samples were tested for antibodies: 14 were positive for hantaviral IgG and 17 were positive for *A. phagocytophilum* IgG antibodies. Of the 14 hantavirus-positive rodents, the majority (12) were — as was to be expected —bank voles and only 2 were wood mice. Both infected wood mice and 7 out of the 12 bank voles were males. Wood mouse is considered the main rodent reservoir for *A. phagocytophilum*, but our results showed that 10 out of the 17 positive samples were bank voles, and 7 out of 17 were wood mice. Seven out of the 10 bank voles and 4 out of the 7 wood mice found positive for *A. phagocytophilum* IgG antibodies were males.

6.5 Discussion

Average abundances of both wood mouse and bank vole doubled in experimental plots with acorn addition relative to control plots (irrespective of tree species), although not significantly so for wood mouse. Likewise, in 2010, one year after the large acorn crop, the abundances of both wood mouse and bank vole were higher compared to the year before, but statistically not significant for wood mouse. Populations of wood mouse and bank vole have been shown to experience higher rates of overwinter survival and, occasionally, winter breeding in mast years. Consequently, the following spring, rodent populations begin to grow rapidly, which results in peak densities in the summer or autumn (Watts 1969, Pucek *et al.* 1993). Pucek *et al.* (1993) also found that the effect of a mast year on rodent abundances was clearest on the combined numbers of mice and voles. Furthermore, we found that the abundance of both rodent species increased in numbers not only in the oak plots but in the pine plots as well. Although our data cannot directly address this issue, we believe that rodents dispersed from the oak plots to the surrounding pine plots following acorn depletion. This assumption is supported by the fact that in two plots where hardly any rodents were caught in 2008 and 2009 (fewer than 5 wood mice or bank voles) more than 50 rodents per plot were caught in 2010.

In the second part of this study, we determined the relationship between immature *I. ricinus* ticks and their rodent hosts and whether the abundance of questing nymphs can be predicted from rodent abundances and habitat variables. Most (98.4 %) attached ticks and all questing ticks belonged to the species *I. ricinus*. Our analysis showed a clear effect of host species and sex on the infestation levels by immature ticks: wood mouse carried more larvae and nymphs than bank vole, while males carried more larvae and had the tendency (not significant) to carry more nymphs than females. The habitat variables (tree species, shrub cover, and herb cover) had no effect on larval and nymphal tick burden on rodents. We did not investigate the seasonal pattern of attached ticks on rodent hosts. However, studies that did look at the seasonal pattern found similar results (e.g., Stanko *et* *al.* 2007, Boyard *et al.* 2008). Possible explanations for this effect of rodent species on tick infestation levels are acquired resistance to tick feeding, which has been reported for voles but not for mice (Dizij & Kurtenbach 1995, Hughes & Randolph 2001), and a greater home range for *Apodemus* mice and the associated greater exposure of mice to questing ticks (Tälleklint & Jaenson 1997). The higher infestation of males has also been linked to their larger home ranges (Tälleklint & Jaenson 1997, Brunner & Ostfeld 2008) and to high testosterone levels, which has been shown to weaken the immune response (Hughes & Randolph 2001).

The abundance of questing nymphs in the forest stands varied between 1 and 196 per 100 m^2, which falls within the range reported by Gray *et al.* (1998). In line with the results of Chapters 3 and 4, the abundance of nymphs was higher in the oak plots than in the pine plots and increased with increasing cover of shrubs. In Chapter 4 we suggested that these differences were to be attributed to differences in habitat use by the ticks' main hosts. Our current study confirms these assumptions and shows that host abundance, besides habitat type, is a determining factor in *I. ricinus* abundance. The best model explaining nymphal abundance included the abundance of wood mouse, bank vole, and larvae in the prior year ($A_{t\text{-}1}$, $M_{t\text{-}1,}$ and $L_{t\text{-}1}$). Moreover, it is interesting to note that, although the abundance of questing nymphs was higher in the oak plots in each year (Fig. 6.4b), the difference in nymphal abundance between pine and oak plots was more pronounced in 2011, i.e., two years after the large acorn crop and one year after the subsequent increase in the size of the rodent populations.

6.6 Conclusion

Several studies, including this one, have shown that both the percentage of infested hosts and the number of ticks per host animal is higher for wood mouse than for bank vole, and because of that, wood mouse is generally considered a better feeding host for larval ticks. However, the abundance of bank vole also appeared an important variable to be included in our analysis, probably because voles were very abundant in the study area and, thus, feed a significant proportion of the tick population. Our results suggest that local variation in abundance of questing *I. ricinus* nymphs depends on the abundance of larvae and rodents in the prior year, and the latter, in turn, is strongly influenced by tree seed production. Due to the population responses of rodents to mast production and the important role of rodents as feeding hosts for larval ticks, temporal fluctuations in acorn production might cause spatial and temporal variations in the abundance of nymphs and in the risk of getting a tick bite two years after mast production. However, it is important to note that larvae feeding early in the year could in principle feed as nymphs in the autumn of the same year, thereby completing the whole tick life cycle in two years instead of three (Randolph *et al.* 2002). This means that mast production could also increase the abundance of questing nymphs one year after a mast year.

[Photograph Gert Arijs]

7 General discussion and conclusions

It is a well-established fact that nature plays a vital role in human health and well-being (Maller *et al.* 2006, Mayer *et al.* 2009). A large body of evidence demonstrates that humans derive both physical and psychological benefits from spending time in (semi-) natural environments. For instance, contact with nature is related to greater physical activity (Saelens *et al.* 2003), positively influences children's development (Maller & Townsend 2006, Hussein 2010), enhances concentration (Kaplan 1995), reduces stress (Hansmann *et al.* 2007, Van den Berg *et al.* 2010), and improves self-esteem and mood (Barton *et al.* 2012). Not surprisingly, many people want to live in green surroundings and have the need to recreate in nature reserves and forests to relax, to engage in physical activities, to recover from stress, and to escape from everyday routine (Praestholm *et al.* 2002, Bell *et al.* 2005, Hansmann *et al.* 2007). Yet, although proximity to nature has many beneficial effects on human health, it also brings people in closer contact with wildlife and their disease-transmitting tick parasites. Especially people living in forested areas, people working in forests, and people visiting forests for recreational purposes (e.g., picnicking, hunting, and harvesting of mushrooms and berries) are at high risk for tick-borne infections (Cisak *et al.* 2005, Linard *et al.* 2007, Šumilo *et al.* 2008).

The growing fear of ticks and tick-borne infections in recent decades has raised some pertinent questions, e.g., which factors are responsible for the emergence of tick-borne diseases and which measures should be taken to prevent disease transmission? To answer these questions, we need to identify the climatic, ecological, landscape, and anthropogenic factors that could have an impact on the host-vector-pathogen relationships and human behavior. Knowing where tick populations occur and how abundant they are, is important for understanding and controlling ticks and the diseases they transmit. Several studies have tried to identify the driving forces for changes in the geographical distribution of ticks and tick-borne diseases. Climate change is considered a major driving force, especially in regions where temperature is a limiting factor for tick survival and development. There is increasing evidence that higher temperatures have significantly contributed to an expansion in the tick's geographic range at extremes of latitude in northern Europe (Jaenson *et al*. 2012b). In the Czech Republic, the expansion of ticks and tick-borne encephalitis towards higher altitudes has also been associated with changes in climate (Daniel *et al.* 2003, 2004, Danielová *et al*. 2008). In the Baltics, significant socio-economic changes are believed to have contributed to the increase in both the abundance of TBEV-infected ticks and the frequency of human contact with ticks, resulting in an upsurge in human cases of tick-borne encephalitis (Šumilo *et al.* 2008). Increased numbers of roe deer (*Capreolus capreolus* Linnaeus, 1758) are also considered an important factor in explaining the spread of ticks and tick-borne diseases. For instance, the northward spread of *I. ricinus* in Sweden was not only related to milder temperatures during winter, but also to an increase in roe deer numbers (Jaenson *et al*. 2012b). In Italy, the emergence of tick-borne encephalitis at higher altitudes was not related to an increase in temperature. Instead, the increase in TBE incidence in humans was attributed to changes in forest structure and in density of roe deer. In Great Britain, a questionnaire study revealed that perceived increases in tick abundance coincided spatially and temporally with reported increases in deer populations at many locations, indicating that deer might be an important determinant of tick abundance (Scharlemann *et al*. 2008).

In western Europe, socio-economic conditions have been relatively stable over the past decades and climate change is not expected to exert a great influence on *I. ricinus* populations. In this densely populated part of Europe, where most of the forest ecosystems have been shaped by human activity for many centuries, anthropogenic factors such as forest management, wildlife management, and changes in land use patterns, are probably driving forces for *I. ricinus* range expansion (Medlock *et al*. 2013). The conversion of structure-poor coniferous monocultures into well-structured mixed forests is expected to have a great influence on the suitability of forests for wildlife (e.g., increased food resources and shelter), which, in turn, might influence tick populations and disease dynamics. Yet, there have been relatively few studies addressing the variation in tick abundance between forest types, and literature specifically addressing the impact of forest conversion on tick populations is lacking. With this PhD thesis, we aimed to fill some gaps in the knowledge concerning the potential impact of forest conversion on *Ixodes ricinus* tick populations. We focused on four different forest types: structure-poor pine stands, structure-rich pine stands, structure-poor oak stands, and structure-rich oak stands. Fieldwork was carried out in the Campine region (northern Belgium). We investigated how the abundance of ticks was influenced by the composition and vertical structure of forests (Chapters 3–5), landscape configuration (Chapter 3), and food availability as influenced by mast seeding (Chapter 6). These findings allowed us to formulate suggestions for forest management and raised several questions to be addressed in further research.

7.1 Forest conversion effects on tick abundance

In this thesis, we provide multiple lines of evidence that forest conversion may increase tick populations. In Chapter 3, a large-scale tick survey in 176 forest stands in 21 forests showed that *I. ricinus* tick abundance was significantly affected by forest composition and structure: the abundance of questing larvae, nymphs, and adults was 2–3 times higher in oak (*Quercus* spp.) stands compared to pine (*Pinus* spp.) stands and twice as high in forest stands with high (> 50 %) compared to low (< 10 %) shrub cover. Consequently, oak stands with high shrub cover yielded 6–7 times more ticks than homogeneous pine stands with low shrub cover. It must be stated that the forest stands were sampled only once due to the time-consuming nature of tick sampling. Thus, we did not take into account the temporal variation in tick abundance. The importance of tree species and shrub cover on tick abundance was confirmed in Chapter 4. This study was carried out in 25 forest stands at two sites. Ticks were sampled in pine and oak stands with and without a significant shrub layer, on several occasions in three successive years, thereby including both a spatial and temporal component. Similar to the results described in Chapter 3, oak trees and shrub cover had a positive effect on the abundance of questing *I. ricinus* larvae, nymphs, and adults. This pattern was observed at both forest sites and on almost every sampling occasion. Overall, larvae were 10 times more abundant, and both nymphs and adults were 3 times more abundant in the structure-rich oak stands compared to the homogeneous pine stands. These results show that structure-rich deciduous forests can support higher tick population levels than structure-poor coniferous forests, which is in line with previous European studies (Gray *et al.* 1998, Estrada-Peña 2001, Lindström & Jaenson 2003).

Chapter 5 describes the results of an experimental study in which we manipulated the forest's structural attributes. Thinning had no immediate effect on local tick abundance. However, it must be stated that the duration of the study was too short to study the response of the understory and shrub layer to thinning and the implications of such a change on tick populations. Shrub clearing, on the other hand, resulted in a significant suppression of all three life stages of *I. ricinus* in the two following years, thereby verifying the results obtained in two previous studies (Chapter 3, Chapter 4).

The observed differences in tick abundance between the forest types are most likely to be attributed to differences in habitat use by vertebrate hosts. This is strongly supported by the fact that the number of deer beds encountered during sampling showed the same association with the forest types as tick abundance (Chapter 4): about 4 times as many deer beds were found in structure-rich oak stands compared to structure-poor pine stands. Although the co-occurrence of high tick abundances and deer beds does not imply causality, it certainly suggests an important role of roe deer in maintaining high levels of tick populations, which is in concordance with the literature (Ruiz-Fons & Gilbert 2010, Jaenson *et al.* 2012b). Oak stands, and especially structure-rich oak stands, are probably more intensely used by roe deer, as these provide more palatable forage and shelter than (structure-poor) pine stands (Maillard & Picard 1987, Tufto *et al.* 1996, Tixier *et al.* 1997, Cornelis *et al.* 1999). Such preferential habitat use by cervids may greatly influence the ticks' reproduction and dispersal, as has been reported from the United States (Wilson *et al*. 1985, 1990). This might also explain the higher tick abundances in fragmented forests with a high amount of forest edge habitat (Chapter 3), which can be explained by the fact that forest edges are important ecotones that provide a good interspersion of forage and cover for a number of key hosts for *I. ricinus* ticks, e.g., rodents and deer (Wahlström & Kjellander 1995, Tufto *et al.* 1996, García *et al.* 1998, Saïd & Servanty 2005, Boyard *et al.* 2008). These findings are in line with other studies emphasizing the importance of the spatial configuration and connectivity of landscape features on the distribution, density, behavior, and population dynamics of ticks and their hosts (Allan *et al.* 2003, Estrada-Peña 2003, Brownstein *et al.* 2005, Ostfeld *et al.* 2005).

In Chapter 6, we found a positive relationship between the abundance of questing nymphs and the prior year's abundance of wood mouse (*Apodemus sylvaticus* Linnaeus, 1758) and bank vole (*Myodes glareolus* Schreber, 1780), indicating that higher levels of rodent populations provide greater feeding opportunities for larval ticks. Wood mice carried more larvae and nymphs than bank voles. Yet, bank voles were more abundant than wood mice. Our results indicate that both rodent species are key hosts for the immature stages. We also showed that rodent abudance was strongly influenced by tree seed production in the previous autumn (Chapter 6), thereby confirming the results of studies carried out in the United States (Ostfeld *et al.* 2001), which stated that temporal fluctuations in acorn production might cause spatial and temporal variations in the abundance of nymphs and in the risk of getting a tick bite two years after mast production years. As rodents are the main reservoir host of *Borrelia burgdorferi* sl, forest management may not only influence the proportion of larvae feeding on rodents but also the infection prevalence in nymphal ticks. Yet, despite the difference in tick abundance between the structure-poor pine stands and the structure-rich oak stands, which is most likely at least partly due to differences in rodent populations, we did not find a significant effect of forest type on the nymphal infection rate with *Borrelia burgdorferi* sl (Chapter 4).

7.2 Recommendations for forest management

Based on the results of this PhD thesis, we formulate some recommendations for forest management.

Forest conversion towards structure-rich deciduous forests contributes significantly to the creation of suitable tick habitats. In combination with an anticipated increase in the recreational use of these forests, we predict that the degree of human-tick contact will increase over the coming decades. If the goal of the management is to promote and enhance forest-based recreation, a comprehensive strategy for reducing the likelihood of exposure to ticks is required, involving surveillance, personal protection, education, land use planning, wildlife management, and vector reduction.

Since the prevention of tick attacks is considered the most effective measure against tick-borne infections, it is important to know where and when ticks are abundant. The first line of defense against tick bites is to avoid high-risk habitats during periods of high tick activity. Surveillance is needed to identify these high-risk areas and periods. Our study may help in identifying potential high-risk areas and periods, thus enabling forest managers to focus their management efforts in a more targeted way. For instance, certain trails could be temporarily closed to public access to avoid people from entering areas where tick abundance is high and public health educational campaigns could be conducted two years following a mast event. We recommend providing information at parking lots and trail heads to raise awareness about taking precautions when entering tick habitat, e.g., wearing protective clothing, application of repellents, walking in the centre of trails to avoid contact with questing ticks on the vegetation, and a thorough examination of the entire body for ticks after outdoor activities.

The results obtained in Chapter 3 support the use of landscape management to control the distribution and abundance of vector ticks. Several studies have demonstrated a positive link between forest fragmentation and both tick abundance and infection prevalence with pathogens (Brownstein *et al.* 2005). Therefore, the transmission of tick-borne diseases such as Lyme borreliosis might be reduced by countering forest fragmentation and by avoiding residential development adjacent to forests in endemic areas (Allan *et al.* 2003, Brownstein *et al.* 2005, Jackson *et al.* 2006). Likewise, much more attention should be paid to the surrounding landscape to avoid potential risk zones (e.g., ecotones) when creating trails and other recreational opportunities such as campsites and picnic areas. Shrub clearing can locally suppress tick populations (Chapter 5) and could be a useful component of an integrated tick control strategy, especially in areas used for recreational activities. Other measures to reduce the local abundance of ticks include mowing vegetation (Wilson 1986), removal of leaf litter (Schulze *et al.* 1995), and deer exclusion by fencing (Gilbert *et al.* 2012).

We wish to emphasize that this work is not a plea against forest conversion, as the various benefits of forest conversion (e.g., biodiversity conservation, watershed protection, amenity values) are widely acknowledged. Rather, we would like to stress the importance of research studying the influence of habitat and landscape composition and structure, forest and wildlife management, and human activities on ticks and tick-transmitted diseases. Such knowledge would greatly help policy makers and people involved in nature and forest management in deciding how to manage habitats and landscapes in order to reconcile ecological needs (e.g., biodiversity) with societal needs (e.g., recreation). The results of this thesis are not only relevant in the light of ongoing forest conversion. The emergence of several infectious diseases in recent decades has forced us to think about how we should manage habitats and landscapes in order to minimize contact with disease-transmitting ticks. For instance, the movement of ticks into new areas depends on the movements of their hosts, and, thus, de-fragmentation of habitats by providing habitat corridors could have an influence on the dispersal of both wildlife and their tick parasites. Moreover, urban green corridors are facilitating a movement of deer into urban areas and there have been repeated problems of deer and ticks in residential gardens (Jameson & Medlock 2010).

7.3 Suggestions for further research

This thesis has contributed to current knowledge concerning the impact of forest conversion on tick abundance and has formulated some recommendations for forest management. Nevertheless, there remain issues that could be addressed in future research.

A limitation of this study is the limited number of forest types studied. We focused on pine and oak forests on nutrient-poor and acidic sandy soils because these are representative for the forests in the Campine region (northern Belgium) and relevant to address the research question. Our investigation could be expanded to include other soil types and other important tree species, such as common beech (*Fagus sylvaticus* L.) and Norway spruce (*Picea abies* (L.) Karst.). Such information is especially relevant in central Europe, where the conversion of spruce monocultures into spruce-beech forests is an important issue with regard to nature-oriented forest management (Matthes & Ammer 2000, Elmer *et al.* 2004).

We showed a positive effect of forest edge length on tick abundance. Although the type of edge (e.g., forest-pasture, forest-agriculture, or deciduous-coniferous) might also be very important, we did not look into that specifically. The importance of ecotones and edge types on wildlife and tick abundance needs to be investigated further, especially taking into consideration that nature management increasingly seeks to create and sustain a diverse habitat mosaic, e.g., for butterfly conservation purposes (New *et al.* 1995), which could be beneficial for several vertebrates, such as roe deer, and tick populations. Furtermore, we stress the need to investigate other landscape variables, e.g., landscape connectivity, as there are numerous habitat connectivity initiatives ongoing in many parts of Europe, which might have a great influence on the dispersal of wildlife and ticks.

Literature often emphasizes the importance of roe deer for the maintenance and dispersal of ticks. Yet, other wildlife may also play an important role, e.g., wild boar (*Sus scrofa* Linaeus, 1758). In certain parts of Germany, for instance, roe deer populations have reportedly remained stable, whereas large increases of wild boar have been linked with increases in *I. ricinus* abundance (Schwarz *et al*. 2009). With populations of wild boar on the rise in several European countries, this certainly deserves further attention.

Shrub clearing was identified here as an important component of an integrated tick control strategy, especially in restricted areas (e.g., recreational areas). Further research needs to be done to evaluate the effectiveness and efficiency of other potentially useful vector control measures: deer management by hunting and fencing (Gilbert *et al.* 2012), host-targeted acaricide application, e.g., the use of acaricide-treated sheep as 'lethal mops' or the use of bait stations to treat deer with acaricides thus preventing ticks to feed (Durland & Childs 2009, Dobson & Randolph 2011, Harmon *et al.* 2011), and biological control of ticks by entomopathogenic fungi, nematodes, and parasitic wasps (Hartelt *et al.* 2008, Collatz *et al.* 2011).

Our study stresses the importance of spatial heterogeneity in forest habitat quality and landscape configuration on tick abundance. However, the epidemiological risk posed by *I. ricinus* ticks depends not only on the abundance of ticks, but also on their infection rate with pathogenic organisms and the degree of contact between infected ticks and humans. The hypothesis that local habitat and landscape features not only influence tick abundance but also the infection rate of ticks has been demonstrated by several authors (Ostfeld et al 2005) and presents a promising avenue for further research. Large-scale forest conversion programs could change the composition and abundance of wildlife communities to the extent that the relative proportion of reservoir-competent and incompetent hosts changes, thereby influencing not only tick abundance but the infection prevalence in ticks as well. Furthermore, by promoting forest-based recreation, forest conversion might bring more people in contact with questing ticks. Further research is needed to better understand the complex interactions between ticks, pathogens, hosts, and humans and how these can be influenced by, e.g., forest conversion. Such knowledge is of prime importance for properly assessing the risk of disease transmission and for designing early warning systems and risk maps.

Summary

Ticks and tick-borne diseases affecting human and animal health have become a subject of growing concern. Lyme borreliosis and tick-borne encephalitis, the most important tick-borne diseases in humans in Europe, are predominantly transmitted by the tick *Ixodes ricinus* (i.e., the vector). Lyme borreliosis is an endemic infection in Belgium and is caused by the bacteria *Borrelia burgdorferi*. Tick-borne encephalitis, which is caused by a virus, does not yet occur in Belgium. The ticks may acquire these two infections either by feeding on infected vertebrate hosts or by co-feeding with infected ticks on uninfected hosts. They can then pass the infection on to other host animals or humans during a following blood meal. Since ticks are typically associated with forested areas, especially people working in forests and people visiting forests for recreational purposes are at high risk of encountering ticks.

By altering the characteristics of forests, e.g., tree species composition and vertical structure, forest management may have a considerable impact on the suitability of forests for tick populations and their main hosts, thus influencing the dynamics of tick-borne infections. This is particularly important in the context of close-to-nature forestry and ongoing forest conversion projects that aim at optimizing the economic, ecological, and social/recreational functions of forests. In response to environmental concerns (biodiversity loss among others) and changing societal needs, one of the main goals of the forest policy in Europe is the conversion of homogeneous plantations to semi-natural forest types. Yet, the effects of such changes on tick populations have scarcely been investigated. In this study, we aimed to increase our understanding of the potential impact of forest conversion on *I. ricinus* tick populations. We focused on the Campine region in the northeast of Belgium, where large areas of homogeneous pine stands are being converted into mixed, uneven-aged stands with multi-layered canopies dominated by native broadleaved species.

First, a preliminary study was conducted to evaluate the efficiency of the entire-blanket and the strip-blanket for the sampling of host-seeking ticks. Both sampling methods were compared in four different understory vegetation types (bracken fern, bilberry, purple

moor grass, and short grass). The entire-blanket appeared to be the best sampling technique for collecting nymphal and adult ticks. The strip-blanket only provided an advantage over the entire-blanket for the sampling of larvae in dense, bracken-fern dominated sites. Based on these results, we decided to use the entire-blanket for the remainder of our study and we avoided sites with a dense understory of bracken fern in order to minimize sampling bias due to differences in vegetation type.

A large-scale survey of *I. ricinus* ticks was conducted at 21 forest sites in a total of 176 stands that varied in dominant tree species (pine *vs*. oak), shrub cover, and herb cover. The main goal of this survey was to investigate the effect of local habitat and landscape variables on tick abundance and to determine the overall *B. burgdorferi* prevalence in nymphs, which are considered the main vector for transmission of the bacteria to humans. *Borrelia*-infected nymphs were found throughout the study area and had an overall mean infection prevalence of 9 %, which is consistent with the mean value obtained for Europe (10 %). At the forest stand level, the dominant tree species and the shrub cover significantly affected tick abundance. The abundances of all life stages were higher in oak stands compared to pine stands, and increased with increasing shrub cover. Overall, the structure-rich oak stands yielded 6 to 7 times more larvae, nymphs, and adults compared to the structure-poor pine stands. Additionally, at the landscape level, a positive effect was found for forest edge length on the abundance of nymphs and adults.

A second observational study was carried out to verify the influence of dominant tree species and vertical forest structure on tick abundance. This study focused on two forest sites (25 forest stands in total), which enabled including a temporal component within the study design to account for possible seasonal fluctuations in tick activity. Tick sampling was carried out on multiple occasions in three successive years in four distinct forest types: structure-poor pine stands, structure-rich pine stands, structure-poor oak stands, and structure-rich oak stands. Additionally, the habitat use by roe deer was assessed in those forest types by counting the number of deer beds encountered during sampling. Our findings confirmed the results obtained in the previous study. We found that the abundance of host-seeking larvae, nymphs, and adults was higher in oak stands compared to pine stands and higher in structure-rich compared to structure-poor forest stands. Overall, larvae were 10 times more abundant in the structure-rich oak stands than in the structure-poor pine stands. Nymphs and adults were 3 times more abundant in the structure-rich oak stands. An identical pattern was observed regarding the number of deer beds. This strongly suggests that the observed differences in tick abundance are at least partly due to differences in habitat use by roe deer. This makes sense, as roe deer is generally considered the main wildlife host of adult ticks and, thus, a key host for the reproduction and dispersal of *I. ricinus*. Differences in forest composition and structure might also influence the *Borrelia* infection prevalence in nymphal ticks by influencing the population dynamics of rodents such as wood mouse and bank vole, which are important hosts of larvae as well as important reservoirs for *Borrelia* spirochetes. Yet, the *Borrelia* prevalence in nymphs did not differ significantly between the structure-rich oak stands and the structure-poor pine stands.

Subsequently, an experiment was carried out in 6 pine stands (with a control plot and an experimental plot in each stand) to study the importance of vertical forest structure on tick populations and to examine whether shrub-removal could be an important component of an integrated tick control strategy. A moderate thinning was carried out in all plots and shrub clearing was performed in the treatment plots. Thinning had no effect on tick abundances, while shrub clearing had an adverse affect on the abundances of all three life stages up to two years post-clearing. These results show that a well-developed shrub layer is indeed an important component of tick habitat and demonstrate that shrub clearing could be applied locally as a tick control measure, for instance in recreational areas where there is a high degree of human-tick contact.

By promoting the use of mast-producing tree species (e.g., oak) that produce large seed crops every few years, forest management might affect the food availability for numerous vertebrates, including rodents, which could have a significant effect on tick abundance and on the incidence of Lyme borreliosis. Therefore, we studied (i) the abundances of wood mouse and bank vole before and after a seed-addition experiment and after a mast year and (ii) the association between these rodents and both attached and questing immature ticks. Sampling was carried out in 6 pine stands and 6 oak stands in three successive years. The majority of the attached ticks were *I. ricinus* larvae (95 %). Wood mice harbored greater numbers of larvae and nymphs than bank voles, and males carried a higher larval burden than females. The abundance of both rodent species increased after the seed-addition experiment and the mast year, although the effect was significant only for bank voles. The abundance of questing *I. ricinus* nymphs depended on the abundance of both rodent species and of larvae in the prior year. These results show that wood mouse and bank vole are indeed key hosts of immature ticks, and that a phenomenon such as a mast year might have a strong impact on both rodent and tick abundance.

Our results provided more insight into the role of forest composition, forest structure, and landscape configuration on the spatiotemporal variation in the abundance of larval, nymphal, and adult *I. ricinus* ticks. We conclude that forest conversion might create suitable habitats for ticks by altering the dynamics between ticks and their key hosts, especially roe deer and rodents such as bank vole and wood mouse. Since we expect forest recreation to increase in the future, we stress the need for intensive information campaigns, tick monitoring and control measures to reduce tick populations and tick-borne diseases.

Samenvatting

Teken en door teken overdraagbare ziektes bij dier en mens zijn een bron van toenemende zorg. Lyme borreliose en tekenencephalitis, de voornaamste ziektes die in Europa door teken op de mens kunnen worden overgebracht, worden voornamelijk overgedragen door de teek *Ixodes ricinus* (i.e., de vector). Lyme borreliose is in België een endemische infectieziekte die veroorzaakt wordt door de bacterie *Borrelia burgdorferi*. Tekenencephalitis, veroorzaakt door een virus, komt nog niet voor in België. Teken kunnen geïnfecteerd raken door het voeden op een dier dat de ziekteverwekker in de bloedsomloop heeft of door het voeden op een dier in de buurt van een geïnfecteerde teek. De teek kan vervolgens de ziekteverwekker overdragen op andere dieren en de mens tijdens een volgende bloedmaal. Teken komen voornamelijk voor in bossen, waardoor vooral mensen die frequent in het bos vertoeven om er te werken of te ontspannen een risicogroep vormen om gebeten te worden door teken.

Door het veranderen van bepaalde boskenmerken, zoals de boomsoortensamenstelling en de verticale structuur, kan het bosbeheer een aanzienlijke invloed uitoefenen op de geschiktheid van het bos als habitat voor teken en hun gastheren, en dus ook op de dynamiek van de ziektes die ze kunnen overdragen. Dit is vooral van belang in de context van natuurgetrouwe bosbouw en de huidige bosomvormingsprojecten, die gericht zijn op het optimaliseren van de economische, ecologische en socio-recreatieve functies van bossen. Een van de belangrijkste doelstellingen van het bosbeleid in Europa is de omvorming van homogene plantages naar half-natuurlijke bossen om zo tegemoet te komen aan de huidige milieuproblemen (o.a. verlies aan biodiversiteit) en gewijzigde maatschappelijke behoeftes. In deze studie hebben we geprobeerd om inzicht te verkrijgen in de mogelijke gevolgen van bosomvorming op tekenpopulaties wat tot op heden uiterst zelden onderzocht werd. Als studiegebied werd geopteerd voor de Kempen in het noordoosten van België, waar talrijke homogene dennenbestanden geleidelijk omgevormd worden tot ongelijkjarige gemengde bestanden met een gevarieerde verticale structuur en met een groot aandeel aan inheemse loofboomsoorten.

Eerst werd een studie uitgevoerd om de efficiëntie na te gaan van twee verschillende sleepdoeken voor het bemonsteren van teken die in de vegetatie op zoek zijn naar een geschikte gastheer. De *entire-blanket* en *strip-blanket* werden vergeleken in vier vegetatietypes (adelaarsvaren, blauwe bosbes, pijpenstrootje en kort gras). De *entire-blanket* bleek de beste bemonsteringstechniek voor het vangen van nimfen en adulten. De *strip-blanket* had enkel een voordel ten opzichte van de *entire-blanket* voor het vangen van larven in gebieden met een dichte begroeiing van adelaarsvaren. Op basis van deze resultaten werd geopteerd om de *entire-blanket* te gebruiken tijdens het verdere verloop van de studie. Ook werd beslist om geen gebieden te bemonsteren met een dichte begroeiing van adelaarsvaren om vertekening van de onderzoeksresultaten ten gevolge van verschillen in vegetatiestructuur te reduceren.

Een grootschalig onderzoek werd uitgevoerd in 21 bossen in een totaal van 176 bosbestanden die varieerden qua boomsoort (eik vs. den) en bedekking van de struik- en kruidlaag. De hoofddoelstelling was het nagaan van het effect van enkele lokale habitat- en landschapsvariabelen op de abundantie van de teek en het bepalen van de prevalentie van *B. burgdorferi* in nimfen (die beschouwd worden als de voornaamste vector). Met *Borrelia*-geïnfecteerde nimfen werden aangetroffen in het ganse onderzoeksgebied met een gemiddelde prevalentie van 9 %, wat overeenstemt met de gemiddelde waarde voor Europa (10 %). Op bestandsniveau hadden de dominante boomsoort en de bedekking van de struiklaag een significante invloed op de tekenabundantie. De abundanties van alle levensstadia waren hoger in eikenbestanden dan in dennenbestanden en namen toe met toenemende bedekking van de struiklaag. In de structuurrijke eikenbestanden werden 6 tot 7 keer meer larven, nimfen en adulten aangetroffen dan in de structuurarme dennenbestanden. Op landschapsniveau bleek de bosrandlengte een positief effect te hebben op de abundanties van de nimfen en adulten.

Een tweede observationele studie werd uitgevoerd om het effect van hoofdboomsoort en verticale structuur op de abundantie van teken te verifiëren. Dit onderzoek richtte zich op twee bosgebieden (in een totaal van 25 bosbestanden) wat ons toeliet om ook een temporele component in de proefopzet te verwerken. Hierdoor kon rekening worden gehouden met mogelijke seizoenale schommelingen in tekenactiviteit. De tekenpopulatie werd regelmatig bemonsterd gedurende drie opeenvolgende jaren en in vier verschillende bostypes: structuurarme dennenbestanden, structuurrijke dennenbestanden, structuurarme eikenbestanden, en structuurrijke eikenbestanden. Daarnaast werd ook het habitatgebruik van reeën geschat door het aantal ligplekken te tellen die werden aangetroffen tijdens de bemonstering. Onze bevindingen bevestigen de resultaten verkregen in de vorige studie. De abundanties van de larven, nimfen en adulten waren hoger in de eikenbestanden dan in de dennenbestanden en hoger in de structuurrijke bestanden dan in de structuurarme bestanden. Larven waren 10 keer meer abundant in de structuurrijke eikenbestanden dan in de structuurarme dennenbestanden. Nimfen en adulten waren 3 keer meer abundant in de structuurrijke eikenbestanden. Een identiek patroon werd waargenomen met betrekking tot het aantal ligplekken van reeën. Dit toont aan dat de waargenomen verschillen in tekenabundantie minstens gedeeltelijk te wijten zijn aan verschillen in het habitatgebruik van reeën. Dit houdt steek aangezien reeën beschouwd worden als de voornaamste in het wild levende gastheren van volwassen teken, en dus een sleutelgastheer zijn voor de reproductie en verbreiding van *I. ricinus*. Verschillen in bossamenstelling en –structuur zouden ook de *Borrelia*-infectiegraad in nimfen kunnen beïnvloeden door het beïnvloeden van de populatiedynamiek van knaagdieren zoals de bosmuis en rosse woelmuis, die niet enkel belangrijke gastheren zijn voor de larve van de teek maar ook natuurlijke reservoirs zijn voor *Borrelia* spirocheten. We vonden echter geen significante verschillen in prevalentie tussen de structuurrijke eikenbestanden en structuurarme dennenbestanden.

Vervolgens werd een experiment uitgevoerd in 6 dennenbestanden (met een experimenteel proefvlak en een controleproefvlak in elk bestand) om het belang na te gaan van de verticale bosstructuur op tekenpopulaties en om te onderzoeken of het kappen van de struiklaag kan ingezet worden als maatregel in het kader van een geïntegreerde aanpak om de teek te bestrijden. Er werd een reguliere dunning uitgevoerd in alle bestanden en de struiklaag werd verwijderd in de experimentele proefvlakken. De dunning had geen effect op de tekenabundanties terwijl het verwijderen van de struiklaag tot twee jaar na de behandeling een negatief effect had op de abundanties van de drie levensstadia. Deze resultaten tonen aan dat een goed ontwikkelde struiklaag inderdaad een belangrijk onderdeel vormt van een voor de teek geschikt habitat en dat de verwijdering van de struiklaag lokaal kan aangewend worden als maatregel, bijvoorbeeld in recreatiegebieden waar er een grote kans is om in contact te komen met teken.

Door het bevorderen van boomsoorten die in sommige jaren uitbundig veel zaden produceren (bijvoorbeeld eik), kan het bosbeheer een sterke invloed uitoefenen op de beschikbaarheid van voedsel voor verscheidene diersoorten, met inbegrip van knaagdieren, wat een groot effect zou kunnen hebben op tekenpopulaties en de incidentie van Lyme borreliose. Om die reden onderzochten we (i) de abundantie van de bosmuis en de rosse woelmuis na een mastjaar en na een experimentele nabootsing van een mastjaar, en (ii) de relatie tussen de knaagdieren en de onvolwassen teken (zowel gastheerzoekende teken als teken die zich hadden vastgehecht aan knaagdieren). Het veldwerk werk uitgevoerd in 6 dennenbestanden en 6 eikenbestanden gedurende drie opeenvolgende jaren. Het merendeel van de teken die van de knaagdieren werden verwijderd, waren *I. ricinus* larven (95 %). Er werden aanzienlijk meer larven en nimfen aangetroffen op bosmuizen dan op rosse woelmuizen, en mannetjes werden in sterkere mate geparasiteerd door larven dan vrouwtjes. Beide knaagdiersoorten namen in aantal toe na het experiment en na het mastjaar, al was dit effect enkel significant voor rosse woelmuis. Bovendien kon een relatie worden aangetoond tussen het aantal gastheerzoekende nimfen in een bepaald jaar en de abundantie van gastheerzoekende larven, bosmuizen en rosse woelmuizen in het vorige jaar. Deze resultaten tonen aan dat de bosmuis en de rosse woelmuis inderdaad sleutelgastheren zijn van de onvolwassen stadia van de teek, en dat een fenomeen zoals een mastjaar een sterke invloed kan uitoefenen op de abundanties van zowel knaagdieren als teken.

De resultaten van deze studie hebben meer inzicht verschaft in de rol van de bossamenstelling en –structuur en de configuratie van het landschap op de ruimtelijk-temporele variatie in de abundantie van I. ricinus larven, nimfen en adulten. We besluiten dat bosomvorming geschiktere leefgebieden kan creëren voor teken door een verandering teweeg te brengen in de dynamiek tussen teken en hun voornaamste gastheren waaronder het ree, de bosmuis en de rosse woelmuis. Omdat we verwachten dat bosrecreatie zal toenemen, wijzen we op de noodzaak van o.a. intensieve sensibilisatiecampagnes, monitoring van tekenpopulaties, en het nemen van maatregelen in het kader van een geïntegreerde aanpak van teken en de ziektes die ze kunnen overdragen.

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Curriculum vitae

Personal data

Name	Wesley G. Tack
Place of birth	Ghent, Belgium
Date of birth	February, 24, 1981
Nationality	Belgian

Education

2001–2005	MSc in Bioscience Engineering, Land & Forest Management Faculty of Bioscience Engineering, Ghent University
1999–2001	BSc in Bioscience Engineering, Land & Forest Management Faculty of Bioscience Engineering, Ghent University
1993–1999	Secondary School, Sciences–Mathematics Koninklijk Atheneum Erasmus Deinze

Professional experience

2012–present	Vector ecologist at Avia-GIS Agriculture and Veterinary Intelligence and Analysis
2008–2011	PhD research at Ghent University Faculty of Bioscience Engineering Department of Forest and Water Management Financed by IWT Flanders
2006–2008	Civil servant Department of Environment and Sustainability, city Eeklo

Scientific publications

Publications in international journals with peer review cited in the Science Citation Index (IF: impact factor in 2011)

Tack W, Heyman P, Cochez C, Baeten L, Bonte D, De Blander E, Madder M, Vanhellemont M, Verheyen K (2013) The influence of acorn mast on rodents and their tick parasites.Ticks and Tick Borne Diseases, *submitted* (IF 2.370)

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National publications with peer review

Tack W, Madder M, Baeten L, Verheyen K (2011) Meer teken door bosomvorming? Eerste resultaten van tekenvangsten in de Kempen. Natuur.focus 10, 161–165

Gruwez R, Vanden Broeck A, De Frenne P, Tack W, Verheyen K (2010) Welke toekomst voor de Jeneverbes in Vlaanderen? Een evaluatie aan de hand van beheerscenario's. Natuur.focus 9, 167–173

National publications without peer review

Tack W, Madder M, Verheyen K (2010. De impact van mastjaren op knaagdieren, teken en de ziekte van Lyme. Antenne 4, 29–31

Scientific activities

Participation in congresses, symposia or workshops

Tack W, Madder M, Verheyen K. Converting pine plantations to mixed-species forests: implications for *Ixodes ricinus* ticks and public health. Poster presentation at the Belgian Biodiversity and Public Health Conference (Brussels, Belgium; November, 30, 2011)

Tack W, Madder M, Verheyen K. Effects of forest composition and vertical structure on the abundance of *Ixodes ricinus* ticks in northern Belgium. Poster presentation at the 12th European Ecological Federation (EEF) Congress (Ávila, Spain; September, 25–29, 2011)

Tack W, Madder M, Verheyen K. Effects of forest composition and structure on Lyme disease risk. Oral presentation at the 41st Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (Oldenburg, Germany; September, 5–9, 2011)

Tack W, Madder M, Verheyen K. Onderzoek naar de relatie tussen immature teken en knaagdieren. Oral presentation at Starters in het Bosonderzoek (Brussels, Belgium, in Dutch; March, 17, 2011)

Tack W, Madder M, Verheyen K. The relationship between forest rodents and immature ticks. Poster presentation at the Netherlands Annual Ecology Meeting (NAEM) (Lunteren, the Netherlands; February, 8–9, 2011)

Tack W, Madder M, Verheyen K. The effect of an acorn surplus on rodent and tick abundance. Poster presentation at the 16th PhD Symposium on Applied Biological Sciences (Ghent, Belgium; December, 20, 2010)

Tack W, Madder M, Verheyen K. Ticks in the Kempen (Belgium) and their relationship to mast-eating rodents. Oral presentation at the symposium TickTactics in the Lowlands (Amsterdam, the Netherlands; April, 27, 2010)

Tack W, Madder M, Verheyen K. Ecological relationships between *Ixodes ricinus* ticks and mast-eating rodents. Oral presentation at the Netherlands Annual Ecology Meeting (NAEM) (Lunteren, the Netherlands; February, 9–10, 2010)

Tack W, Madder M, Verheyen K. Impact van de omvorming van voormalige dennenplantages op het voorkomen van de schapenteek (*Ixodes ricinus* L.). Oral presentation at Starters in het Bosonderzoek (Brussels, Belgium, in Dutch; March, 19, 2009)

Tack W, De Pus C, Ducheyne E, De Wulf R. Evaluating the use of seasonal parameters for a supervised classification in a semi-arid rangeland of Northern Australia. Poster presentation at the 26th Asian Conference on Remote Sensing (ACRS) (Hanoi, Vietnam; November, 7–11, 2005)

Participation without presentation

Consequences of wildlife introductions (Tervuren, Belgium; October, 7, 2011)

Genetica in natuurbeleid en –beheer (Brussels, Belgium; November, 18, 2010)

Phytomanagement van metaal-verontreinigde gronden in de Kempen (Ghent, Belgium; September, 22, 2010)

Climate change impact on ticks and tick-borne diseases (Brussels, Belgium; February, 6, 2009)

Supervision of MSc students

2010–2011	De Blander Eline. Het belang van een hoge muizenstand voor het voorkomen van teken en de ziekte van Lyme. Supervisors: Prof. dr. Dries Bonte, Prof. dr. ir. Kris Verheyen
2009–2010	Schoonjans Karolien. De verspreiding van de schapenteek (*Ixodes ricinus*) en de besmettingsgraad met *Borrelia burgdorferi* in de Kempen. Supervisor: Prof. dr. ir. Kris Verheyen

Review tasks for international journals

2012: Parasites & Vectors (1)

2012: Journal of Ecology and the Natural Environment (1)