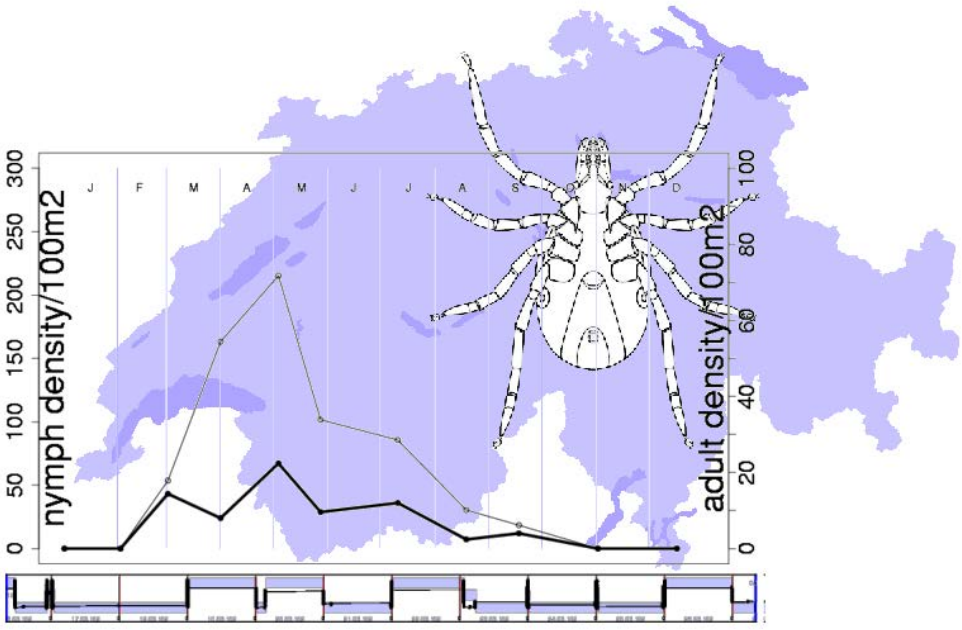
UNIVERSITÉ DE NEUCHÂTEL FACULTÉ DES SCIENCES INSTITUT DE ZOOLOGIE

Computer-assisted Laboratory Observations and Field Studies of the Host-finding Behaviour of the Tick *Ixodes ricinus* (Acarina: Ixodidae): Ecological Implications of Climate and Light

JEAN-LUC PERRET



THÈSE PRÉSENTÉE À LA FACULTÉ DES SCIENCES DE L'UNIVERSITÉ DE NEUCHÂTEL POUR L'OBTENTION DU GRADE DE DOCTEUR ÈS SCIENCES

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We choose to go to the moon in this decade and do the other things, not because they are easy, but because they are hard, because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one which we intend to win, and the others, too.

John F. Kennedy, Rice University, 12 September 1962

IMPRIMATUR POUR LA THESE

Computer-assisted Laboratory Observations and Field Studies of the Host-finding Behaviour of the Tick *Ixodes ricinus* (Acarina: Ixodidae): Ecological Implications of Climate and Light

de M. Jean-Luc Perret

UNIVERSITE DE NEUCHATEL

FACULTE DES SCIENCES

La Faculté des sciences de l'Université de Neuchâtel sur le rapport des membres du jury,

Mmes L. Gern (directrice de thèse),
S. Randolph (Oxford UK),
MM. P. Guerin (co-directeur de thèse) et
J. Bouvier (St-Aubin FR)

autorise l'impression de la présente thèse.

Neuchâtel, le 12 mars 2003

Le doyen:

F. Zwahlen

Abstract

Ixodes ricinus is a tick vector of pathogens. It is widely distributed in the forests of Switzerland. In nature this tick irregularly appears on the vegetation for "questing" periods during which it stays immobile on the low vegetation, ready to grab a passing host. We developed a computer-assisted tracking system which allowed us to continuously follow the behaviour of nymphs during ten-day periods in light and dark conditions without any host stimuli. Using this system, we experimentally demonstrated that the alternation of questing and quiescence in a humid atmosphere happens in the absence of any host. We experimentally proved that the duration of questing periods was limited by dessicating conditions, while the duration of quiescence periods was not. We discovered that quiescence was often interrupted by walks which did not necessarily lead to questing and that nearly all tick walks occurred during darkness, even when the light cycle was changed. We suggest that this behaviour corresponds to an "appetitive search" for hosts.

Using our tracking system, we also showed that *B. burgdorferi* sl (the etiologic agent of Lyme borreliosis) influences the host-finding behaviour of infected nymphs: infected nymphs interrupted their quiescence more often and walked for longer distances than uninfected nymphs, under the most dessicating conditions only.

In the field we measured the density of questing *I. ricinus* at different locations in Western Switzerland from 1999 to 2001. We showed how the density of this tick varies within and among years at different locations in Western Switzerland. We observed that activity onset and activity increase of *I. ricinus* in spring was dependent upon winter temperature while the occurrence of a peak of activity in autumn was dependent upon the spring-summer temperature. We also demonstrated that high saturation deficits inhibit tick questing in the field. The study of tick phenology at 3 different altitudes revealed that *I. ricinus* was present at 900m at a lower density than at the lower altitudes, but with a very stable phenology.

These results demonstrate the important impact of climate on the phenology of *I. ricinus* by its influence on the development and the behaviour of this important disease vector. Finally these results describe behavioural adaptations enabling *I. ricinus* to survive over a very broad geographic distribution despite its high sensitivity to dessication.

Résumé

Ixodes ricinus est une tique vecteur de pathogènes. Elle est largement distribuée dans les forêts de Suisse. Dans la nature cette espèce apparaît irrégulièrement sur la végétation pendant des périodes appelées "quête". Durant cette quête les tiques restent immobiles sur la végétation prête à attraper un hôte. Nous avons développé un système de suivi automatique assisté par ordinateur qui nous a permis de suivre en continu le comportement des nymphes d'*I. ricinus* pendant 10 jours en conditions de lumière variantes sans stimulus d'un hôte. Nous avons démontré experimentalement que la quête sur la végétation et la quiescence au sol alternent en l'absence de tout stimuli d'un hôte. Nous avons également démontré que la durée de la quête est limitée par les conditions dessèchantes, alors que la durée de la quiescence au sol ne l'est pas. Nous avons découvert que la quiescence était souvent interrompue par des déplacements qui n'aboutissaient pas forcément à la quête. Enfin nous avons constaté que pratiquement tous les déplacements des tiques étaient nocturnes, même lorsque le cycle de lumière était modifié. Nous suggérons que ce comportement est une forme active de recherche de l'hôte.

A l'aide du système de suivi automatique des tiques, nous avons également montré que l'infection par *B. burgdorferi* sl (l'agent étiologique de la maladie de Lyme) influence le comportement de recherche d'un hôte chez les nymphes: les nymphes infectées ont interrompu leur quiescence plus souvent et se sont déplacées sur de plus grandes distances que les nymphes non infectées mais dans les conditions les plus dessèchantes seulement.

Entre 1999 et 2001, nous avons mesuré la densité de tiques en quête dans différents lieux de Suisse Occidentale. Nous avons mis en évidence les variations de densité de tiques en quête entre les lieux et entre les années. Nous avons constaté que le début et l'augmentation de l'activité des tiques au printemps dépendaient de la température en hiver, alors que l'apparition d'un pic en automne dépendait de la température à la fin du printemps et au début de l'été. Nous avons également constaté que des déficits de saturation élevés diminuent la densité de tiques en quête. Finalement, l'étude de la phénologie à trois altitudes différentes a révélé qu'*I. ricinus* est présente à 900m avec une densité plus faible, mais une phénologie plus stable qu'à une altitude moins élevée.

Ces résultats mettent en évidence l'impact très important du climat sur la phénologie d'*I. ricinus* par son influence sur le développement et sur le comportement de cet important vecteur de pathogènes. Finalement ces résultats montrent des adaptations comportementales qui permettent à *I. ricinus* d'avoir une distribution très large malgré sa sensibilité à la dessication.

Contents

Chapter 1

Introduction

Hard ticks (Ixodidae) are highly specialised blood-sucking parasites. During their life cycle (figure 1.1) ticks only feed three times on vertebrate hosts. Once as a larva, once as a nymph, and once as a female (figure 1.1). Males may attach to the host and take small blood meals but do not grow in size. After a tick has engorged, it moults to the next stage, or if it is a female, it lays eggs.

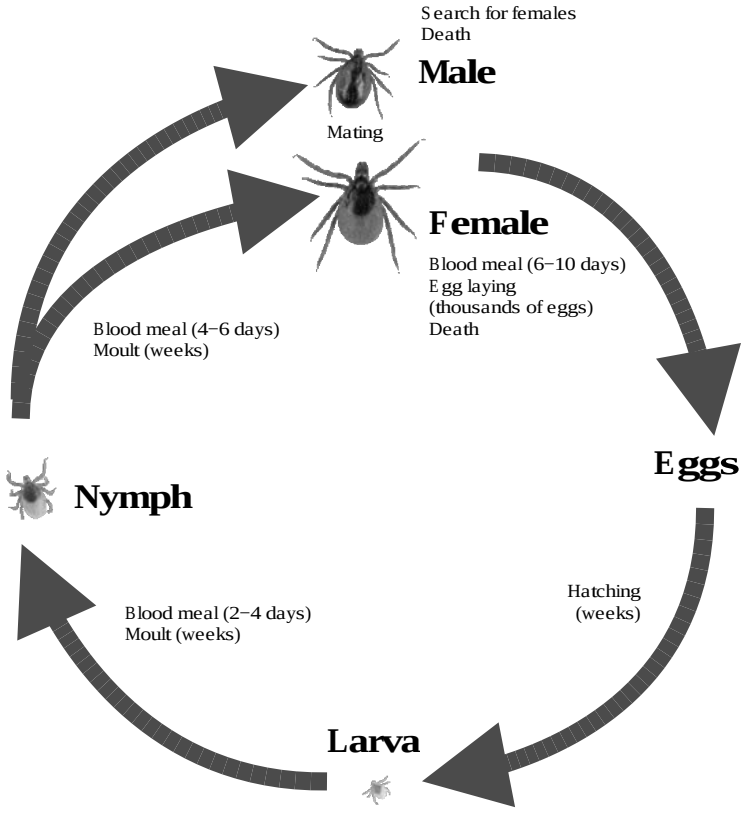


Figure 1.1: **Hard ticks life cycle**
(figure: Jean-Luc Perret 2002, photos: Institute of Zoology, Neuchâtel)

There are more than 800 species of hard ticks known worldwide (Camicas et al., 1998).

These species have various strategies for host-finding and for feeding. Some species (*Boophilus sp.*) find their hosts as a larva and do not leave until the female is engorged, thus moulting on the host. Other species drop off the host after every blood meal and so have to find a host three times during their life cycle (ex: *Amblyomma sp.*, *Ixodes sp.*).

Three-host ticks spend most of their lifetime away from the host (Needham and Teel, 1991), developing on the ground, and seeking a host. Finding a host is therefore a crucial step for the tick life cycle. Different strategies exist in different tick species. Some ticks parasitize only one or a small group of species. In this case they live in close association with their host, staying mainly on the hosts and in the host nesting places. These ticks are called nidicolous or endophilic ticks (Sonenshine, 1993) (ex: *Ixodes vespertilionis* on bats). Other three-host ticks have a much broader host range but also live in close association with their hosts (ex: *Ixodes hexagonus*). Finally some tick species have a broad range of potential hosts and do not live in very close association with their hosts, thus potentially feeding on different species at each life stage. These ticks are called non-nidicolous or exophilic ticks (Sonenshine, 1993). This is the case of the tick we studied in this work: *I. ricinus* (Linné, 1758).

I. ricinus geographical distribution ranges from North Africa to Scandinavia and from Ireland to Russia (Gern and Humair, 2002). Other species, taxonomically very close to *I. ricinus* are found in Asia: *Ixodes persulcatus* and in America: *Ixodes scapularis* (formerly called *Ixodes dammini* (Oliver et al., 1993)) and *Ixodes pacificus*.

I. ricinus represents the main species biting humans in Western Europe. A study in Germany showed that 98% of the ticks attached to humans belonged to this species (Liebisch and Liebisch, 1997). In addition as we will explain below this tick is a vector of pathogens responsible for human and animal diseases.

I. ricinus in Switzerland

I. ricinus is widely distributed in forests below 700m altitude (Kaltenrieder et al., 1985) all over Switzerland (Aeschlimann, 1972) (Mermod et al., 1973) (Mermod et al., 1974) (Mermod et al., 1975) (Aeschlimann et al., 1986) (Miserez et al., 1990) (Zhioua et al., 1994) (Péter et al., 1995) (Humair and Gern, 1998) (Perret et al., 2000) (Jouda et al., in press) (Klaus-Hügli et al., 2002). It was reported to reach a maximum altitude of 1450m (Cotty et al., 1986). In Switzerland as well as in other countries in Europe *I. ricinus* was found to feed on a large range of vertebrates hosts including small, medium and large sized mammals, birds and reptiles (MacLeod, 1932)(Aeschlimann, 1972)(Humair et al., 1993a)(Humair et al., 1993b). In the Swiss plateau *I. ricinus* has been shown to be active from March to November (Mermod et al., 1973) (Mermod et al., 1974) (Gigon, 1985).

Survival of ticks

The tick lifespan is limited by energy which they only obtain from blood meals. Tick survival and more generally arthropod survival is also determined by their ability to maintain water balance (Needham and Teel, 1986) (Needham and Teel, 1991). Tick survival is only possible if water loss does not exceed water intake. The relative humidity at which water loss equals water gain is

called the critical equilibrium humidity (CEH) (Knülle and Wharton, 1964) (Machin, 1979). Ticks like *I. ricinus* do not drink liquid water (Kahl and Alidousti, 1997) (Kröber and Guerin, 1999) but lose and acquire water in the atmosphere (figure 1.2). *I. ricinus* is able to retrieve water from an atmosphere containing at least 85-95% relative humidity (Lees, 1946) at the cost of energy loss: this is called active water sorption (O'Donnell and Machin, 1988). This absorbtion of water from unsaturated atmospheres is possible through the production of a hyperosmotic solution on the hypostome (Gaede and Knülle, 1997). The relative humidity above which water sorption is possible is called the pump threshold (O'Donnell and Machin, 1988). As water loss in arthropods is dependent upon temperature (Needham and Teel, 1986), CEH is also dependent upon temperature. Therefore at relative humidities below the pump threshold, long-term survival of ticks should depend upon saturation deficit[1] rather than upon relative humidity. Above this threshold tick survival will depend upon the energy available for water sorption.

During mobility, respiration and thus spiracle opening increases in ticks (Lighton et al., 1993), resulting in increased water-loss (Rudolph and Knülle, 1979) (Knülle and Rudolph, 1982) (Needham and Teel, 1986). In nature different microclimatic conditions are available for ticks to choose from so that tick life expectation is not only linked to energy use and water balance management but also to tick behaviour.

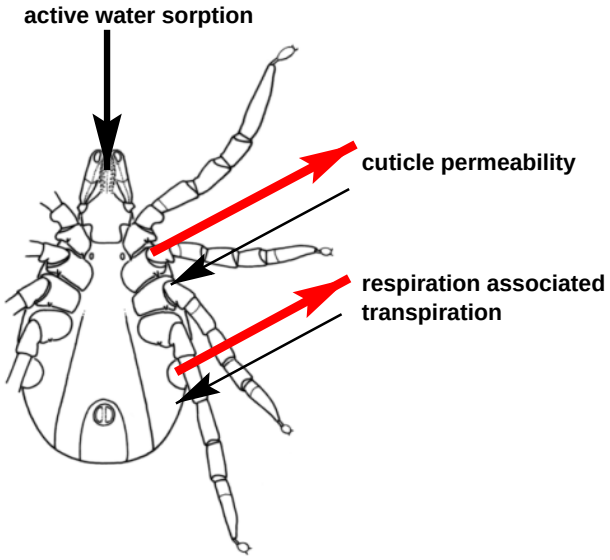


Figure 1.2: **Tick water balance**
arrows qualitatively indicate water flux
(figure: Jean-Luc Perret 2002, tick drawing: Yves Borcard, Institute of Zoology, Neuchâtel)

[1] saturation deficit: water vapor deficit in the atmosphere expressed in mmHg. Since the water vapor amount at saturation is dependent on temperature, saturation deficit is also dependent on temperature (see section 2.2.1.3 on page 24 for further details)

Host-finding behaviour

The host-finding behaviour is the succession of actions undertaken by unfed ticks to get a successful bloodmeal. The host-finding behaviour of *I. ricinus* has been studied since the thirties. It is known that *I. ricinus* individuals repeatedly appear at the tips of vegetation during periods called questing. During such periods ticks wait on the vegetation (Lees and Milne, 1951) at variable heights (Gigon, 1985) (Mejlon and Jaenson, 1997) for a potential host. During questing, ticks experience various climatic conditions, often under their critical equilibrium humidity, so that they lose water (Needham and Teel, 1986). At some point, ticks leave their questing site and move to the litter zone (Lees, 1946) (Milne, 1950) (Lees and Milne, 1951). In nature ticks cannot be observed when they have left their questing place. It was however inferred that after questing *I. ricinus* undergoes a period of quiescence (Lees and Milne, 1951) on the ground where ticks must find a locally humid atmosphere (Lees and Milne, 1951) to rehydrate by active water sorption (Rudolph and Knülle, 1979) (Gaede and Knülle, 1997) (Kahl and Alidousti, 1997). Rehydration in *I. ricinus* requires maximum 24 hours (Kahl and Alidousti, 1997) while typical quiescence in *I. ricinus* lasts for many days (Lees and Milne, 1951) (Gigon, 1985). It is therefore **unclear what ticks do when they are not questing**.

The duration of questing was measured in an open landscape in Great-Britain by Lees and Milne who observed marked adults daily for months on end (Lees and Milne, 1951). They found that *I. ricinus* adults quest for an average of 8-10 days during 3-4 questing events of 2-3 days each, over a period of 35 days. In similar experiments in a lowland forest in Switzerland, Gigon (Gigon, 1985) found that *I. ricinus* adults quest for an average duration of about 40 days in 2.2 questing events of 17 days each over a period of 60 days. Gigon (Gigon, 1985) attributed the discrepancies observed in the questing behaviour between Great-Britain and Switzerland to the different microclimatic conditions experienced by ticks in an open landscape in Great-Britain, and in a forest in Switzerland. He suggested that the forest in Switzerland may have provided less dessicating microclimatic conditions thus enabling longer questing periods than in the open landscape observed in Great-Britain.

Due to their small size, nymphs cannot be individually marked, so that no individual measurements of questing duration in nymphs is available. However Gigon (Gigon, 1985) measured average data for questing duration in a lowland forest of Switzerland and found that on the average total questing duration in nymphs was 11 days within 33 days.

Lees and Milne (Lees and Milne, 1951) and Gigon (Gigon, 1985) all showed big variations in the questing behaviour among individual ticks. However all these authors stated that dessication of questing ticks is the limiting factor for questing duration. We therefore chose to investigate this question experimentally under controlled climatic conditions.

Diurnal rythms in tick activity were also studied by Lees and Milne (Lees and Milne, 1951) who visually observed individually marked adult *I. ricinus* every 2 hours (approximately) during 24 hours. They observed an increased descent to the litter zone during the night, as temperature fell below 10°C. During the day the number of ticks ascending was equal to the number of ticks descending. During this experiment ticks were regularly exposed to host stimuli produced by the observer, so that it is unclear whether Lees and Milne observed the real questing behaviour of ticks or just as a reaction to host stimuli produced by the observer. On the other hand, Gigon

(Gigon, 1985) in Switzerland, observed that most vertical movements in *I. ricinus* adults occurred between 18h and 10h the next morning again in the presence of a human observer. Finally, in *I. scapularis* adults, Carroll et al., using a video camera observing over 48h that most movements occurred during darkness (Carroll et al., 1998). We therefore were interested to follow the host-finding behaviour of *I. ricinus*, especially nymphs, continuously, in both light and darkness under controlled climatic conditions.

The paths followed by moving ticks in nature is difficult to study, especially after questing as ticks disapear into the litter zone (Gigon, 1985). In contrast, the paths followed by climbing ticks was described by Lees and Milne (Lees and Milne, 1951) and by Gigon (Gigon, 1985). They showed that climbing ticks walk up and down grass stems many times before stopping at a questing place. The choice of a questing place may also imply host stimuli, as in *I. scapularis* adults which have been shown to choose their questing places according to chemostimuli left by mammalian hosts on vertical stems (Carroll et al., 1998). Finally the questing height of *I. ricinus* may be influenced by saturation deficit (Randolph and Storey, 1999), vegetation structure (Gigon, 1985) (Mejlon and Jaenson, 1993) and the size of preferred hosts (Gigon, 1985) (Mejlon and Jaenson, 1993).

Since humans are potential hosts for ticks, our knowledge of the host-finding behaviour of *I. ricinus* is limited by our inability to observe them without interfering with their behaviour. We cannot distinguish the behaviours occurring in the absence of hosts from the behaviours occurring as a response to host stimuli.

A general framework for the host-finding behaviour in blood-feeding arthropods was proposed by Hamilton and Hurd (Hamilton and Hurd, 2002). They stated that blood-feeding insects go through 4 successive behaviours leading individuals from a state of hunger to host location, host acceptance and initiation of the blood meal.

Appetitive search: after feeding and moulting, the imperative for blood-feeding arthropods to feed becomes increasingly urgent. Appetitive search is initiated by the state of hunger. Appetitive search will increase the arthropod's probability of encountering host-derived cues. This behaviour was recognised in mosquitoes (Lehane, 1991) and in tsetse-flies (Vale, 1980).

Activation and orientation: activation starts when the arthropod comes into contact with a host cue, switching from a non-directional behaviour to a host oriented response. Host cues may be olfactory, visual, thermic and mechanical.

Attraction: attraction is the behaviour leading the arthropod from a distance to the host. It may imply responses to odours, visual cues, heat and water vapor.

Landing and probing: landing and probing occur within a more or less short distance from the host and leads the arthropod on the host and permits the recognition of host suitability for feeding.

In this framework the word landing refers mainly to flying insects. We therefore named the landing step "grasping" to fit the situation pertinent to ticks (figure 1.3).

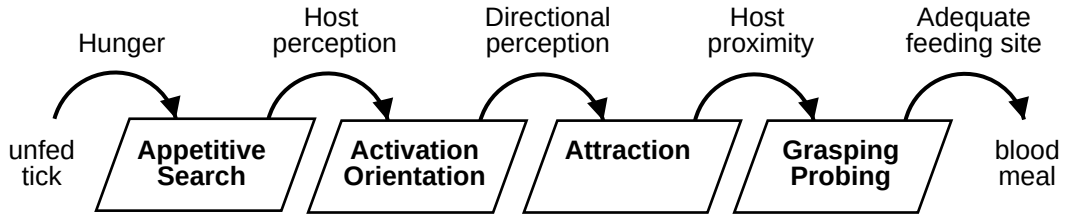


Figure 1.3: **Host-finding process of ticks (adapted from (Hamilton and Hurd, 2002)).**
(figure: Jean-Luc Perret 2002)

These steps may be influenced by endogenous factors (age, nutritional and reproductive status, host preference), by exogenous factors (light, temperature, humidity, wind speed, vibrations, availability of hosts), and by parasites harboured by these arthropods (Hamilton and Hurd, 2002).

We can now evaluate our knowledge of the host-finding behaviour in *I. ricinus* within this framework: It is **unclear whether questing periods in *I. ricinus* are a form of appetitive search** occurring spontaneously as a result of hunger, or whether questing corresponds to behaviours triggered by hosts. When *I. ricinus* is questing, it remains immobile on the vegetation. At the approach of a host, the tick's foremost legs are raised and the tick shows orientation movements (Lees, 1948) (Lees and Milne, 1951). This behaviour corresponds to the "activation and orientation" step described above. Activated individuals may then grasp a passing host if it is close enough. In *I. ricinus* "grasping" occurs randomly. This is shown by the fact that ticks even grab a piece of tissue (flag) dragged over the low vegetation. Under these conditions attraction is limited to the detection of a passing object. It is however unclear if oriented walks towards a nearby host can occur in *I. ricinus*. Indeed horizontal movements in *I. ricinus* is a matter of debate (see below). The recognition of host-suitability only occurs on the host itself. In-vitro feeding experiments have shown that heat, texture of the surface, and chemical stimuli are necessary and sufficient for *I. ricinus* to attach to an articifial membrane (Kuhnert, 1995) (Kuhnert et al., 1995).

The process of finding a host is crucial for tick survival and reproduction. A single engorged female tick lays hundreds or thousands of eggs (Graf, 1978b) so that ticks experience a very fast selection process which is measured by the reproduction rate (r) . **Since host-finding is so crucial, ticks would be expected to display behavioural adaptations to maximize their contact with hosts**, thereby maximising their reproduction rate.

So our interest was to study the host-finding behaviour of *I. ricinus*, a subject that has been studied in the past as described previously. However, most studies implied the presence of an observer, either continuously during a short period, or occasionally during longer periods. The data available are sporadic records of the number of visible ticks, on the assumption that visible ticks were questing and that invisible ticks were quiescent. The observer being present produces a lot of potential stimuli that may be detected by ticks as the presence of a potential host. Indeed *I. ricinus* nymphs have been shown to be activated by a human as far as 3 meters away (Vassallo and Perez-Eid, 2002). The number of visible ticks may thus reflect a mixture of

appetitive search and host attraction responses to the observer's presence. In fact it is even unknown if tick questing and quiescence alternation is even driven by hunger, or if it only occurs in the presence of hosts or stimuli left by hosts. Due to the small size of *I. ricinus* nymphs they are particularly difficult to study, so most studies on *I. ricinus* questing behaviour have focused on marked adults. However, *I. ricinus* nymphs are numerous in the field and are potential vectors of diseases (see below). They therefore represent a threat for humans and animals.

Individual ticks can stay immobile for hours or even days, so continuous observation of single individuals is hardly possible without automation. Finally, humans are not able to see ticks in the dark. For these reasons we developed an automated video tracking system to follow the movements of *I. ricinus* nymphs continuously independent of any host stimuli under controlled climatic conditions and under both light and dark conditions.

I. ricinus horizontal movements

The existence of horizontal movements in ticks of the *I. ricinus* complex is a matter of discussion. While it was admitted for a long time that horizontal movements are short if any, recent findings indicate otherwise. Lees and Milne (Milne, 1950) (Lees and Milne, 1951) have reported very short horizontal movements (a few centimeter), if any, in their study on *I. ricinus* adults. However it must be noticed that these ticks were placed in vegetation patches and were under the regular influence of host stimuli delivered by the observer identifying and counting marked ticks. In contrast to these results, and using a group of ticks marked with a single color, Gigon (Gigon, 1985) showed that horizontal movements could reach up to 2 meters in adult *I. ricinus*. The existence of horizontal movements in *Ixodes* ticks was also proven by the fact that *Ixodes* ticks can be caught using carbon dioxide traps (Gray, 1985) (Falco and Fish, 1991) (Schulze et al., 1997). Using this technique *Ixodes dammini* adults were found as far as 1.8 meters from their release site over 6 days (Falco and Fish, 1991). In *I. ricinus*, using the same technique, Gray recorded horizontal movements of 1 meters in nymphs and 3 meters in adults over 7 days (Gray, 1985). Recently an experiment showed that *I. scapularis* ticks actively choose their questing site according to chemostimuli left by mammalian hosts (Carroll et al., 1998). This suggests that ticks do not climb randomly onto a grass stem, but that their horizontal movements are influenced by host stimuli. In the repeated observations of Lees and Milne (Milne, 1950) (Lees and Milne, 1951), ticks were regularly under the influence of host stimuli. Under these conditions the area of the experiment may have appeared as a very suitable place for host encounter to the ticks, thereby reducing their horizontal movements. Once again these considerations show the importance to control the impact of the observer in tick behaviour studies so as to be able to discriminate between true appetitive search and reactions due purely to the presence of a host.

Moving towards the host or towards a place where host encounter probability is maximised might not be the only reason for horizontal movements. Indeed to rehydrate, *I. ricinus* needs a humid atmosphere. To find such an atmosphere ticks might have to move horizontally. In addition *I. ricinus* adults mostly copulate away from the host from March to May (Graf, 1978a). To find a mate these ticks must therefore move horizontally. All these reasons show that *I. ricinus* ticks might move longer distances than was initially thought by Lees and Milne in their pioneering

work. But to be able to study these horizontal movements we must be able to control the factor "host stimuli" delivered to the ticks.

Phenology of *I. ricinus*

In the field, the density of questing ticks can be estimated by pulling a white flannel flag[2] on low vegetation. Ticks grab the flag and the density of questing ticks per 100m^2 can be estimated. It is important to bear in mind that flagging does not sample among ticks in quiescence. Flagging is therefore not a means to estimate tick population density, but only a means to estimate the questing tick density. We will thus refer to the *questing tick density* rather than to the tick density in the context of field sampling. However the tick density measured with a flag (Q) can be related to the tick population density (P) by equation 1.1.

$$Q = P * p_q * f_q + P * p_w * f_w \tag{1.1}$$

where p_q is the proportion of questing ticks, f_q is the flag success among questing ticks, p_w is the proportion of walking ticks and f_w is the sampling success among walking ticks.

By repeatedly sampling the density of questing ticks we can draw a time plot of the questing tick density. **We call the shape of the questing tick density curve over time the phenology of ticks**.

Throughout its wide distribution *I. ricinus* phenology varies considerably (Gray, 1991). Indeed, in Algeria, *I. ricinus* shows a unimodal pattern with maximum nymph density in winter (Yousfi-Monod and Aeschlimann, 1986). In Ireland *I. ricinus* nymphs and adults show a bimodal pattern reaching its maximum density in spring (Gray, 1984), while in the Crimea the bimodal phenology reaches its maximum in autumn (Korenberg, 2000). In England, both, a unimodal spring peak with a maximum tick density in spring and bimodal peaks were shown to occur simultaneously (Randolph et al., 2002). In Switzerland the maximum density of *I. ricinus* nymphs and adults was recorded in spring (Mermod et al., 1973) with a possible secondary peak occurring occasionally in autumn (Perret et al., 2000). Finally in Sweden the phenology of *I. ricinus* was reported to be variable among years (Tälleklint and Jaenson, 1996). Variations in the phenology of *I. ricinus* may be associated with major variations in biotic (host species, density and behaviour, and vegetation structure) as well as abiotic factors (climate). The temperature interval in which *I. ricinus* could be collected on the grass was already measured in Great-Britain by Macleod in 1932 (MacLeod, 1932). He suggested that ticks can be collected when temperatures exceeded 4.5-7°C and did not exceed 15.5-21.1°C. Similarly the same author (Macleod, 1939) showed that sheep infestation in different areas of Scotland was maximal when temperature was between 4.5 and 21°C. He also showed that ticks can be collected during winter when climatic conditions were mild (MacLeod, 1936). *I. scapularis* in the USA, could be collected during winter as temperature increased over 4°C (Duffy and Campbell, 1994).

In laboratory conditions Macleod (MacLeod, 1935) measured a negative geotropism when

[2]The reason for the flag white colour is to help find dark ticks on a the white background

temperature was between 12°C and 25°C, and a positive geotropism below and above this range when temperature was especially warm or cold. He therefore explained the summer decrease in sheep infestation by a lower negative geotropism reducing the chance for ticks to climb to grab a host. In other words, a lower proportion of questing ticks when temperature were high during the summer.

In Switzerland the onset of tick activity in spring was shown to be related to temperature: both in 1972-1973 in the Staatswald (Mermod et al., 1974) and in 1996-1998 in Neuchâtel (Perret et al., 2000). Therefore, while the general descriptions of *I. ricinus* phenology on a continental scale is adequate, there might be variations from year to year. Indeed, the onset of tick activity and the presence of an autumnal peak of density in nymphs varied in Neuchâtel between 1996 and 1998 (Perret et al., 2000). We therefore wanted to verify whether such differences occur simultaneously in different areas of Western Switzerland, and whether such variations can be related to the various climatic conditions occurring in Western Switzerland.

I. ricinus as a vector of pathogens:

I. ricinus is not only an ectoparasite of vertebrates, but also plays the role of a vector for other parasites. In Switzerland, *I. ricinus* has been shown to transmit bacteria like *Borrelia burgdorferi*, the etiologic agent of Lyme Borreliosis (Burgdorfer et al., 1982), viruses like the tick-borne encephalitis virus (TBE) (Matile, 1982) (de Marval, 1994), rickettsia like *Rickettsia helvetica* (Péter, 1981), (Beati et al., 1993), *R. slovaca*, *Coxiella burnetii* (Aeschlimann et al., 1979) and *Anaplasma phagocytophila* (previously called *Ehrlichia phagocytophila*) (Pfister et al., 1987). *I. ricinus* may also transmit protozoa like *Babesia divergens*, the causative agent of bovine babesiosis (Gern, 1984). Finally *I. ricinus* may also carry trypanosomes (*Trypanosoma theileri*) and nematodes (*Dipetalonema rugosicauda*) (Aeschlimann et al., 1979). In Switzerland two main human pathogens are transmitted by *I. ricinus*. The tick-borne encephalitis virus which is confined within patches in the Eastern and Northern part of the country (Matile, 1982) (Swiss Federal Office of Public Health) (de Marval, 1994), and *B. burgdorferi*, the etiologic agent of Lyme Borreliosis on the other hand, which is widely distributed over the whole of Switzerland (Aeschlimann et al., 1986) (Miserez et al., 1990) (Jouda et al., in press) (Péter et al., 1995) infecting 5-48% of *I. ricinus* ticks (an average infection prevalence of 3% was found in larvae (Zhioua et al., 1994)). *B. burgdorferi* was found to be genotypically heterogeneous (Barbour, 1988) (Barbour, 1989) (Postic et al., 1990) (Baranton et al., 1992). In Switzerland 5 genospecies of *B. burgdorferi* sl were identified: *B. burgdorferi* sensu stricto, *B. afzelii*, *B. garinii*, *B. valaisiana* and *B. lusitaniae* (Hu et al., 1994) (Humair et al., 1995) (Péter et al., 1995) (Humair and Gern, 1998) (Jouda et al., in press). It was shown that some specific associations exist between reservoir host species and *Borrelia* genospecies (Humair and Gern, 1997) (Humair and Gern, 1998) (Humair, 1998) (Kurtenbach et al., 1998) reviewed in (Gern and Humair, 2002) and (K. Kurtenbach et al., 2002). In Switzerland, birds have been found preferentially infected with *B. valaisiana* and *B. garinii* (Humair et al., 1998) while rodents were found preferentially infected by *B. afzelii* and *B. burgdorferi* sensu stricto (Humair et al., 1995) (Hu et al., 1997) (Humair and Gern, 1997).

Both ticks and infecting parasites experience a selection pressure. This pressure may how-

ever be different for ticks and for their parasites. Ticks on one hand depend upon strict energy use and behaviour to maintain their water-balance and to manage the duration of their life-long host-finding behaviour. Parasites transmitted by ticks on the other hand may experience another selection pressure to increase their own reproduction rate (r). Under these conditions the host-finding behaviour may change because of parasite infections (Hamilton and Hurd, 2002). Species of trematodes (*Dicrocoelium dendriticum*), trypanosomes (*Trypanosoma brucei*), protozoans (*Plasmodium sp.*), bacteria (the plague bacillus, *Yersinia pestis*) all manipulate vector's behaviour to increase their own reproduction rate. These vectors include ants, tsetse flies, mosquitoes and fleas. Few studies have addressed this question in ticks. However Alekseev and Dubinina (2000) showed that in Russia, *B. burgdorferi* infected *I. persulcatus* ticks could only be collected after 11:00 am (Alekseev and na, 2000). The impact of such a behaviour on the reproduction rate of ticks and spirochetes is difficult to assess. In Switzerland *I. ricinus* ticks can be collected at any time during the day (personal observation), however the results obtained by Alekseev and Dubinina suggest that spirochetes may be able to control tick behaviour. Two other experimental studies demonstrated an impact of *B. burgdorferi* infection on the behaviour of *I. scapularis*, *I. persulcatus* and *I. ricinus*. Lefcort and Durden measured an increased phototaxy (over 1h) and an increased attraction for vertical surfaces in *B. burgdorferi* infected *I. scapularis* nymphs (Lefcort and Durden, 1996). Their study also showed that infection by *B. burgdorferi* in adult *I. scapularis* did not influence the phototaxy, but reduced the adult ability to overcome obstacles, increased their avoidance of vertical surfaces and reduced their questing height. These measurements were made between 1 and 5 hours following the manipulation of ticks. The behaviour observed by Lefcort and Durden may therefore reflect the tick reaction to host stimuli, and may not reflect the real questing behaviour of these ticks. The impact of these contradictory results in nymphs and adults on their fitness and on the fitness of the transmitted pathogen is difficult to interpret. Another set of experiments was realised by Alekseev et al. with *I. persulcatus* and *I. ricinus*. They measured tick mobility by letting ticks walk during 3 minutes on a tilted surface and by calculating a "mobility index" which consists of a linear combination of speed, turn counts, walking direction and number of falls from the tilted surface (Alekseev, 1999) (Alekseev et al., 2000). Their results showed that *B. burgdorferi* infected *I. persulcatus* and *I. ricinus* have a lower mobility index. Again, it is difficult to relate these results measured over a very short duration (3 minutes) just after tick manipulation to the fitness of ticks or to the fitness of *B. burgdorferi* in nature. The main reason for this difficulty being once again the impossibility to distinguish a potential appetitive search of ticks from an activation and orientation response in the presence of a host, potentially followed by an attraction towards the host.

Considering our poor understanding of the host-finding behaviour of *I. ricinus* we investigated several aspects of this behaviour based on the following hypothesis: The host-finding probability of *I. ricinus* ticks is influenced by the duration of questing, by the duration of quiescence, and by walking activities. We therefore studied the host-finding behaviour of *I. ricinus* under both experimental and natural conditions.

The present work will focus on the biology of *I. ricinus*. Unless otherwise specified, the word tick therefore refers to *I. ricinus* only.

Aims

The aims of this study were to clarify the host-finding behaviour of *I. ricinus* nymphs, in particular the occurrence of an appetitive search in this tick. Then we wanted to study the impact of climate and the impact of *B. burgdorferi* sl on this behaviour. Finally we were interested in the phenology of ticks in the field and wanted to investigate the relation between the phenology of ticks and climate in Western Switzerland.

Three different approaches were used:

- The first one involved the continuous observation of tick behaviour under controlled climatic conditions, in order to study spontaneous appetitive search in *I. ricinus*. We wanted to verify that questing and quiescence occur in *I. ricinus* nymphs in the absence of host stimuli, and that the duration of questing periods are related to saturation deficit (Perret et al., 2000). By undertaking continuous observations of ticks under laboratory conditions we also wanted to study tick activity when in nature ticks disappear from the tips of vegetation and are invisible to humans.

- The second approach used to study the relation between climate and tick behaviour was to follow the proportion of questing ticks in polyamide mesh delimited arenas in the field and to relate this proportion to climatic conditions.

- The third approach was to verify that *I. ricinus* survival is related to saturation deficit when relative humidity is below the pump threshold for active water sorption.

- Finally, we studied the relations between the phenology of ticks and climate in the field. To do so, we measured the phenology of ticks in three sets of locations: in Neuchâtel, on an altitude gradient, and in regions with different climates at low altitudes in Western Switzerland.

As a help for the reader, a glossary containing definitions of terms can be found in appendix F and a list of abbreviations can be found in appendix G.

Chapter 2

Materials and Methods

2.1 General comments on statistical analysis

Unless otherwise specified, all statistics described in the following sections were performed using R[1] for Linux V0.90.0 (Ihaka and Gentleman, 1996) running on a SuSE 6.1 Linux® computer (Pentium® processor). Date and time variables were processed with the help of the "chron" package. Statistical tests were performed using the base functions or the additional functions provided by the "ctest" package. Circular data analysis was perfomed with the help of the "circdes" function written by Jacqueline Moret[2] (Including statistics for the Rayleigh test). The significance level of Rayleigh tests was evaluated according to the table given in "Circular statistics in Biology" (Batschelet, 1981).

2.2 Field experiments

2.2.1 Climatic data

2.2.1.1 Mesoclimatic data

Climatic data were provided by the Swiss Meteorological Institute. Data were extracted from the SMA[3] database on the "wawona" server at the ETHZ, using a homemade Fortran program (see file loadclimatthese.for on the cd-rom). Basically the program writes a flat file (comma separated fields) containing the following fields for each hour: minimum temperature, average temperature, maximum temperature and average humidity. The program needs the year of interest (4 digits), a name for the output file, and the station number. The following table is a subset of station numbers for the existing Meteosuisse automatic meteorological stations:

[1] R is a language which is similar to the S language which was developed at Bell® Laboratories (formerly AT&T® , now Lucent Technologies®) by John Chambers and colleagues. R is available as Free Software under the terms of the Free Software Foundation's GNU General Public License in source code form. See http://www.R-project.org/

[2] Institute of Mathematics, University of Neuchâtel

[3] Schweizerische Meteorologische Anstalt

No	Place	Code	CH1903 coordinate	Altitude (m)
2	Payerne	PAY	562150/184855	490
7	Aigle	AIG	560120/130630	381
23	Neuchâtel	NEU	563150/205600	485
25	Interlaken	INT	633070/169120	580
31	Genève-Cointrin	GVE	498580/122320	420
37	Viège/Visp	VIS	631150/128020	640
51	Changins	CGI	507280/139170	430

Subset of the Meteosuisse Automatic Meteorological Stations (ANETZ).
CH1903: swiss grid coordinates (see 2.2.2.5 on page 29)

The file produced was then retrieved using ftp[4] and processed further locally. Dates and times were added with the program "addtime.perl" and finally, the program "clh2cld.perl" calculated daily minimum, average, and maximum values for temperature, relative humidity and saturation deficit (see 2.2.1.3).

The whole procedure resulted in two files: .clh containing hourly values, and .cld containing daily values. Figures were drawn using R V0.90.0.

The detailed procedure for data retrieval can be found in appendix E on page 219.

2.2.1.2 Microclimatic data

Microclimatic data were recorded using a squirrel 1200 logger manufactured by Grant Instruments (Shepreth, UK[5]). The usual setting included the use of the following probes:

- Temperature probe at 60 cm above ground (type U thermistor)
- Temperature probe at 10 cm above ground (type U thermistor)
- Relative humidity probe at 10 cm above ground (type L Vaisala HPM31)
- Temperature probe 10 cm in the ground (type U thermistor)

Detailed squirrel settings are given in table D.1 in appendix D on page 217. Data were downloaded from the squirrel through the serial interface with the DOS transfer program provided by Grant Instruments.

2.2.1.3 Saturation deficit

Saturation deficit is a measurement of the "drying power" of the air. It can be calculated using the following empirical formula (Randolph and Storey, 1999):

[4] File Transfer Protocol
[5] imported by Tectron Ag, Fortunastrasse 3, 8636 Wald, Switzerland

$$SD = \left(1 - \frac{RH}{100}\right) * 4.9463 * 2.71828^{(0.0621*T)} \quad (2.1)$$

where SD is the saturation deficit in mm Hg, T is the temperature in degree Celsius and RH is the relative humidity in percent. Saturation deficit decreases linearly with relative humidity and increases non linearly with temperature.

2.2.1.4 Meso-Microclimate comparison

To estimate the difference between the mesoclimatic variables measured by the Swiss Meteorological Institute in Neuchâtel with the microclimate inside the forest North of Neuchâtel, we recorded air temperature at 10 and 60cm above ground and air relative humidity at 60 cm above ground in the experimental garden at the Neuchâtel University botanic garden (coordinates CH1903: 561550/205575). Measurements were made continuously during three periods: 21.3 - 25.4.2000, 14.8 - 5.9.2000 and 3.10 - 25.11.2000. Due to water condensation on the outside protection of the relative humidity probe, measurements at high relative humidity were inaccurate, so that the values used for the comparison between micro and mesoclimatic recordings of relative humidity and saturation deficit only included the hours when relative humidity was below 100%.

2.2.2 Tick sampling in the field

2.2.2.1 Method

Sampling of questing *I. ricinus* was made by pulling a 1m^2 white terry flag over the vegetation. A wood stick was used to pull the flag on the sides of the user's walking path (figure 2.1). **At each sampling location ticks were always sampled from the same surface**. Individual ticks were counted and collected on the flag every 25 meters (except for the extended surface sampling in Neuchâtel from 1998 to 2002 where ticks were collected every every 18 meters). These sampling units were called a "drag". The optimal efficiency for tick sampling by flagging was shown to be reached with drag length of 10 meters (**?**). A longer drag length was however used during this study to enable comparisons with data available in Neuchâtel in 1996 and 1997. Instars and sexes were kept in separate tubes. Sampling areas located along a footpath were sampled alternatively on each side of the path (except for the extended surface sampling in Neuchâtel where ticks were only collected on the uphill side every 25 meters (in 1996 and 1997) or 18 meters (from 1998 to 2002)). Sampling was not undertaken during rainy days and during days with wind reaching 11 km/h in the forest.

Figure 2.1: **Flagging technique**
Tick sampling technique (not a real sampling site) using a white flag.
(photo: Jean-Luc Perret 2002)

2.2.2.2 Questing tick density

The questing tick density was defined as the number of individuals collected per 100m^2 (m^2 abbreviated sqm in some figures).

2.2.2.3 Phenology of ticks

The phenology of ticks was evaluated by repeatedly sampling a single area. The phenology was represented graphically in the form of timeplots of the quesing tick density. On these timeplots nymphs were represented with a thin line while adults were represented with a bold line. To be able to compare questing tick density between locations and between years, several descriptive statistics were derived separately for nymphs and adults (figure C.1). These statistics were based on the repeated observations of the questing tick density and on the two following assumptions: the evolution of the questing tick density between measurements can be considered as linear and the beginning of tick activity from the 31. of January until the first measurement is linear.

Different descriptive statistics were calculated from the phenology of nymphs and adults. A subset of the most usefull statistics is presented below, and an exhaustive list of these statistics is given in appendix C on page 151. We distinguished between descriptive statistics for the whole year, for the spring peak and for the autumn peak of questing tick density.

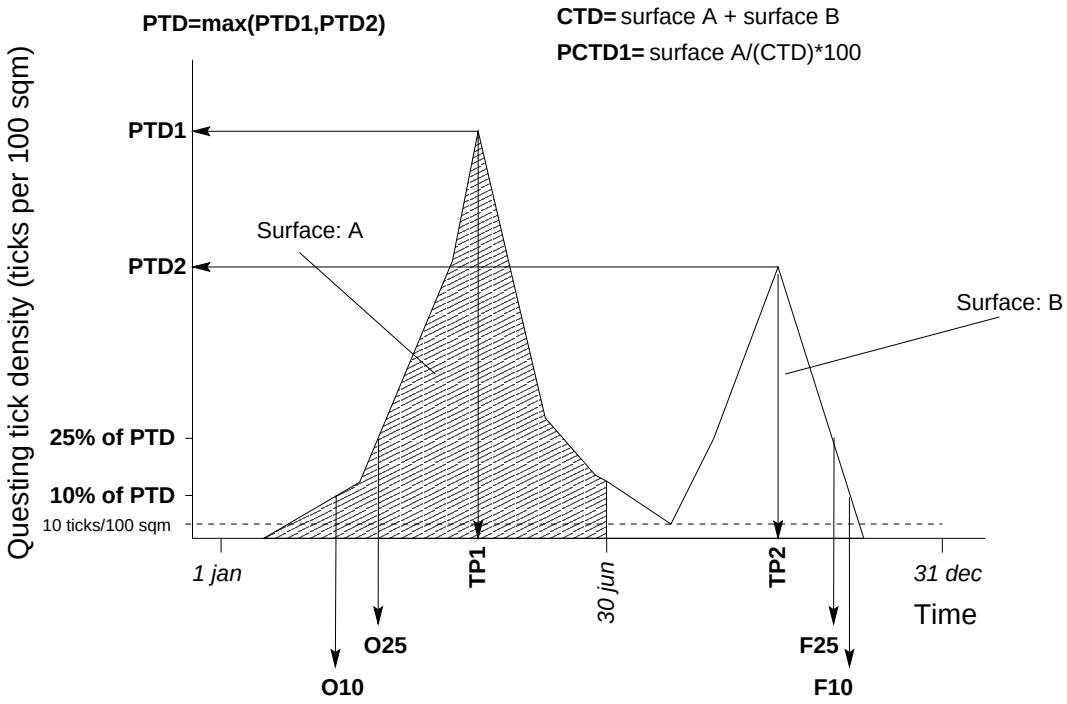


Figure 2.2: **Subset of the descriptive statistics calculated to describe tick phenology** for details see text.
(figure: Jean-Luc Perret 2002)

Descriptive statistics for the whole year:

Timing of peak (TP) : The timing of peak was defined as the sampling date corresponding to the maximal questing tick density over one year, calculated separately for nymphs (TPN) and adults (TPA).

Peak tick density (PTD) : Maximal tick density recorded during one year, calculated separately for nymph (PTDN) and adult (PTDA) peak density

Onset 25% (O25) : First date when tick density increased above 25% of the peak tick density, calculated separately for nymphs (O25N) and for adults (O25A).

Onset 10% (O10) : First date when questing tick density increased above 10% of the peak tick density, calculated separately for nymphs (O10N) and adults (O10A).

Fall 25% (F25) : Last date when questing tick density decreased below 25% of the peak tick density, calculated separately for nymphs (F25N) and adults (F25A).

Fall 10% (F10) : Last date when questing tick density decreased below 10% of the peak tick density, calculated separately for nymphs (F10N) and adults (F10A).

Cumulative tick density (CTD) : The cumulative tick density (in number of ticks per 100m^2 and per year) was estimated by integrating the questing tick density curve over one year[6]. This statistic was calculated separately for nymphs (CTDN) and adults (CTDA).

Proportion of cumulative tick density in the first half-year (PCTD1) : The proportion of cumulative tick density during the first half-year (in %) was calculated as the following ratio:

$$\%\ tick\ presence\ in\ first\ half\ year = \frac{\int_{1\ January}^{30\ June} questing\ tick\ density\ curve}{\int_{1\ January}^{31\ December} questing\ tick\ density\ curve} * 100$$

This statistic was calculated separately for nymphs (PCTD1N) and for adults (PCTD1A).

Proportion of cumulative tick density in the second half-year (PCTD2) : The proportion of cumulative tick density during the second half-year (in %) was calculated as 100-PCTD1

This statistic was calculated separately for nymphs (PCTD2N) and for adults (PCTD2A).

Descriptive statistics for the spring peak:

Timing of peak 1 (TP1) : the sampling date corresponding to the maximal questing tick density before June 30, calculated separately for nymphs (TP1N) and adults (TP1A).

Peak tick density 1 (PTD1) : maximal tick density recorded before June 30, calculated separately for nymphs (PTD1N) and adults (PTD1A).

Descriptive statistics for the autumn peak:

Timing of peak 2 (TP2) : the sampling date corresponding to the maximal questing tick density after F1.25N or if there is no spring peak, after June 30, calculated separately for nymphs (TP2N) and adults (TP2A).

Peak tick density 2 (PTD2) : maximal tick density recorded after June 30, calculated separately for nymphs (PTD2N) and adults (PTD2A).

[6] Leap years induce a slight error in the estimation of the cumulative tick density, but this error was considered as negligible and ignored

2.2.2.4 Phenology in relation with climate

The descriptive statistics defined above for the phenology of ticks were related to descriptive statistics for the climate. Statistics on climate were based upon hourly average measurements recorded by automatic weather stations of Meteosuisse (see 2.2.1 on page 23). We tried to relate the onset of tick activity in spring (O10, O25 and TPN) with temperature. To do so we calculated the cumulated hourly average temperature from the 1st of January to the 1st of February, 1st of March, 1st of April and 1st of May. We were also interested to correlate the importance of the autumn peak of activity with temperatures during and after the spring peak. To do so we calculated the cumulated hourly average temperature from the 1st of March to the 1st of July.

2.2.2.5 Geographic coordinates

The geographic coordinates of locations in Switzerland were expressed using the Swiss grid system CH1903 as defined by the "Office fédéral de la Topographie" in Bern. Briefly the system uses a Bessel 1841 ellipsoïd projection with its fundamental point at the old observatory of Bern (7° 26' 22.50" North and 46° 57' 08.66" East according to the ETRS89 reference system) and a meter based grid with the coordinate Y = 600'000 m and X = 200'000 m at the fundamental point in Bern. Coordinates are written with the format: XXXXXX/YYYYYY where X and Y are the coordinates in meters. This grid is printed on Swiss topographical maps (1:25000 and 1:50000) and is available in most GPS receivers.

2.2.2.6 GPS receiver

To measure coordinates in the field a Garmin®12 GPS receiver was used.

2.2.2.7 Tick sampling areas

Ticks were sampled monthly in the field during three main experiments. First, to have a precise measurement of the questing tick density over time, an extended surface in Neuchâtel (figure 2.3). Second, a set of 10 areas located over Western Switzerland were sampled monthly within 48h to test the spatio-temporal variability in the density of questing ticks (figure 2.3). Finally ticks were sampled monthly along an altitude gradient to test the impact of altitude on the density of questing ticks (Chaumont, figure 2.3). Sampling locations were all chosen in deciduous dominant forests below 800m (except for the altitude gradient), and according to the goal of the investigation, the proximity of a Meteosuisse meteorological station, the existence of previous records on the presence of ticks and accessibility (in the case of the 10 locations in Western Switzerland).

Extended surface sampling in Neuchâtel: To assess the spatio-temporal evolution of questing tick density in the forest North of Neuchâtel, an extended surface was sampled monthly from March 1996 to August 2002 (descriptive statistics needing a full year of sampling were calculated with the data covering the period 1996-2001) (figure 2.3). Sampling took place between 10h and

17h. In 1996 and 1997 a 375m^2 surface, divided into four consecutive zones, was sampled (locations 40-43 in table 2.2). From 1998 to 2002 the monthly sampled surface was extended to a 1049m^2 path (including 250 meters of the path used in 1996 and 1997) also divided into four zones (locations 36-39 in table 2.2). Climatic data were measured in Neuchâtel by Meteosuisse (station number 23 in table 2.2.1.1 on page 23).

Sampling in Western Switzerland: To assess spatial variability in the density and phenology of questing *I. ricinus*, we sampled 10 different areas in Western Switzerland on a monthly basis from the beginning of 1999 to the end of 2001 (figure 2.3). These locations were located in deciduous dominant forests between 400 and 800 meters above sea level: Vaumarcus, Bavois, Eclépens, Bois-de-Portes, Brochus-Creuson, St-Livres, Bière, Aigle, Viège[7] and Interlaken (figure 2.3, locations 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 in table 2.2). To enable the collection of all samples within 2 consecutive days, a relatively small surface (150m^2, 100m^2 at Bavois) was sampled at each location (sampling occurred between 08h30 and 20h00. The forest in Vaumarcus (location 6) was destroyed during the winter of 1999-2000 due to the N5 motorway construction. The study area in the destroyed forest was still sampled until the 19th October 2000 to check for tick presence under extreme conditions (trees were removed; remaining branches were chopped into 1-30 cm sized pieces and left on the ground).

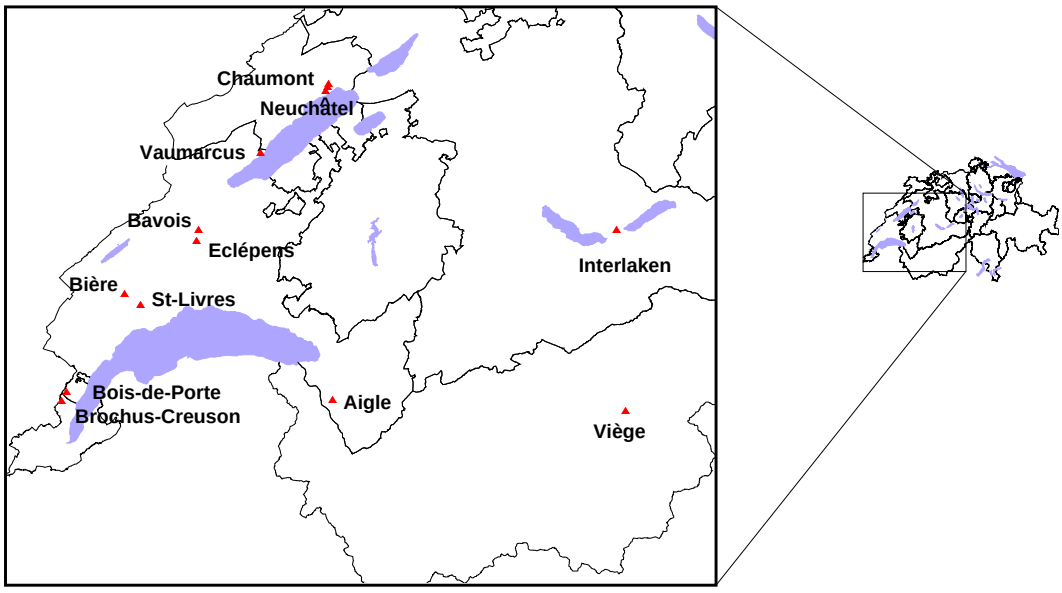


Figure 2.3: **Location of the sampled areas in Western Switzerland.**
figure: Jean-Luc Perret 2002, cartographic background: GEOSTAT, Office fédéral de la statistique, Office fédéral de la topographie, groupe SIGNE.

[7] Viège and Visp are respectively the french and the german name for the same town in the canton of Wallis/Valais

Code	Location	Coordinates CH1903	Altitude (m)	Surface (m^2)	Picture figure	Meteosuisse station
13	Aigle	563994/128790	420	150	2.4	Aigle
7	Bavois	531907/171132	440	150	2.5	Payerne
12	Bière	513845/155263	800	100	2.6	NA
9	Bois-de-Portes	499894/130664	480	150	2.7	Cointrin
10	Brochus-Creuson	498900/128450	440	200	2.8	Cointrin
3	Chaumont High	563121/207718	900	150	2.14	NA
4	Chaumont Middle	562670/207125	740	150	2.15	NA
5	Chaumont Low	562287/206035	620	125	2.16	NA
8	Eclépens	531336/168437	485	150	2.9	Payerne
15	Interlaken	632431/171149	620	150	2.10	Interlaken
36	Neuchâtel Zone 1b	563017/206063	520	437		Neuchâtel
37	Neuchâtel Zone 2b	562650/205987	570	210		Neuchâtel
38	Neuchâtel Zone 3b	562764/206105	585	245		Neuchâtel
39	Neuchâtel Zone 4b	562975/206140	560	157		Neuchâtel
40	Neuchâtel Zone 1a	563017/206063	520	125		Neuchâtel
41	Neuchâtel Zone 2a	562890/206000	535	125		Neuchâtel
42	Neuchâtel Zone 3a	562975/206140	560	75		Neuchâtel
43	Neuchâtel Zone 4a	563040/206155	550	50		Neuchâtel
11	St-Livres	517778/152534	680	150	2.11	Changins
6	Vaumarcus	546669/190400	480	150	2.12	NA
14	Viège/Visp	634552/125852	780	150	2.13	Visp

Table 2.2: **Location of the tick sampling areas**
CH1903: Swiss grid coordinates (see 2.2.2.5 on page 29) (Neuchâtel Zone 1-4a were used from 1996-1997 and Neuchâtel Zone 1-4b were used from 1998-2002)

Swiss grid coordinates (CH1903): 563994/128790
Altitude: 420m
Canton: VD
Date of photograph: June 1999

Figure 2.4: **Aigle**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 531907/171132
Altitude: 440m
Canton: VD
Date of photograph: June 2000

Figure 2.5: **Bavois**.
(photo: Jean-Luc Perret 2000)

Swiss grid coordinates (CH1903): 513845/155263
Altitude: 800m
Canton: VD
Date of photograph: June 1999

Figure 2.6: **Bière**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 499894/130664
Altitude: 480m
Canton: VD
Date of photograph: June 1999

Figure 2.7: **Bois de Portes**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 498900/128450
Altitude: 440m
Canton: VD
Date of photograph: June 1999

Figure 2.8: **Brochus-Creuson**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 531336/168437
Altitude: 485m
Canton: VD
Date of photograph: June 1999

Figure 2.9: **Eclépens**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 632431/171149
Altitude: 620m
Canton: BE
Date of photograph: June 1999

Figure 2.10: **Interlaken**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 517778/152534
Altitude: 680m
Canton: VD
Date of photograph: June 1999

Figure 2.11: **St-Livres**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 546669/190173
Altitude: 480m
Canton: VD
Date of photograph: June 1999

Figure 2.12: **Vaumarcus**.
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 634552/125852
Altitude: 780m
Canton: VS
Date of photograph: June 1999

Figure 2.13: **Viège/Visp**.
(photo: Jean-Luc Perret 1999)

Sampling along an altitude gradient: To assess variations in tick phenology with respect to altitude, three sampling areas were selected at different altitudes: 900, 740 and 620 meters. These areas were located on the south exposed face of Chaumont mountain, North of Neuchâtel, in a forest reaching 1000 meters above sea level (figure 2.3). For the two highest locations there were six consecutive drags of 25 meters ($150m^2$) and 5 drags for the lowest altitude ($100m^2$). They were sampled alternatively on both sides of the footpaths during the same afternoon on a monthly basis from March 1999 until November 2001. The locations are described in more details in figures 2.14, 2.15 and 2.16.

The cumulative tick density was calculated for 1999, 2000 and 2001 separately for nymphs and adults. To test the existence of a relation between tick density and altitude we used the non-parametric Jonckheere test (Siegel and Castellan, 1988). Similarly, we tested the existence of a relation between altitude and the maximum tick density in spring by using the Jonckheere test.

Swiss grid coordinates (CH1903): 563121/207718
Altitude: 900m
Canton: NE
Date of photograph: June 1999

Figure 2.14: **Chaumont Highest Altitude**
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 562670/207125
Altitude: 740m
Canton: NE
Date of photograph: June 1999

Figure 2.15: **Chaumont Middle Altitude**
(photo: Jean-Luc Perret 1999)

Swiss grid coordinates (CH1903): 562287/206035
Altitude: 620m
Canton: NE
Date of photograph: June 1999

Figure 2.16: **Chaumont Lowest Altitude**
(photo: Jean-Luc Perret 1999)

2.2.2.8 Spatial distribution of *I. ricinus* at local scale

The horizontal distribution of ticks was measured between March 1999 and August 2002 by the extended surface sampling in Neuchâtel (see 2.2.2.7 on page 29). We were interested to test whether ticks were randomly distributed along the sampling path. To test the null hypothesis of random distribution, the number of ticks collected was recorded every 18 meters, and the Poisson index (Vassallo et al., 2000) was calculated for each sampling session. Briefly the Poisson index (I) of a sample of size N is the variance (V) of the sample divided by the mean (M): $I = \frac{V}{M}$. The null hypothesis of $I = 1$ was tested using $(N-1)I$ which follows a χ^2 distribution with $N-1$ degrees of freedom. As the test was repeated for each month, the Bonferroni correction was also applied and a significance level of 0.001 was chosen.

In addition, an autocorrelation function was calculated for each sampling session. Since 44 autocorrelation functions were calculated (for each sampling session) the Bonferroni correction was applied and a significance level of 0.001 was chosen.

All calculations were done using R for Linux V1.4.1 (Ihaka and Gentleman, 1996) running on a SuSE 7.1 Linux® computer (Pentium® processor) and an additional package called "ts".

2.2.3 Field arenas

Six field arenas were built to observe tick activity in the field. These arenas were made of a cylindric polyamide mesh (500 μm grid, Sefar-Nitex, Sefar AG, CH-9410 Haiden) closed with a plastic sheet on the top. The arenas were driven into the ground using an open plastic cylinder at the bottom (see figure 2.17). The 6 arenas were set up in the forest located North of the experimental garden at the University of Neuchâtel botanic garden (coordinates CH1903: 561550/205575). Ticks were placed in 3 arenas at the end of March 2000 (21.3 2000), and in 3 other arenas during the second half of May (17.5.2000). A total of 426 nymphs, 54 females and 83 males were placed in the arenas (instars were mixed in all arenas, see table 2.3). Forty nymphs were added to arenas 4 and 5 on the 23rd of October 2000 (2.3) (these additional ticks were collected on the same day in the forest close to the botanical garden). Observations lasted from the 21.3.2000 to the 22.12.2000. The number of ticks visible in the arenas was recorded 216 times from the 22 March 2000 to 22 December 2000 (twice daily, morning and afternoon over 74 days and once daily over 68 days). Results are presented as timeplots of the proportion of visible ticks (nymphs and adults separately). In order to highlight the evolution of this proportion, a non parametric smoothing of the data (Friedman's supersmoother) was applied using the default options of the function "supsmu" of the package "modreg" in R for Linux V0.90.0. The number of ticks visible in the morning and in the afternoon was compared using the paired Wilcoxon test. The numbers of visible nymphs and adults were compared using the unpaired Wilcoxon test for each observation of the arenas.

no arena	date of ticks introduction	No. nymphs	No. females	No. males
1	21.3.2000	126	10	17
2	24.3.2000	53	9	26
3	24.3.2000	67	5	10
4	17.5.2000	60+40*	10	10
5	17.5.2000	60+40*	10	10
6	17.5.2000	60	10	10
total		426+80*	54	83

Table 2.3: **Distribution of ticks in the field arenas**

* added on 23 October 2000 (see text for further details).

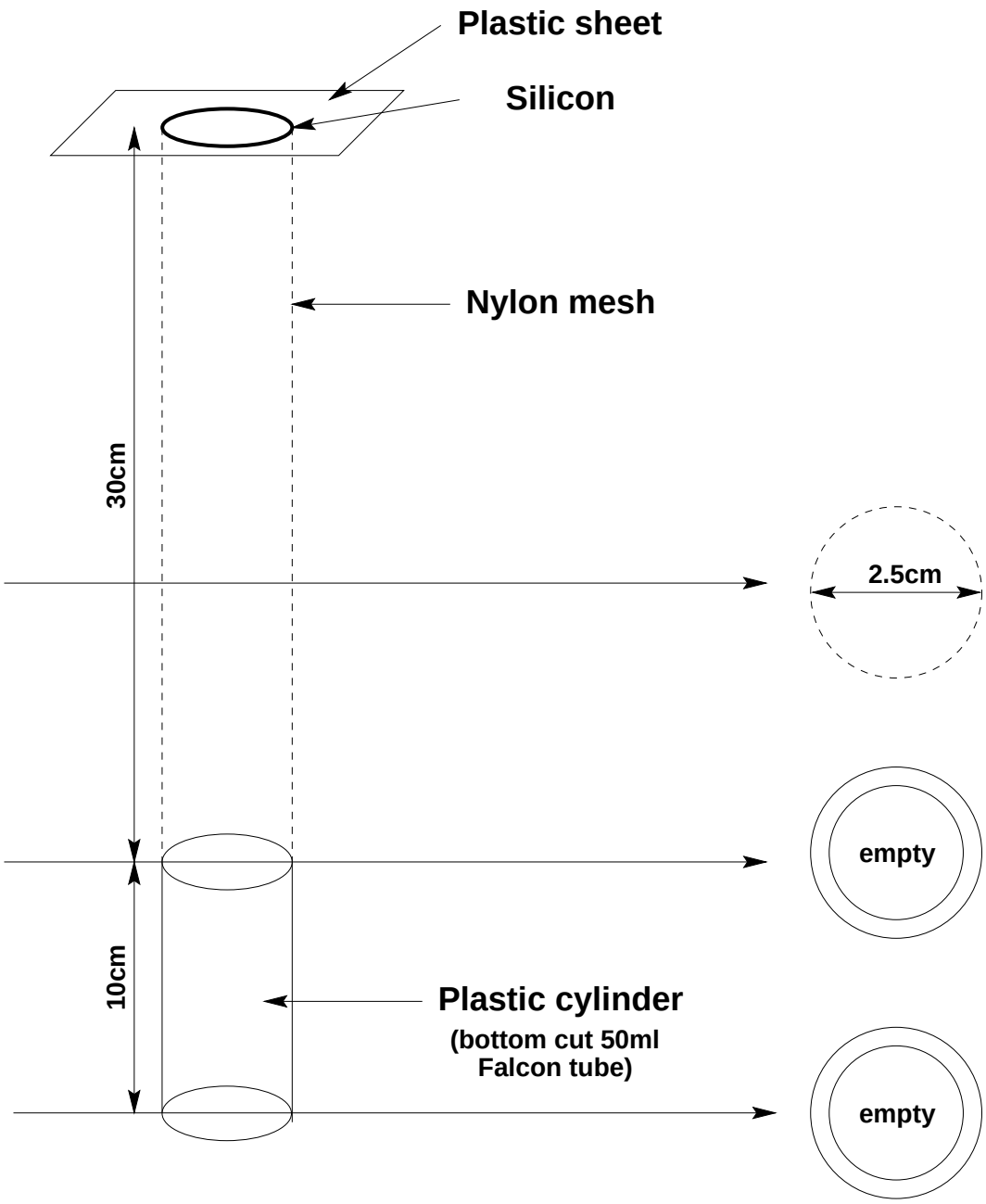


Figure 2.17: **Field arenas**.
The arenas were made of a cylindric polyamide mesh (500 μm). The top was closed using a plastic sheet and the bottom was glued to a plastic cylinder (open at both ends) which was driven into the ground.
(figure: Jean-Luc Perret 2002)

2.2.4 Tick recruitment by Beagle dogs

In order to test the correlation between the density of questing ticks in the field and the tick recruitment rate of a vertebrate host, we organised walks with Beagle dogs in the forest North of Neuchâtel («Bois-Gentil»), in collaboration with Novartis Animal Health (Saint-Aubin, FR). During these walks the questing tick density, the path walked and the resulting infestations of dogs were measured. These experiments took place on the 3rd and 7th of April 2000 and on the 6, 7, 8, 15 and 16 May 2002. The walked path was measured using a Garmin®12 GPS. Tracks were imported in a Linux computer using gpsman (V4.0, 21.12.1999). Walked distance, average speed and duration of the walk were calculated from the recorded tracks using the same software. Questing tick density on the vegetation was measured over 200m^2 in a sampling area in «Bois-Gentil» (North of Neuchâtel, coordinates: 562510/205991, altitude: 600m) (see 2.2.2 on page 26 for the sampling method). The area sampled for questing ticks was avoided during the walks with the dogs.

Immediately after the walks and 24h after the walks, dogs were visually examined for attached ticks at the Novartis Animal Health research center in Saint-Aubin (FR).

Based on questing tick density, distance walked and host surface, we calculated the expected number of ticks met by each dog during the walks. To do so the contact surface of dogs with the vegetation was estimated to be 50 cm^2 and the following formula was used:

$$MET = FD * W * DS \tag{2.2}$$

where MET is the expected number of ticks met by dogs during the walk, FD is the questing female tick density collected by flag sampling (individuals/m^2), W is the walked distance (meters), and DS is the dog surface in (0.5 m^2) [8].

[8] On the 7 and 16 May 2002 the questing tick density was not measured by flagging. To calculate the expected number of ticks met by the dogs, we used the questing female density recorded one day before.

2.3 Laboratory experiments

2.3.1 Tick survival

In order to compare male and female *I. ricinus* survival under different climatic conditions, ticks were collected in the field between the 9th and the 23rd of May 2001 on the Chaumont Mountain (locations 3 and 4, section 2.2.2.7 on page 29). Ticks were kept in water saturated atmosphere at ambient temperature until the 29th of May 2001 when they were transferred into the survival test arenas (figure 2.18). Saturated solutions of salts at the bottom of the arenas provided different relative humidities in the upper part of the arenas. These test arenas containing either 10 males or 10 females each were placed in incubators (constant dark) at different temperatures (14°C, 25°C, 28°C, 34°C), thus resulting in 20 tested climatic conditions (table 2.4). The number of dead ticks were counted over 10 days at various intervals (after 20, 28, 44, 52, 68, 76, 93, 119, 145, 165, 187 and 240 hours of incubation). The mean survival time of ticks was calculated for males and females for each tested climatic condition.

relative humidity	11%	32-33%	75%	83-85%	97-98%
salts / temperatures	$LiCl$	$MgCl_2$	$NaCl$	KCl	K_2SO_4
14°C	10.5	7.9	2.9	1.7	0.25
25°C	20.8	15.7	5.7	3.7	0.63
28°C	25.1	19.0	7.0	4.5	0.80
34°C	36.4	27.8	10.2	7.0	1.35

Table 2.4: **Water saturation deficits (mm Hg) tested for the estimation of *I. ricinus* adult survival times**.

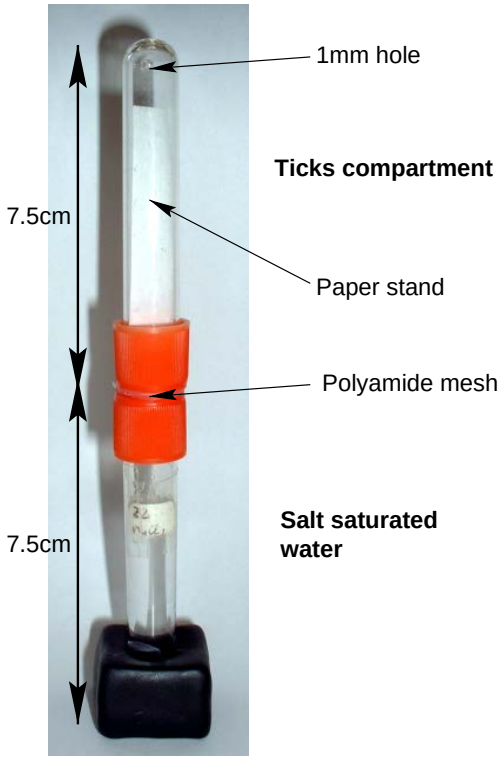


Figure 2.18: **Survival test arena**.
The arenas were made of two plastic culture tubes (Milian VSS751) with their screw caps perforated and separated by a polyamide mesh. The tubes were glued together by their caps using silicone.
(figure: Jean-Luc Perret 1999)

2.3.2 Continuous vertical movement tracking

2.3.2.1 Experimental setup

I. ricinus nymph movements were recorded with an experimental setup (figure 2.19). Transparent plastic arenas were built to confine the ticks and enable their tracking. The arenas are composed of vertical grooves (100x6x1.2 mm) in which tick nymphs can walk (figure 2.21). The grooves are delimited by plastic, and by a polyamide mesh (500 μm grid, Sefar-Nitex, Sefar AG, CH-9410 Haiden) on one face. Water is provided by a wet cotton wick at the bottom of the grooves (simulating water saturated atmosphere in the ground). Ten ticks can be placed simultaneously in the arena. Nymphs collected in the forest North of Neuchâtel were kept for a minimum of 1 week in a water saturated atmosphere at room temperature. Ten nymphs were then transferred into the grooves of the tracking system. They were finally placed in a climatic chamber where light, humidity and temperature were controlled. A constant infrared source (CES Ag Dübendorf (ordering no: 20177) WFL2-LED30 950nm 50° 30W) was used to provide observation light during darkness. Philips toucam pro webcams were modified as follows: the built-in "infrared-block" filter was replaced by an "infrared-pass" filter (Kodak 87, Figure 2.20 on page 48). This modification allows the continuous observation of ticks under changing light conditions. The webcam was connected by the USB interface to an Intel based computer[9] running SuSE Linux 7.1. As a result ticks appeared on the computer screen as white spots on a dark background.

[9] Pentium II 450 Mhz

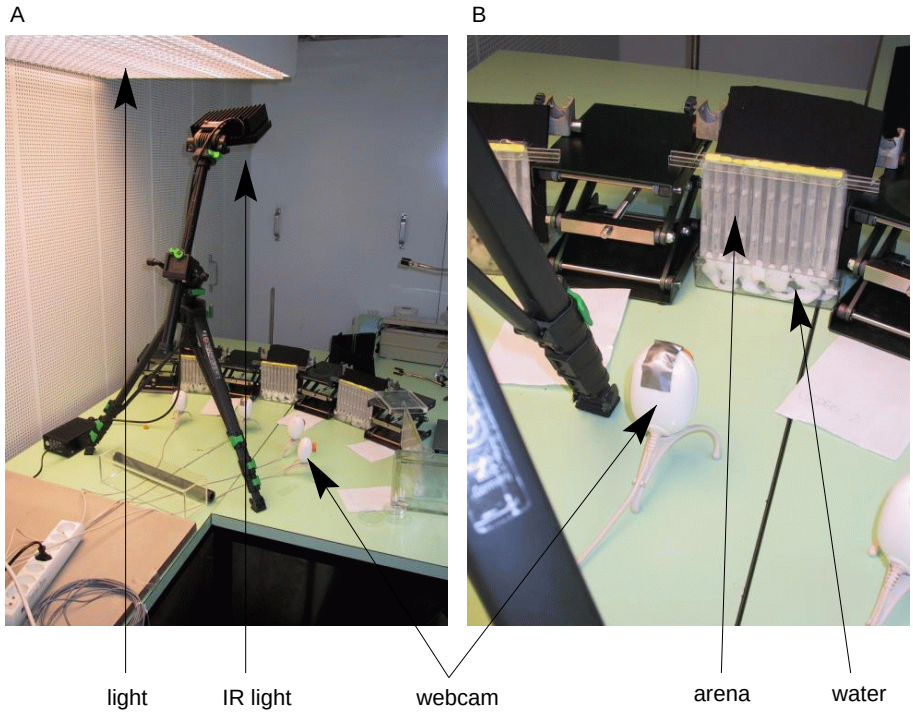


Figure 2.19: **Computer-assisted tracking system**.
Webcams were used to observe the vertical movements of *I. ricinus* nymphs in vertical arenas. An infrared light provided constant IR illumination, while the visible light could be varied in intensity. Water saturated atmosphere was provided at the bottom of the arenas by a wet cotton wick.
(photos: Jean-Luc Perret 1999)

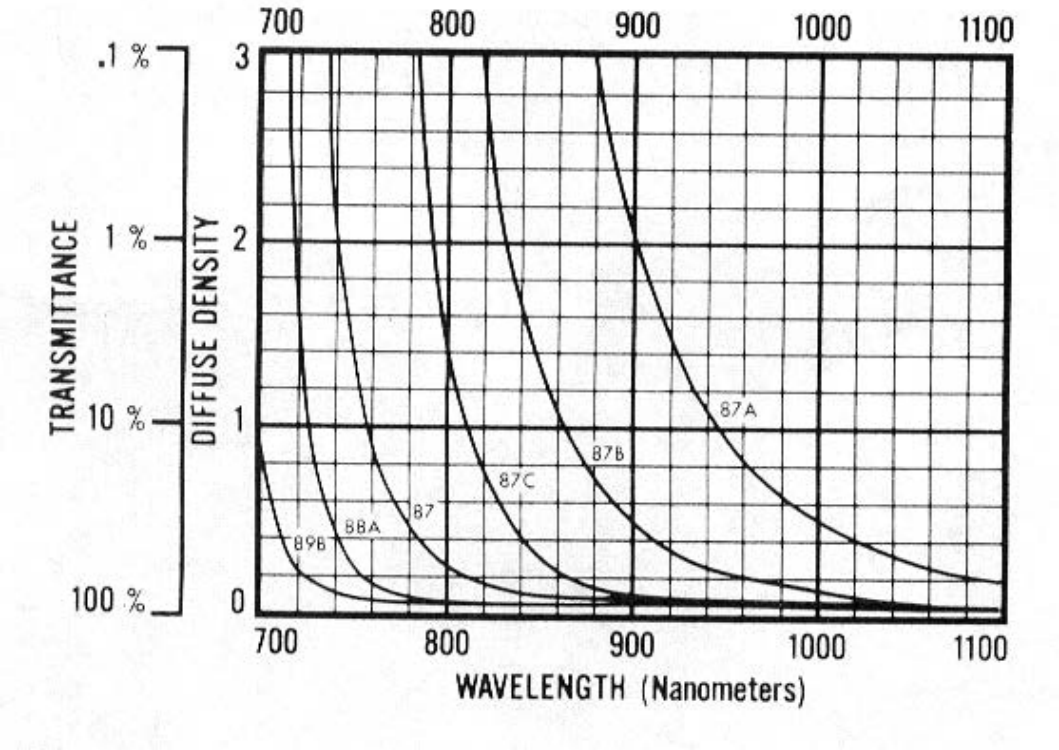


Figure 2.20: **Transmittance of Kodak Infra-Red pass filters**.
We used the model 87 in front of our Philips toucam pro webcam.
(figure: Kodak technical documentation)

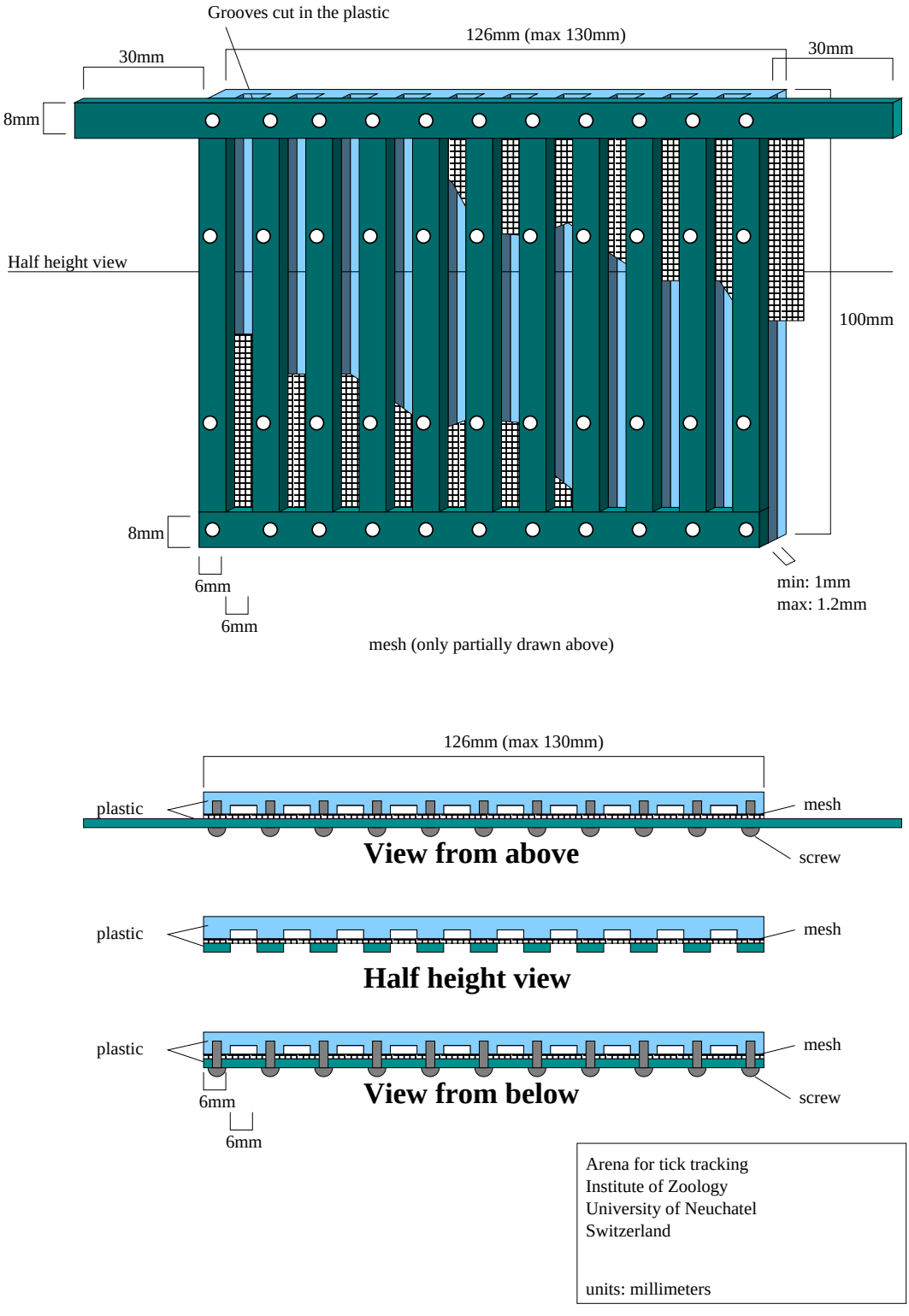


Figure 2.21: **Vertical tracking test arena**.
The arenas were made of transparent plastic and polyamide mesh.
(figure: Jean-Luc Perret 2002)

2.3.2.2 Tracking software (camtud3)

A homemade program was developed in C++ to track tick positions. The program was called camtud3 (camera tracking with USB device version 3)[10]. Many **observation zones** can be defined within the image provided by the camera. Within these zones, tick position detection was based on motion detection, i.e. tick position was only detected during tick movements. Image analysis was realised using the following procedure:

- acquire gray images every σt seconds. The last image is called the **current image**.
- substract the greyscale values of a **reference image** acquired ΔT seconds before the current image from the current image resulting in a **difference image**.
- search for differences in the observation zones. This step was made by scanning the observation zones and looking for a difference greater than a noise threshold μ. When the first difference pixel was found, a **potential target** was defined. Difference pixels within a distance Ω from the target geometric center were successively added to the target. After scanning the whole observation zone the geometric center of targets (x/y coordinates), their surfaces, the date and time were stored in a separate file for each observation zone.

The parameters σt and ΔT were optimised experimentally based on tick walking speed and given the constraint that the amount of data has to be kept in manageable amounts. σt was set to 3 seconds and ΔT was set to 30 seconds. Given the size of nymphs, Ω was set to 10 pixels. Finally, according to the noise threshold level, μ was set to 40.

The output of the camtud3 program are "track files", namely one text file for every observation zone containing the date, time, x and y positions of the detected moving targets each 3 seconds. In addition, a sequential number attributed to each detected target[11], and the surface of the targets were also recorded. Targets with a minimum surface of 2 pixels were used for the analysis. These settings enabled detection of tick positions when ticks moved faster than $\frac{1 pixel}{30 seconds}$. Given the primary magnification used in our experiments, this means a minimal speed of 1mm per minute.

2.3.2.3 Track file processing

The tracks obtained for each tick were preprocessed before analysis. The preprocessing involved successively 1) the removal of outlier points[12] and 2) splitting the tracks into individual **events**.

[10] An early prototype of the tracking system used a video camera and a framegrabber together with a windows 95 computer. The tracking program was written in Visual Basic. This system was abandonned for the benefit of webcams due to the possibility to connect more than one webcam to a single Linux computer without an expensive framegrabber. Image quality of the Philips toucam pro (640x400) was equivalent to the framgrabber and provided more stable gray levels.

[11] If only one target was detected it received number 0, if more than one spot was detected they received numbers starting from 1

[12] An automatic outlier detection procedure was used to remove isolated points lying far away from the last and the next recorded position

For each event its nature, start time, end time, and duration was estimated from the tracks. Events were defined as follows:

QUESTING: tick immobile for more than 2 hours at a position higher than 1.5 cm (outside the humid zone produced by the wet cotton, see figure 2.22)

QUIESCENCE: tick immobile for more than 2 hours at a position lower or equal to 1.5 cm (within the humid zone produced by the wet cotton)

WALKING: tick walked more than 3 cm, or changed its position by more than 1.5 cm.

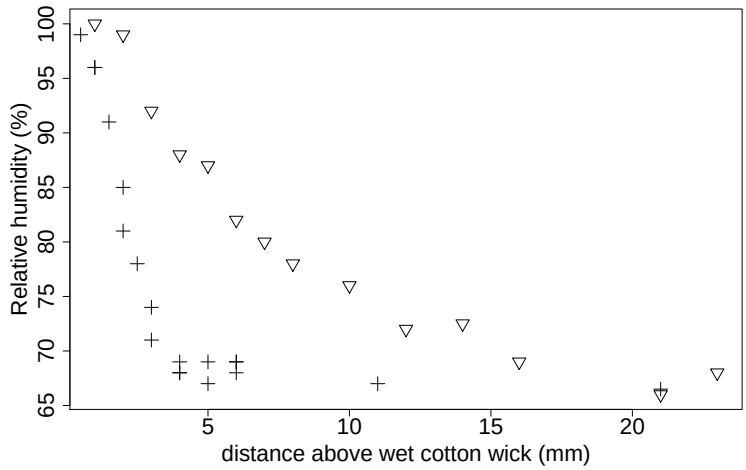


Figure 2.22: **Water diffusion in the atmosphere above a wet cotton wick**
Relative humidity as a function of distance above a wet cotton wick. $+$: RH above cotton outside vertical channels, ∇: RH above cotton in vertical channel (RH in the room was 68% as recorded by the same probe).
(figure: Jean-Luc Perret 2002)

Experiments included many **periods** of observation for which varying conditions were tested (climate, light,...) (table 2.5). Events for which start and end time could not be precisely determined were removed from further analysis (for example because the event had not ended at the end of the observation period). Unless otherwise stated, events which overlapped between two different periods were also removed from further analysis.

2.3.2.4 Calculation of transition matrices

The first order transition matrix gives the probability of an event type X being followed by an event type Y. The second order transition matrix gives the probability of an event type X being followed by any event, itself followed by an event type Y (Haccou and Meelis, 1992). These matrices were calculated based on the succession of events observed for groups of ticks tested under each set

of experimental conditions. Calculations were made using a specially written function in R for Linux V1.4.1 (Ihaka and Gentleman, 1996).

2.3.2.5 Experiments

Ticks were collected in the forest North of Neuchâtel and were kept in water-saturated atmosphere prior to all experiments[13]. The same ticks were kept throughout an **experiment**. In each experiment ticks were successively exposed to different **periods** of homogeneous environmental conditions (temperature, relative humidity and light cycle). To remove any behavioural bias following the host stimuli delivered by the observer to the ticks when they were introduced in the arenas, the data recorded from the time of tick manipulation until the next morning at 08:00 (period 1) were not considered in the analysis.

Exp.	Start (date)	Period	Temp. (°C)	RH (%)	Light Cycle (L:D)	nb of nymphs	duration (days)
exp 3	21.5.2001	2	25	60	14:10	9	10
	31.5.2001	3	25	60	0:24	9	5
	5.6.2001	4	25	60	8:4	9	10
exp 4	29.6.2001	2	25	60	14:10	9	10
exp 5	13.7.2001	2	25	85	14:10	9	10
exp 7	12.10.2001	2	15	85	14:10	30	10
exp 8	6.11.2001	2	25	85	14:10	40	10
exp 10	16.3.2002	2	25	60	14:10	40	11
	27.3.2002	3	25	60	0:24	40	11
	7.4.2002	4	25	60	8:4	40	15
	22.4.2002	5	25	60	0:24	40	1*
	23.4.2002	6	25	60	0:24	40	13
	6.5.2002	7	25	60	14:10	40	4
	10.5.2002	8	25	60->85	14:10	40	1/2
	11.5.2002	9	25	85	14:10	40	13

Table 2.5: **Experimental conditions tested during continuous video tracking experiments.** Each experiment was divided into periods during which climatic conditions and light cycle were kept constant.
(* after entry to the climate chamber)

[13] tick collection dates: exp 3: 9 May 2001; exp 4: 28 May 2001; exp 5: 28 May 2001; exp 7: 25 September 2001; exp 8: 27 September 2001; exp10: 11 March 2002

2.3.2.6 Statistical analysis

The distribution of start and end time over the hours of the day (coded over 24h in seconds) were analysed using circular statistics. The null hypothesis of uniform distribution during the 24h of the day was tested using the Rayleigh test (Batschelet, 1981).

The null hypothesis of identical distributions of event durations was tested using the Wilcoxon test (Siegel and Castellan, 1988). The relation between event durations and saturation deficit was evaluated using the Jonckheere test (Siegel and Castellan, 1988). The reason for the choice of these non parametric tests was their insensivity to deviations from normality (see section 3.3.2).

2.3.3 Tick examination for *B. burgdorferi* infection

Immunofluorescence: To examine for infection by *B. burgdorferi*, ticks (longitudinally cut) were squashed using a pair of tweezers on 12.5 mm^2 spots on glass slides. Preparations were dried for 24 hours at 37°C and fixed in acetone for 10 minutes. Slides were then treated as previously described (Gern et al., 1999) with a fluorescein isothiocyanate-conjugated polyclonal antibody prepared from a pool of Lyme borreliosis patient sera. Each individual spot was visually examined for the presence of spirochetes using a fluorescence microscope.

2.3.4 Locomotion compensator

Tick mobility was recorded on a locomotion compensator: a motion controlled sphere which can compensate for the movements of an animal placed at the top of it (Kramer, 1976). This system measures speed, direction and duration of tick walk. An air flow with controlled humidity constantly flowed tangentially to the top of the sphere. Mobility tests were run by Dr Connor McMahon[14]. Adult *I. ricinus* were placed on the locomotion compensator for 6 minutes. Climatic conditions were ambient temperature (around 20°C), 70% relative humidity and total darkness. Air speed was 0.2 meter/second. All ticks were adult *I. ricinus* collected in the field between June and July 2001 and kept in water saturated atmosphere before the analysis. Descriptive statistics on the tracks were calculated as described by McMahon and Guerin (McMahon and Guerin, 2000) Briefly the no move time (NMT) represents the duration (in seconds) during which ticks were immobile[15]. The mean speed (l.speed) is the mean walking speed calculated when ticks were moving. The time in cone (tcon) represents the duration in seconds during which ticks were moving upwind[16]. Finally, the proportion of the distance in cone (l.c.l) represents the proportion of the path walked upwind. After the locomotion test, ticks were examined individually for *B. burgdorferi* sl infection by direct immunofluorescence staining as described in paragraph 2.3.3, above. We therefore could link the behaviour of each individual tick on the locomotion compensator to its infection status. To compare the descriptive statistics obtained for infected and uninfected ticks we used the Wilcoxon-Mann-Whitney test.

[14] Department of Animal Physiology, Institute of Zoology, University of Neuchâtel
[15] ticks moving at a speed of at least 0.1mm/sec were considered mobile
[16] the wind direction was defined as a cone of 120 degrees around due upwind

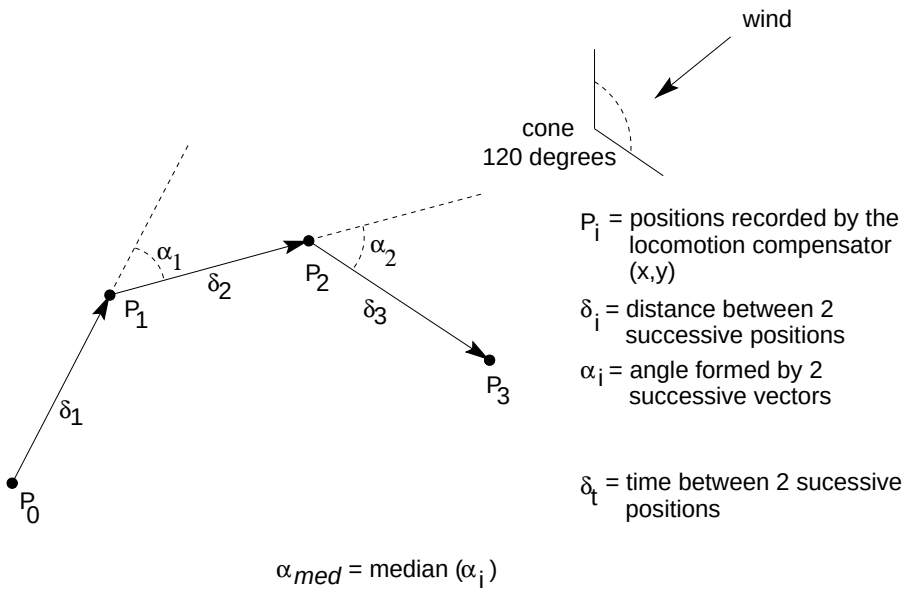


Figure 2.23: **Calculations on the tracks recorded by the locomotion compensator**
The cone delimits directions considered to be walking upwind.
Angles are expressed in ° , distances in mm and durations in seconds.
(figure: Jean-Luc Perret 2002)

2.3.5 Choice of quiescence locus

The ability of ticks to choose a favorable quiescence locus was tested in an environment providing heterogeneous water supply. A plastic test arena (12 x 12 cm surface, 8 cm high) was built for this test (figure 2.24). On one side, the plastic arena was closed by a polyamide mesh (500 μm grid, Sefar-Nitex, Sefar AG, CH-9410 Haiden) to enable gas exchange with the environment. One hundred eppendorf tubes (500μl were driven into the arena floor (on a regular 10x10 grid). Eight of these eppendorf were inverted and glass stems were added on top of them, providing vertical stems (1mm diameter x 6cm high) to the ticks. The remaining 92 eppendorfs contained alternatively dry and humid cotton wicks (water was provided by a 1mm hole at the bottom of the humid tubes). The top of the arena was closed by transparent plastic (sealed with modelling clay). Thirteen *I. ricinus* field collected nymphs (kept in a water saturated atmosphere prior to the experiment) were introduced onto the ceiling in the arena at 14:00 and their position was visually registered after 30 minutes, 2h, 6h, 18h, 24h and 68h. The arena was maintained in a climate chamber where conditions were kept at 25°C and 80% RH with light constantly on. After 26 hours the climate chamber was switched off (light off) and the relative humidity dropped to 25% RH and temperature to 22°C. After 68 hours the position of ticks was recorded for the last time. The registered position included the vertical position (on the floor or elsewhere), and for ticks which were on the floor, the neighborhood of a humid zone.

Figure 2.24: **Quiescence locus choice arenas**.
The arena was made of plastic and polyamide mesh. The bottom of the arena contained a grid of alternatively wet and dry tubes, as well as 8 vertical stands. The ceiling was closed with a transparent plastic plate (not shown).
(photo: Jean-Luc Perret 2002)

Chapter 3

Results

3.1 *I. ricinus* survival

The survival of field collected adult *I. ricinus* under different climatic conditions was evaluated over 10 days. Ten males and ten females were kept in two separate tubes at 5 different humidities and at 4 different temperatures, resulting in 20 tested climatic conditions (section 2.3.1 on page 44). The time of death of male and female *I. ricinus* was recorded by visual inspection[1]. Results are shown in figure 3.1 as a function of time.

The first observation is that the survival of males and females was not identical. Females survived much longer than males whatever the climatic conditions (grouped climatic conditions: $p = 7.105e^{-15}$ Wilcoxon test). At the end of the observation period (240h) all 200 males were dead while 21 of the 200 females were still alive. Twenty of the 21 surviving females were held at 97% relative humidity and one at 85%, most of them ($n = 19$) were held between 25 and 28°C ($n = 2$ at 15°C).

The mean survival time of females (excluding females kept at 97% RH) was inversely proportional to saturation deficit ($R^2 = 0.50$) (figure 3.2). With lower correlations, female mean survival time was also positively related to relative humidity ($R^2 = 0.27$) and negatively related to temperature ($R^2 = 0.42$). Similarly, male mean survival time was inversely proportional to saturation deficit ($R^2 = 0.63$, with a power function) and with lower correlations to temperature ($R^2 = 0.48$) and relative humidity ($R^2 = 0.29$) (figure 3.2).

In order to check if survival time of *I. ricinus* larvae and nymphs was also related to saturation deficit we reanalysed data published by Macleod in 1935 (MacLeod, 1935) (figure 3.3). His data also show that larvae and nymphs survival time is related to saturation deficit rather than to relative humidity or temperature alone. A more detailed comparison between the survival time of nymphs and adults using these data is prevented by the use of different measurements of survival time (we used the mean survival time, while Macleod used the time to 100% death) and by the different origins of the tested ticks (we used field collected adults while Macleod used ticks of similar ages). The increased R^2 observed for the relation between saturation deficit and survival in larvae and nymphs of similar ages compared to adults of mixed ages may therefore

[1] after 20, 28, 44, 52, 68, 76, 93, 119, 145, 165, 187 and 240 hours of incubation

indicate that tick aging affects the resistance to dessication of *I. ricinus* ticks.

These results confirm that both male and female *I. ricinus* survival time is under the influence of humidity and temperature, which are both included in saturation deficit. The survival time of field collected females was however longer than the survival time of field collected males. Aging may affect the survival of ticks under dessicating conditions.

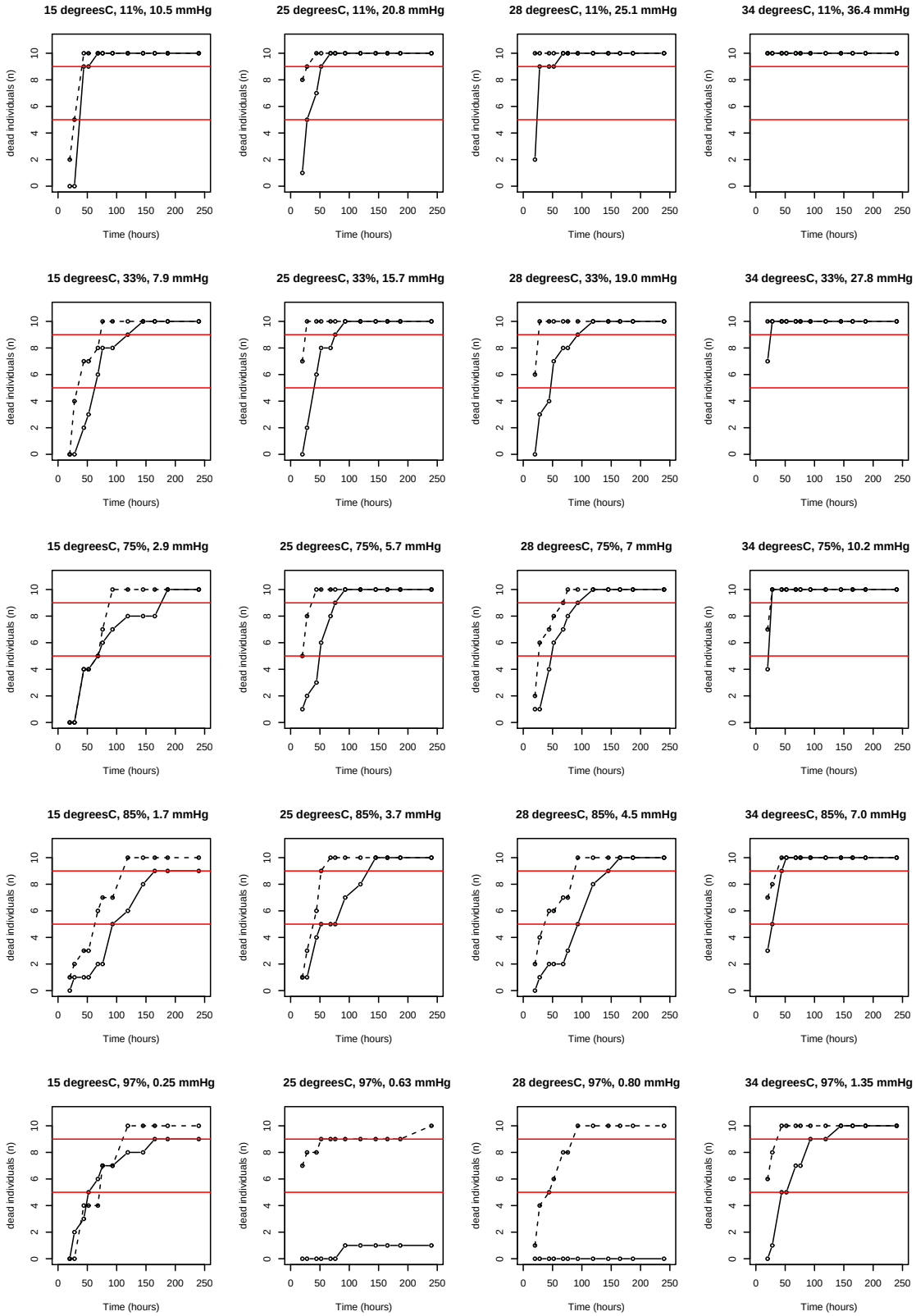


Figure 3.1: **Mortality of adult *I. ricinus*** under different climatic conditions. Temperatures (°C), relative humidities (%) and saturation deficits (mmHg) are printed above each graph. (dotted lines: males, continuous lines: females). Horizontal lines indicate 50% and 90% mortality. Tick mortality increased with increasing saturation deficit. Most females, but not males, survived at 97% relative humidity and 25°C-28°C.

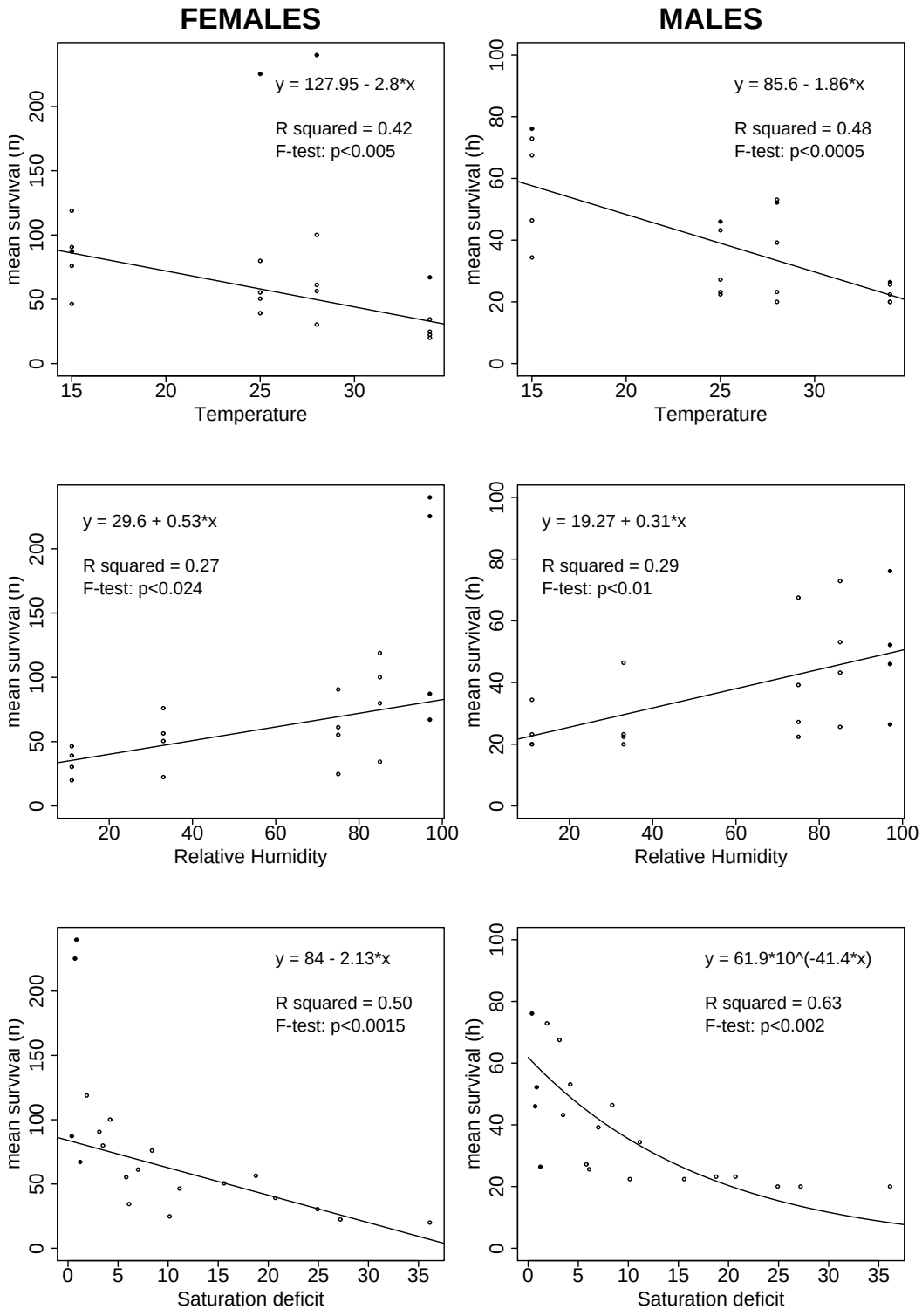


Figure 3.2: **Mean survival time of field collected adult *I. ricinus*** as a function of temperature (°C, top row), relative humidity (%, middle row) and saturation deficit (mmHg, bottom row). The vertical scale is different for females and for males (● indicates the survival time of ticks placed at a relative humidity of 97%.)

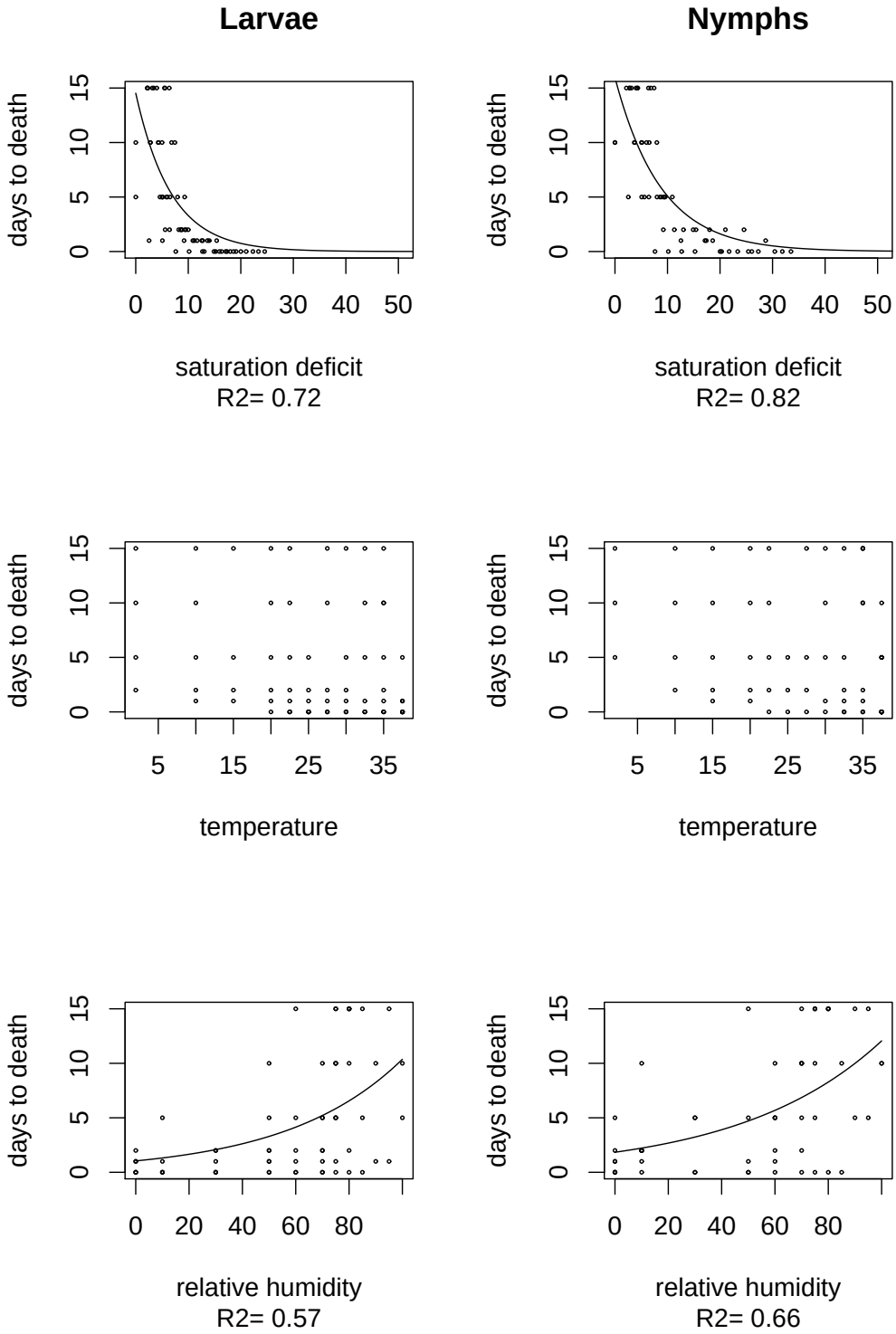


Figure 3.3: **Survival time of *I. ricinus* larvae and nymphs (100% mortality)** as a function of saturation deficit (mmHg, top row), temperature (°C, middle row), and relative humidity (%, bottom row). The fitted lines represent models of the form $y = a * 10^{x/b}$ (Reanalysed from Macleod 1935).

3.2 *I. ricinus* quiescence locus

In the preceeding chapter we observed that 50% of *I. ricinus* ticks die within 48h at 25°C and 85% RH (i.e. 3.5 mm Hg saturation deficit) (see 3.1 on page 57). Under natural conditions ticks may locally find water-saturated loci where they may rehydrate. We therefore checked whether *I. ricinus* nymphs indeed choose their quiescence place according to humidity. To do so we built an arena providing vertical stems and a checkerboard of alternatively dry and wet tubes at the bottom of the arena[2]. The whole arena was placed in a climate chamber in which the climatic conditions were kept at 25°C and 80% relative humidity. After 26 hours the climate chamber was switched off and the temperature decreased at 22°C and the relative humidity dropped at 25% (i.e. 14.5 mm Hg saturation deficit).

The position of 13 field collected nymphs was observed 6 times over 68 hours, starting at 14:00. In each observation we counted the number of ticks present on the floor, and the humidity of the nearest tube (wet or dry). The number of nymphs recorded on the floor increased over time (figure 3.4), as well as the proportion of ticks nearest or in the wet tubes[3]. After 24 hours 6/13 ticks were on the floor and 5/6 were in or nearest to wet tubes. Forty four hours later the position of the nymphs was checked for the last time. Twelve out of thirteen nymphs were on the floor and 11/12 were close by or in the humid tubes.

These results show that indeed *I. ricinus* nymphs are able to choose their quiescence locus according to microclimatic conditions, and that they do so, especially when conditions are very dessicating.

[2] we drove little eppendorf tubes. These tubes were filled with cotton wicks. A little hole was made at the bottom of every second tube. As the whole arena was placed over water, the perforated tubes let water come in while the unperforated tubes remained dry

[3] the ticks were usually on the side of the eppendorf tube, above the wet cotton, without touching it

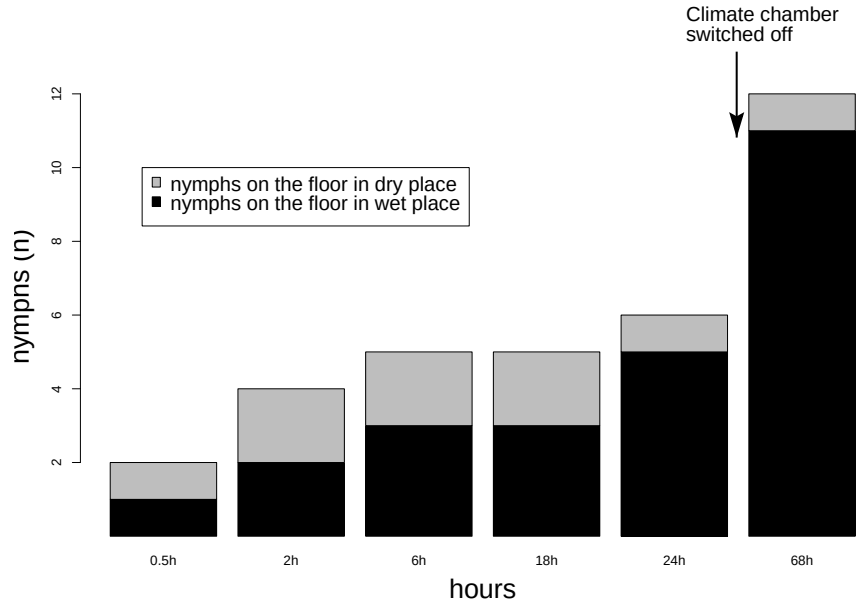


Figure 3.4: **Number of nymphs on the arena floor and their position relative to the humid zones**

3.3 *I. ricinus* questing behaviour

The questing behaviour of *I. ricinus* was studied in two different ways. First ticks placed in arenas under natural conditions were observed on a daily basis. Secondly, under controlled laboratory conditions, tick behaviour was continuously recorded by computer-assisted observations.

3.3.1 Field arenas

In order to study the proportion of ticks questing under natural conditions, ticks placed in mesh delimited arenas in the forest North of Neuchâtel (see figure 2.17 in section 2.2.3 on page 40) were observed from the end of March to the middle of December 2000 twice per day in spring and summer and once per day in autumn. Ticks visible on the sides and top of the arenas were counted. Knowing the number of ticks in the arenas at the start of the experiment, the proportion of ticks visible in the arenas was estimated (figure 3.5). From the three arenas set up in March only one could be used for nymphal observation because little holes let nymphs escape (not adults). Therefore 126 nymphs (in one arena) and 77 adults (in three arenas) were observed from the 22.3.2000 to the 22.12.2000. On the 17.5.2000 3 arenas were added, totalising 306 nymphs in four arenas and 137 adults in six arenas observed from 17.5.2000 to 23.10.2000, at which point 40 newly collected nymphs were added to 2 arenas which were observed until 22.12.2000.

At any time during the whole year, the proportion of questing adults was significantly higher (mean: 24%) than the proportion of questing nymphs (mean: 12%) ($p < 0.000001$, Wilcoxon tests). No significant difference was found between the proportion of questing ticks in the morning and afternoon (paired Wilcoxon tests: p=0.94 for adults and p=0.54 for nymphs).

The proportion of questing ticks (nymphs and adults) decreased stepwise with time (figure 3.5). The proportion of visible adults partially recovered after each decrease (top graph). In contrast, the proportion of visible nymphs was strongly reduced during a single short period in June and did not recover even partially (second graph). Decrease in the proportion of visible ticks was strongly related either to a peak in saturation deficit or to a drop in maximal relative humidity (dotted arrows in figure 3.5). In autumn no increase in the proportion of questing ticks occurred as saturation deficit decreased. However the 80 autumn collected nymphs were able to quest in the arenas.

This demonstrates that highly dessicating conditions reduce the proportion of questing ticks. It also demonstrated that in 2000 in Neuchâtel ticks active in spring did not reappear in autumn. Finally it demonstrates that proportionally more adults than nymphs are questing at any time, and that adults are found later during the year than questing nymphs.

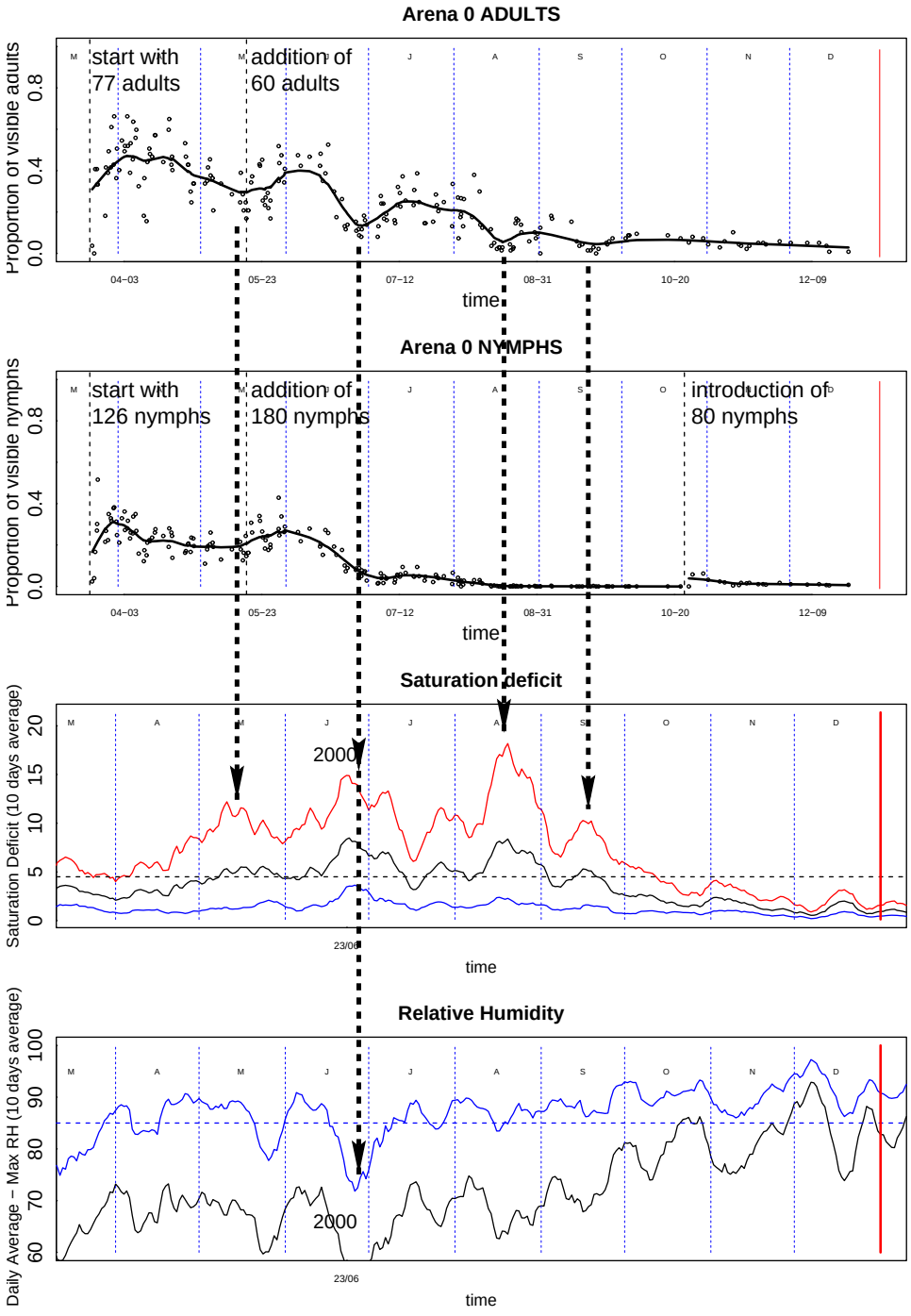


Figure 3.5: **Proportion of visible ticks in all field arenas during 2000**
The top 2 graphs show the proportion of ticks visible in the arenas. In these two graphs, the continuous line is a non parametric smoothing of the data. The third graph shows the 10 days average of daily maximum, average and minimum saturation deficit (dashed line: 4.5 mmHg saturation deficit) . The bottom graph shows the 10 days average of daily average and maximum relative humidity (dashed line: 85% relative humidity) .

3.3.2 Continuous video tracking

3.3.2.1 Design of the tracking system

Our understanding of tick host-seeking behaviour is limited by the difficulty of observing ticks without interfering with them. Indeed as humans are potential hosts for *I. ricinus* their presence may influence tick behaviours. In addition, these behaviours last many hours or even days so continuous observation of single individuals over days is hardly possible without automation. Furthermore humans are not able to see in the dark. For these reasons we designed and built an automated video tracking system with the following requirements:

- Spatially precise tracking of individual nymphs (precision: a few mm).
- Continuous tracking for up to months (time resolution of a few seconds).
- Independence to slowly changing light intensity, ability to track ticks even during darkness.

A single computer running SuSE Linux 7.1 (Kernel 2.2.18) with 4 USB controllers[4] was used to grab images from 4 USB ToUCam PRO Philips Webcams[5]. The infrared (IR) block filters of the webcams were replaced by Kodak IR pass filters (see page 48). This turned the webcams into higly sensitive infrared cameras providing 640x400 pixel images at a framerate of 15 images/s, or 25 images/s at a resolution of 320x200 pixels. The scene was illuminated using a constant infrared source (CES ag Dübendorf (ordering no: 20177) WFL2-LED30 950nm 50° 30W). The IR pass filter in front of the camera insured that a maximum of IR and a minimum amount of visible light passed to the cameras. However a small part of visible light still passed the filter and was added to the IR by the camera. The autogain of the webcams was switched on so that the exposition was automatically adjusted, correcting most light variations. Nevertheless, some fluctuations in the image exposition remained during transitions from darkness to light. The tracking algorithm was thus designed to be robust to gradual changes in light conditions. This algorithm was implemented in a software called "camtud3 which is described in 2.3.2 on page 46.

Using this system, the position tracking of IR reflective objects was possible under any visible-light intensity provided that changes in light intensity were gradual. Ticks are IR reflective and appeared as white spots on a dark background.

In order to keep ticks in the focus plane of the camera we built transparent plastic arenas (see figure 2.21 on page 49) consisting of vertical channels with a transparent side toward the cameras and a nylon mesh on the opposite side. The tracking task was thus reduced to the detection of 2mm moving objects in a vertical channel under slowly changing light conditions.

[4] 2 USB ports on the motherboard plus one additional USB card with two ports

[5] A first tracking system was designed with a frame grabber (Miro DC30+) and a black and white video camera. The system was running on a MS-Windows 95 computer with a tracking software written in MS-Visual Basic 6. Unfortunately the system was not very stable and had to be rebooted every 24 hours in order to avoid image acquisition problems. In addition, only a single camera could be used which only permitted the observation of a few nymphs simultaneously. Finally the image processing in Visual Basic was very slow and only enabled the treatment of one picture every 3 seconds. For all these reasons the design of the tracking system was completely redesigned in C++ under Linux

Image size (pixels)	One camera (msecs)	Four cameras (msecs)
640x400	400	not possible
320x200	100	800
160x100	50	200

Table 3.1: **Time resolution achieved for position detection by camtud3 running on a Pentium II 450 computer (time required between two positions in msec)**

The time resolutions achieved by the system using a Pentium II 450 Mhz computer is given in table 3.1. To minimize the amount of recorded positions, and considering the relatively slow walking speed of ticks, we analysed one image every 3 seconds.

To measure the position-error rate of the tracking system we built a mechanical arm which moved a target linearly in a predictible manner. Ten thousand positions were measured without any error. We therefore conclude that the error rate is inferior to 1 in 10'000 positions.

3.3.2.2 Tick tracking

The continuous video tracking we developed enables us to follow the movements of animals continuously under changing light conditions (up to complete darkness). The first hypothesis we wanted to confirm with this system was that ***I. ricinus* spontaneously undergoes (in the absence of any host stimulation) alternating quiescence and questing periods**. To test this, 58 *I. ricinus* nymphs, in three separate experiments, were placed under continuous digital video observation for 10 days in vertical channels as described in section 2.3.2 at 25°C and 60% RH with a 14:10 L:D cycle (in a climate chamber). Water was provided at the bottom of the vertical channels by a wet cotton wick. Relative humidity inside the channels decreased gradually and was equivalent to the chamber conditions 2cm above the wet cotton (figure 2.22 on page 51).

The raw data obtained for one individual tick and the corresponding interpretation are given in figure 3.6 as an example. From the succession of events we calculated the first order transition matrix, that means the probability of changing to event Y after undergoing event X (see 2.3.2.4 on page 51). From this matrix (table 3.2) we observed that nymphs always undergo a walking period after or before questing and quiescence. As ticks have to move to change from quiescence to questing this information is not very interesting. We can also observe that once a nymph is walking, the probability of questing when it stops is 0.18 while the probability of quiescence is 0.82. Since ticks always undergo a walking event between quiescence and quiesting, the second order transition matrix (the probability of changing to event Y two events after undergoing event X) is more informative (table 3.3). The probability of observing a quiescence event two events after a questing is extremely high (0.93), indicating the need for nymphs to enter quiescence after questing. The opposite is not true. Indeed the probability of having another questing event 2 events after quiescence is proportionally quite low (0.21). Increasing RH to 85% changed the transition probabilities slightly (table 3.4 on page 68). Indeed, under more humid conditions nymphs were less inclined to start quiescence after questing (0.80), and were more inclined to start questing after being quiescent (0.36). However these differences were not significantly

different from the transition probabilities observed at 60% RH (Fisher's exact test: $p = 0.21$).

		2nd event		
	event type	QUESTING	WALKING	QUIESCENCE
1st event	QUESTING	0	1	0
	WALKING	0.18	0	0.82
	QUIESCENCE	0	1	0

Table 3.2: **First order transition matrix at 25°C 60% RH**
(probability for event of type Y to follow event of type X) for 58 nymphs observed over 10 days ($n = 33$ questing events, 147 quiescence events and 172 walking events)

		3d event		
	event type	QUESTING	WALKING	QUIESCENCE
1st event	QUESTING	0.07	0	0.93
	WALKING	0	0.99	0.01
	QUIESCENCE	0.21	0	0.79

Table 3.3: **Second order transition matrix at 25°C 60% RH**
(probability for event of type Y to follow event of type X with an event of any type in between) for 58 nymphs observed during 10 days ($n = 27$ questing events, 123 quiescence events and 171 walking events)

		3d event		
	event type	QUESTING	WALKING	QUIESCENCE
1st event	QUESTING	0.20	0	0.80
	WALKING	0	1	0
	QUIESCENCE	0.36	0	0.64

Table 3.4: **Second order transition matrix at 25°C 85% RH**
(probability for event of type Y to follow event of type X with an event of any type in between) for 89 nymphs observed during 10 days ($n = 54$ questing events, 124 quiescence events and 207 walking events)

When no wet cotton was provided at the bottom of the channels nymphs walked continuously and died after showing an intense walking activity (figure 3.7), within 12-83h and after having walked between 5-31 meters.

We thus showed that *I. ricinus* nymphs undergo questing and quiescence periods without any host stimuli and that nymphs almost always undergo quiescence after questing while the opposite is not true since quiescence was often interrupted by walking events which did not lead to questing.

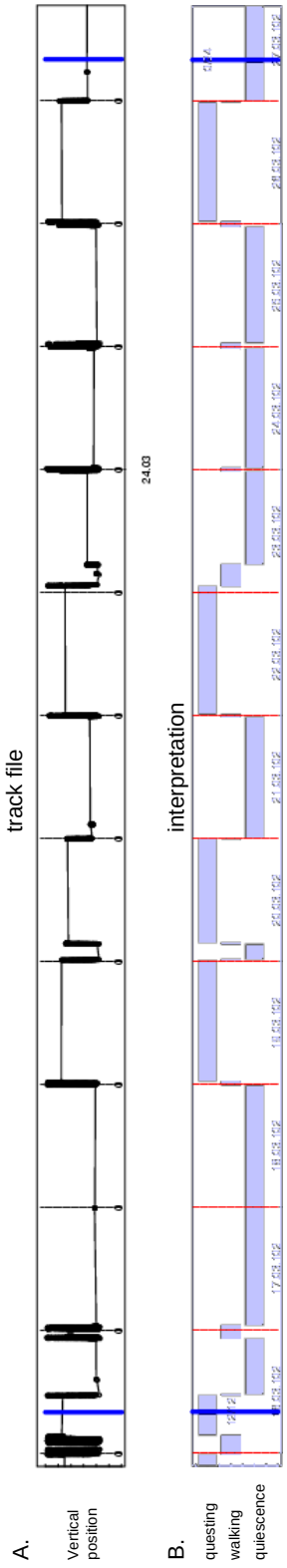


Figure 3.6: **Timeplot of the vertical movements of an individual *I. ricinus* nymph and interpretation of the timeplot**
A: Raw data: timeplot of a nymph vertical position, B: Track interpretation

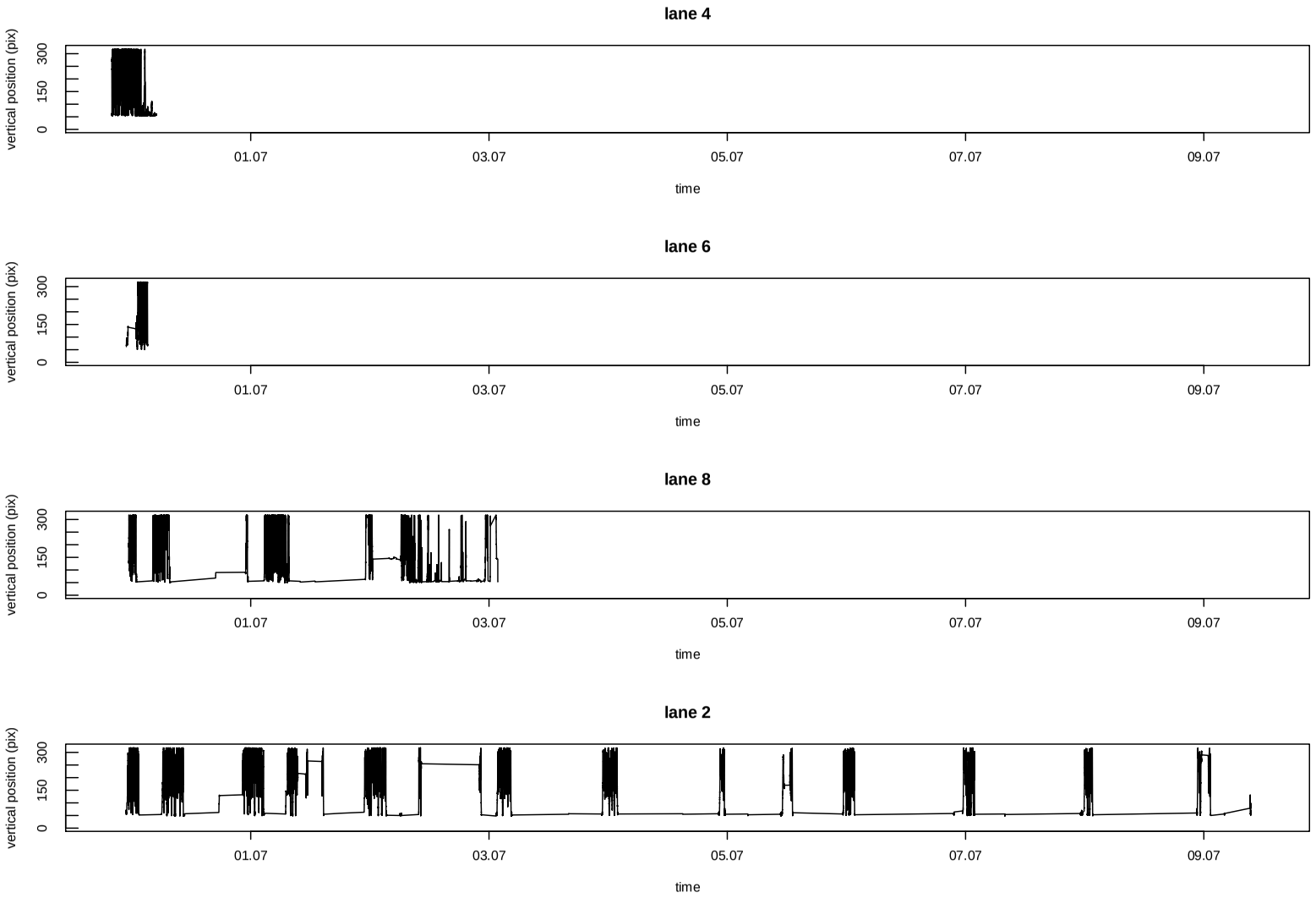


Figure 3.7: **Vertical movements of individual *I. ricinus* nymphs**
3 top graphs: channels with a dry cotton; **bottom graph:** channels with a humid cotton.

During our observations at 25°C and 60% RH (58 nymphs in three separate 10 day experiments) ticks did not move uniformly during the day (Rayleigh test: $p < 0.001$). In fact most of the walking events occurred during darkness (89%) in a 14:10 LD cycle. Only 5% of all movements occurred at maximal light intensity when light intensity was maximal during 42% of the time (the remaining 6% of movements occurred during the transitions between light and darkness) (figure 3.8a). It was thus hypothesised that tick movement was triggered by the light cycle. To test this we left the nymphs in total darkness during 5 days and changed the photoperiod to a 8:4 LD cycle. Again movements were not uniformly distributed (Rayleigh test: $p < 0.001$). Eighty seven percent of all movements (49 nymphs[6]) occurred during total darkness, and only 1% occurred at maximal light intensity when maximum light intensity represented 33% of the time (figure 3.8b).

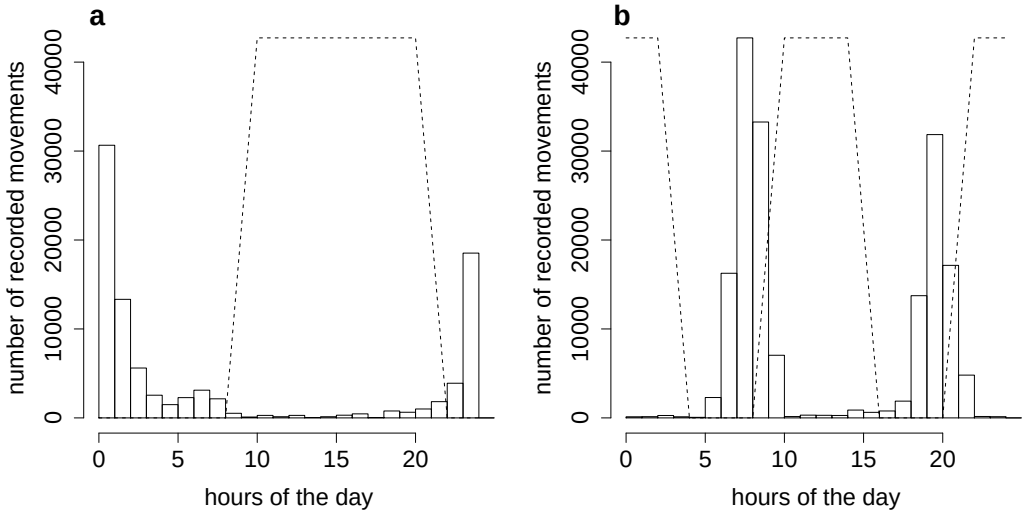


Figure 3.8: **Histograms of *I. ricinus* nymph movements (actograph)** recorded at intervals of 3 seconds over 10 days; **a** 14:10 L:D and **b** 8:4 L:D at 25°C, 60% RH. Light cycles are indicated by the dotted lines with full intensity beginning at 10:00 in a, and at 10:00 and 22:00 in b; $n = 49$ nymphs.

[6] 9 nymphs were not tested under the 8:4 L:D cycle

The duration of questing in the field was suspected to be related to dessicating conditions (Gigon, 1985), possibly saturation deficit, but questing duration was not measured experimentally in relation to dessicating conditions. To test this hypothesis we followed different groups of ticks with the computer-assisted tracking system under three different climatic conditions: at 9.3mm Hg ($n = 58$ nymphs), 3.5mm Hg ($n = 50$ nymphs) and 1.9 mm Hg ($n = 27$ nymphs). The mean duration of questing events was inversely related to saturation deficit (figure 3.10): 19.4 hours, 25.9 hours and 39.8 hours at 9.3mm Hg, 3.5mm Hg and 1.9mm Hg respectively (Jonckheere test $p < 0.05$, questing events: $n = 33$, $n = 44$, $n = 66$ respectively). On the contrary, the mean duration of quiescence was not related to saturation deficit (28.6 hours, 17.2 hours and 20.64 hours at 9.3mm Hg, 3.5mm Hg and 1.9mm Hg, Jonckheere test $p = 0.93$, quiescence events: $n = 148$, $n = 106$ and $n = 68$, respectively, figure 3.9).

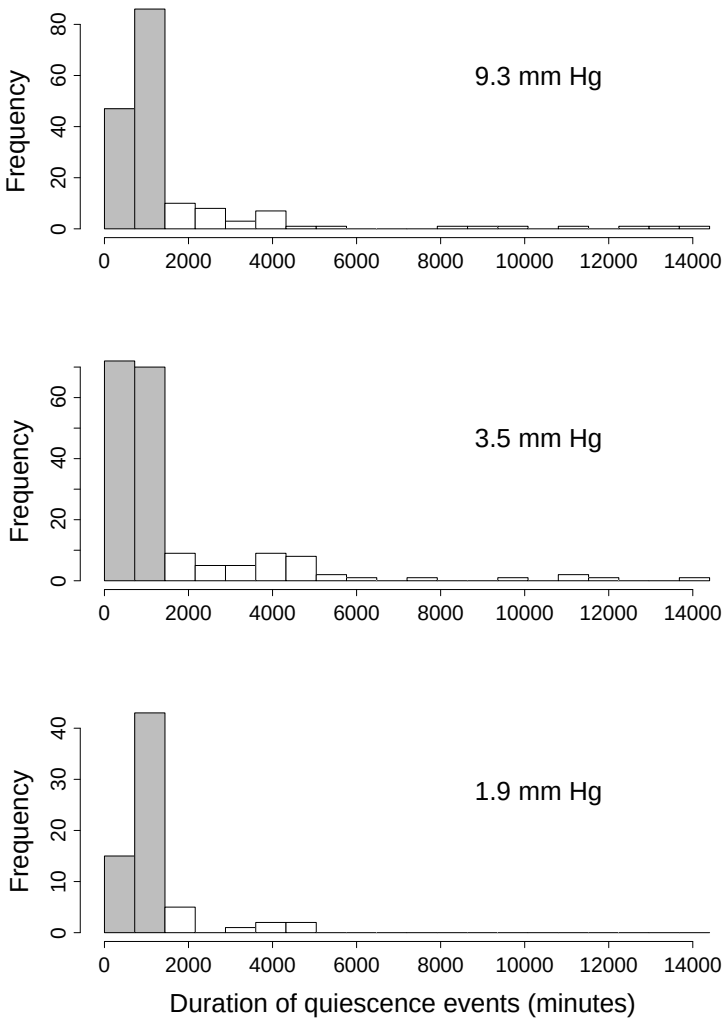


Figure 3.9: **Histograms of the duration of quiescence events by *Ixodes ricinus* nymphs** at saturation deficits of 9.3, 3.5 and 1.9 mm Hg; Most events are shorter than 24 hours (gray bars). Quiescence duration is not related to saturation deficit (p = 0.93 Jonckheere test, see text for details).

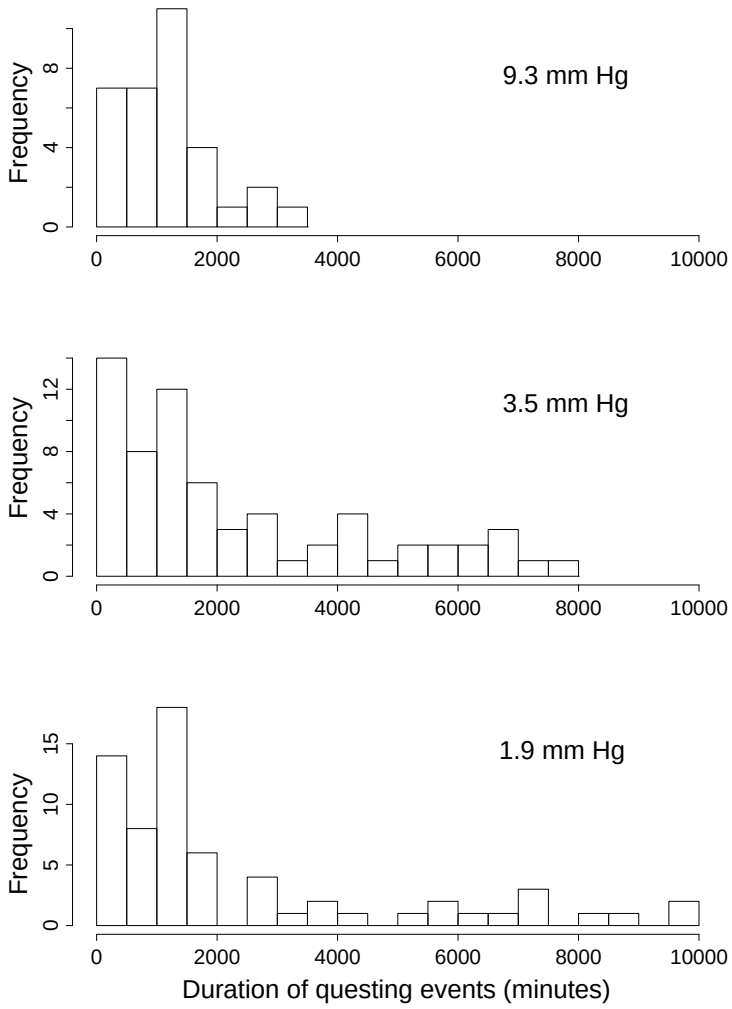


Figure 3.10: **Histograms of the duration of questing events by *Ixodes ricinus* nymphs** at saturation deficits of 9.3, 3.5 and 1.9 mm Hg. Questing duration decreases with increasing saturation deficit ($p < 0.05$ Jonckheere test, see text for details).

In these experiments under different climatic conditions, we also examined tick movements to verify that ticks still move preferentially during darkness under all conditions. Indeed, movements associated with the start and end of quiescence and questing events occurred mainly during darkness under all three climatic conditions (figure 3.11, Rayleigh test: $p < 0.005$ for the start and end times of all event types under all conditions).

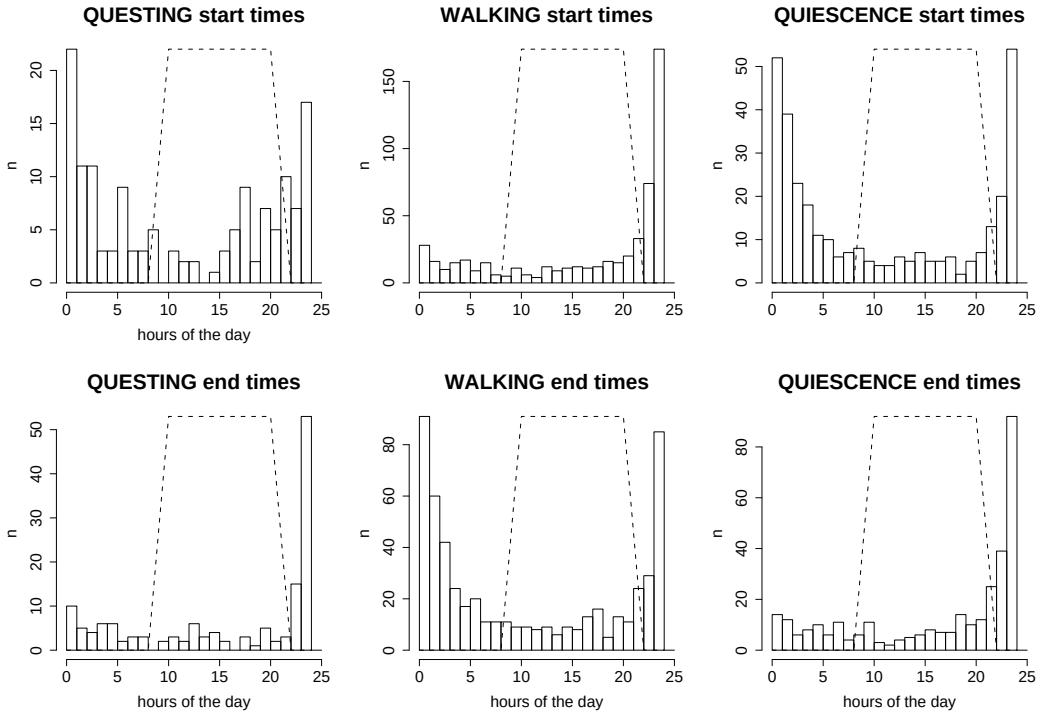


Figure 3.11: **Pooled frequency plots of *I. ricinus* nymphal questing, walking and quiescence start (top) and end times (bottom) recorded over 10 days in a 14:10 L:D cycle, under three climatic conditions.**

The light cycle is indicated by the dotted line (black line = darkness), with full intensity beginning at 10h. Totals of 204 quiescence, 93 questing and 321 walking events were recorded for 95 nymphs.

The number of ticks walking increased during darkness. What about the number of quiescent and questing ticks? Under all climatic conditions in the 14:10 L:D cycle and over 10 days, the number of questing nymphs during light or dark remained globally constant, with an instability when ticks changed their activity during darkness (figure 3.12). The number of quiescent nymphs on the other hand strongly decreased during darkness, when nymphs started to walk. These results emphasize that nymphs interrupt quiescence during darkness to start walking. The same result was observed under the 8:4 L:D cycle (figure 3.13).

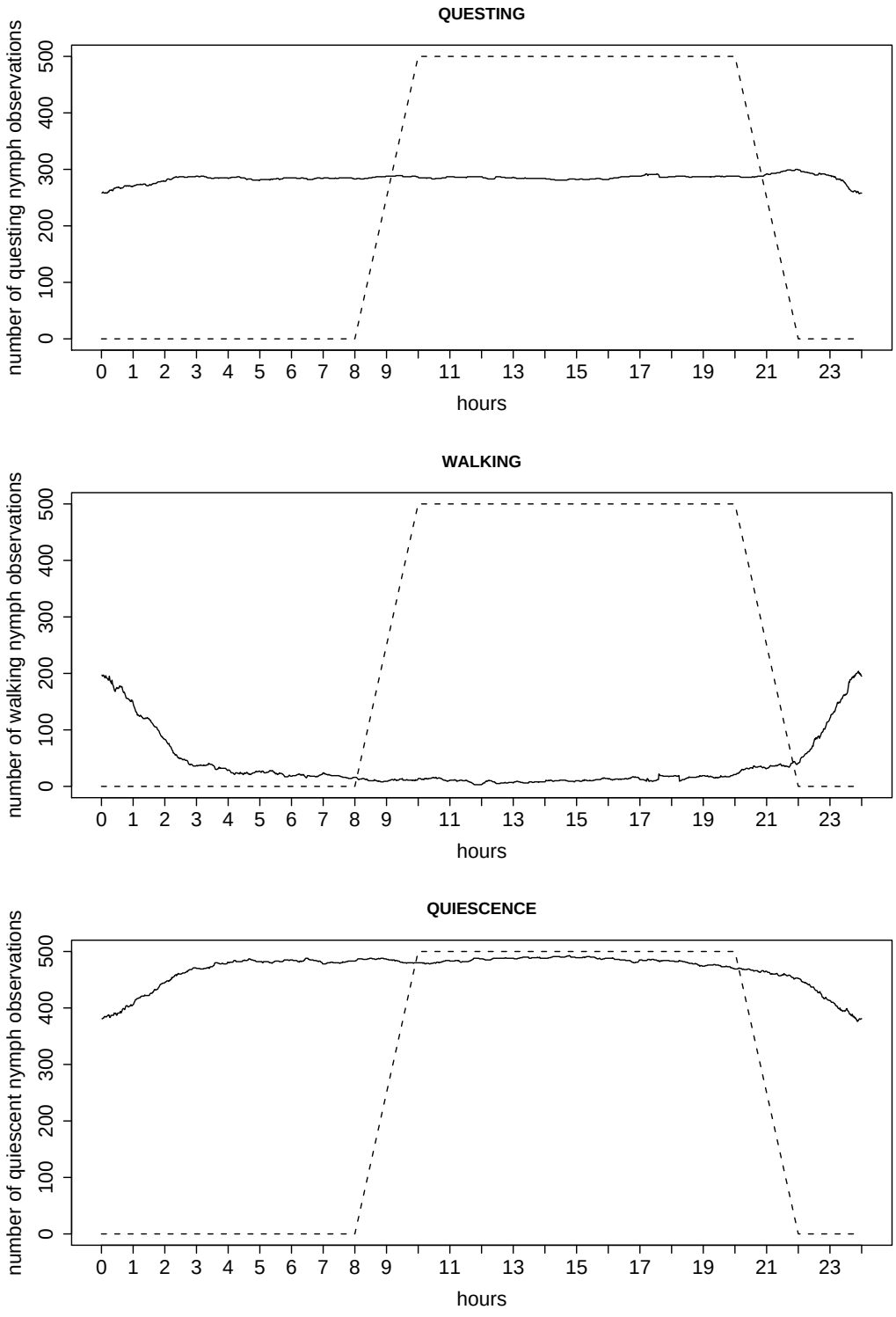


Figure 3.12: **Daily activity of *I. ricinus* nymphs**
observed over 10 days under all tested climatic conditions (425 quiescence events, 743 walking events and 183 questing events). The light cycle is indicated by the dotted line (reaching maximum intensity at 10:00).

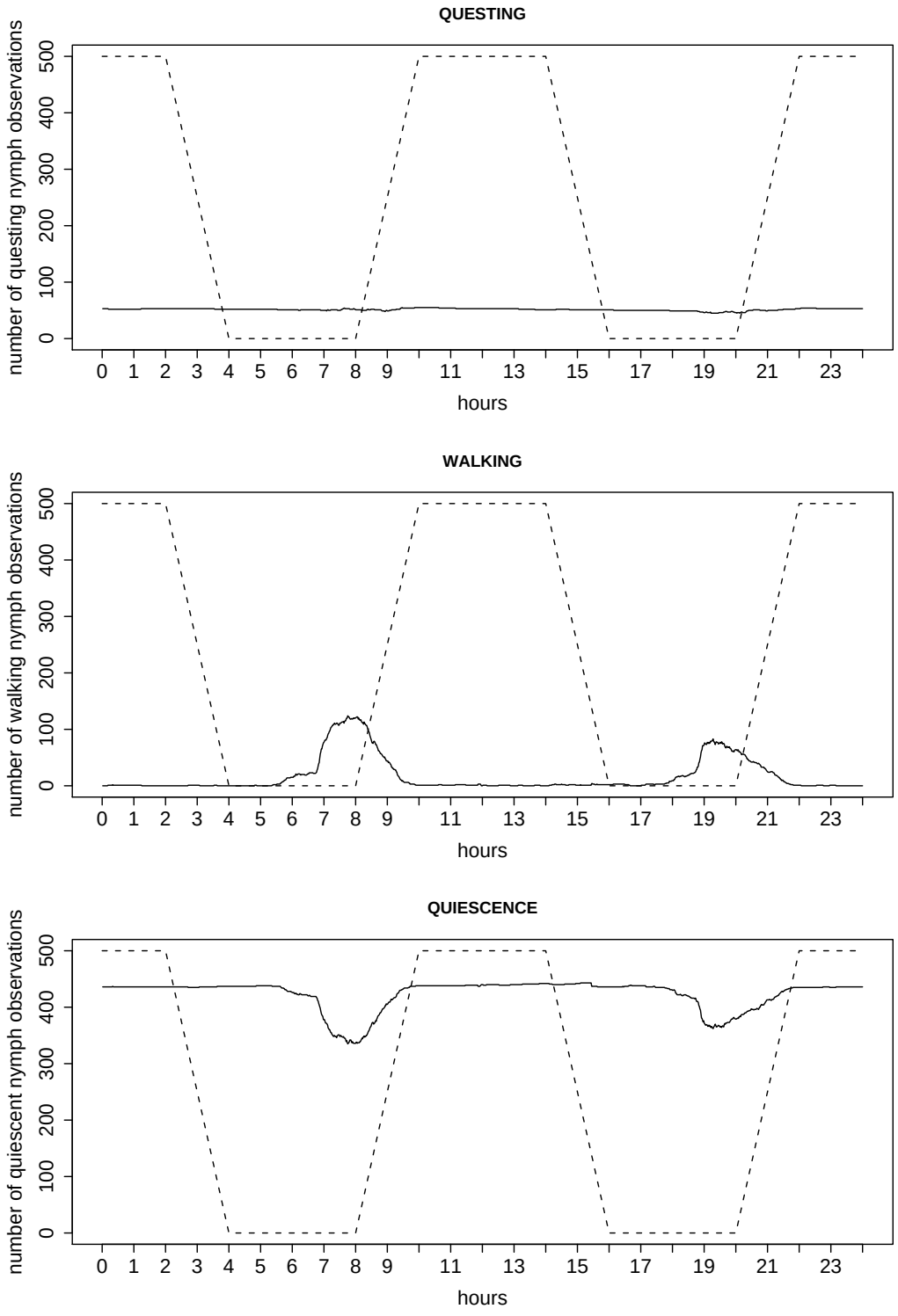


Figure 3.13: **Daily activity of *I. ricinus* nymphs** observed over 10 days under all tested climatic conditions (375 quiescence events, 453 walking events and 44 questing events). The light cycle is indicated by the dotted line (reaching maximum intensity at 10:00 and 22:00).

In summary we showed that questing duration is limited by dessication while quiescence duration is not. We also showed that quiescence was often interrupted by walking events which did not necessarily lead to questing. Finally we showed that most movements in *I. ricinus* nymphs occurred during darkness.

The distance walked after questing interruption was not related to saturation deficit (Jonckheere test: $p = 0.28$, table 3.5) and reached an average of 32 cm and a maximum of 2.92 meters. In contrast, the distance walked after quiescence interruption was positively related to saturation deficit (Jonckheere test: $p = 1.17e-07$, table 3.5, figure 3.14) and reached a maximum of 9.65 meters at 9.3mm Hg. This means that the drier the conditions, the further nymphs walked after being quiescent.

T	RH	SD	average distance after questing mean, median (1st and 3rd quartiles)	average distance after quiescence mean, median (1st and 3rd quartiles)
°C	%	mm Hg	cm	cm
15	85	1.9	34,12 (7-38)	44,21 (9-54)
25	85	3.5	26,30 (7-31)	46,27 (12-55)
25	60	9.3	40,21 (8-36)	110,43 (23-123)

Table 3.5: **Distance walked by ticks after questing and after quiescence under three different climatic conditions (SD: saturation deficit)**

We then examined whether the increases in the distances walked after quiescence under dessicating conditions were due to increases in speed or to increases in the duration of the walking events. After quiescence the walking speed was positively related to saturation deficit (Jonckheere test: $p < 0.001$, table 3.6), as well as the duration of the walking events (Jonckheere test: $p = 0.021$).

The more dessicating the conditions, the faster and the longer nymphs walked after quiescence, thus covering longer distances.

T	RH	SD	average speed after questing mean, median (1st and 3rd quartiles)	average speed after quiescence mean, median (1st and 3rd quartiles)
°C	%	mm Hg	cm/min	cm/min
15	85	1.9	0.67, 0.67 (0.48-0.84)	0.44, 0.43 (0.31-0.56)
25	85	3.5	1.85, 1.4 (0.79-1.98)	1.69, 1.25 (0.77-1.69)
25	60	9.3	0.99, 1.08 (0.42-1.4)	1.16, 1.1 (0.68-1.57)

Table 3.6: **Walking speed of ticks after questing and after quiescence under three different climatic conditions (SD: saturation deficit)**

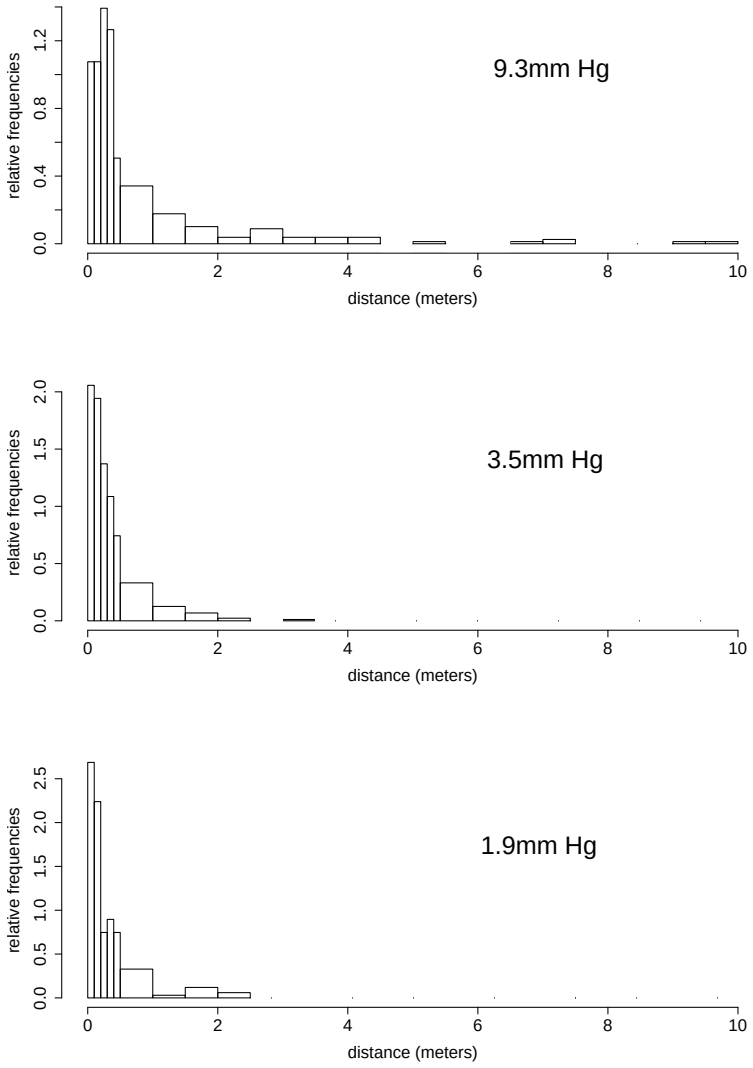


Figure 3.14: **Histogram of the distances walked after quiescence at 3 different saturation deficit.**

3.4 Impact of *B. burgdorferi* infection on tick behaviour

Many parasites influence the behaviour of their insect vectors (Hamilton and Hurd, 2002). We were therefore interested to investigate the effect of a tick-borne bacteria, *B. burgdorferi* sl, on the behaviour of a tick vector, *I. ricinus* in our case. We first investigated the effect of *B. burgdorferi* infection on the locomotion of adult *I. ricinus* just after they got a host stimulus (tick manipulation by a human). To do so we used a locomotion compensator. Second we investigated the effect of *B. burgdorferi* infection on the host-finding behaviour of *I. ricinus* under different climatic conditions and in the absence of any host stimuli. To do so, we used the computer-assisted tracking system. The ticks used below were ticks collected in the field (North of Neuchâtel) during June-July 2001, held for a minimum of one week in a water saturated atmosphere prior to observations.

3.4.1 Impact of *B. burgdorferi* infection on locomotion

We hypothesised that *I. ricinus* infection by *B. burgdorferi* sl could change its walking behaviour. We therefore examined the walks of 69 field collected adults during 6 minutes on a locomotion compensator (see 2.3.4 on page 53). From these 69 *I. ricinus* adults 24 were infected with *B. burgdorferi* sl.

None of the measured parameters showed any significant difference between infected and uninfected ticks (table 3.7, figure 3.15): the time spent moving, the walking speed, the time spent moving upwind, ant the proportion of the path walked against the wind were all unaffected by the infection of ticks by *B. burgdorferi* sl.

We conclude that the locomotion activity of adult *I. ricinus* on a horizontal surface, immediately after reception of a host stimulus, was not affected by *B. burgdorferi* sl infection.

statistic	description	median uninfected	median infected	Wilcoxon test p value
nmt	no move time (s)	0.5	1.0	0.21
l.speed	mean speed (mm/s)	2.28	1.88	0.48
tcon	time upwind (s)	11.5	2.0	0.32
L.C.L	proportion of distance in cone (%)	0.1	0.016	0.37

Table 3.7: **Descriptive statistics for the walks of 24 infected and 45 uninfected adult *I. ricinus* on the locomotion compensator over 6 minutes.**

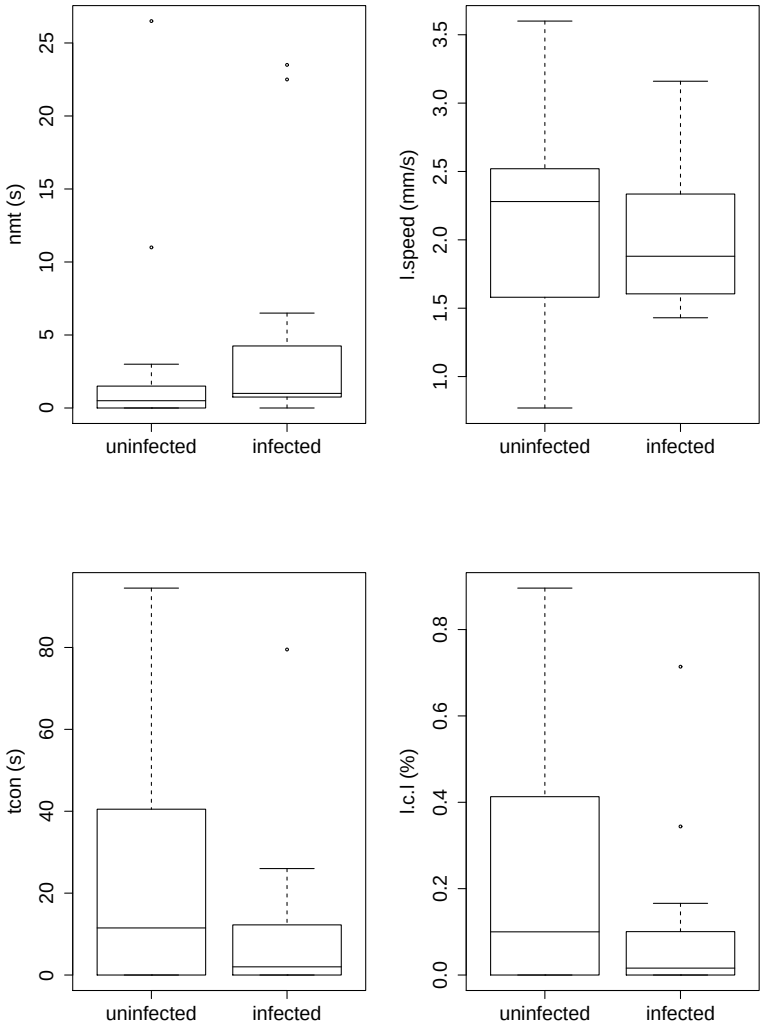


Figure 3.15: **Boxplots of the descriptive statistics recorded for 6 minute walks of 24 *B. burgdorferi* infected and 45 uninfected *I. ricinus* adults on a locomotion compensator.** Top left: time spent immobile (s); top right: mean speed (mm/s); bottom left: time walking upwind (s); bottom right: proportion of the distance walked upwind(%).

3.4.2 Impact of *B. burgdorferi* infection on host-finding behaviour

Using the computer-assisted tracking system we were able to study the impact of *B. burgdorferi* infection not only on the mobility of ticks, but also on the questing behaviour without host stimuli. In these experiments on tick questing behaviour we used *I. ricinus* nymphs collected in the forest North of Neuchâtel. Since this is an endemic area of Lyme borreliosis with about 25% of nymphs infected with *B. burgdorferi* sl (unpublished data), we studied the behaviour of these nymphs prior to the determination of their infection status by immunofluorescence. We were interested to see whether the infection by *B. burgdorferi* had an impact on the behaviour of nymphs. We were particularly interested in the number and the duration of questing, quiescence and walking events as well as in the average speed during walking events.

3.4.2.1 Walking

The behaviour of *I. ricinus* nymphs was observed under 3 different saturation deficits: 9.3, 3.5 and 1.9 mm Hg. The impact of the infection status[7] on tick behaviour[8] was assessed separately for each climatic condition.

9.3mm Hg Saturation deficit: (25°C and 60% RH) 33 nymphs were examined for their behaviour and their infection status. Ten of them were infected with *B. burgdorferi*. The number of walking events over 10 days was higher in infected nymphs (median = 7) and in uninfected nymphs (median = 2, Wilcoxon test: $p = 0.027$). The duration of these walking events was significantly longer in infected nymphs (median = 60.6 minutes) than in uninfected nymphs (median = 49 minutes, Wilcoxon test: $p = 0.022$), and over 10 days the total walking duration was higher in infected nymphs (median duration=7.8 hours) than in uninfected nymphs (median duration = 3.11 hours, Wilcoxon test: $p = 0.038$). However, during the walking events the average walking speed was similar between infected nymphs (0.94 cm/min) and uninfected nymphs (1.13 cm/min, Wilcoxon test: $p = 0.06$). Since the walking duration was increased in infected nymphs and the speed was similar between infected and uninfected nymphs, the total distance walked over 10 days was increased in infected nymphs (median = 409 cm) as compared to uninfected nymphs (median = 101 cm, Wilcoxon test: $p = 0.03$).

At 9.3mm Hg infected nymphs walked longer and more often than uninfected nymphs.

3.5mm Hg Saturation deficit: (25°C and 85% RH) 32 nymphs were examined for their behaviour and their infection status. Ten of them were infected with *B. burgdorferi*. The number of walking events over 10 days was similar in infected nymphs (median = 2.5) and in uninfected nymphs (median = 2, Wilcoxon test: $p = 0.58$). The duration of these walking events was longer, but not significantly, in infected nymphs (median = 66.9 minutes) than in uninfected nymphs (23.6 minutes, Wilcoxon test: $p = 0.06$). Over 10 days the total walking duration was significantly higher in infected nymphs (median = 3.2 hours) than in uninfected nymphs (median =

[7] uninfected or infected as determined by immunofluorescence

[8] as defined in paragraph 2.3.2 on page 46

1.8 hours, Wilcoxon test: $p = 0.036$). The walking speed during walking events was similar between infected nymphs (median = 0.8 cm/min) and uninfected nymphs (median = 0.72 cm/min, Wilcoxon test: $p = 0.39$). Since the walking duration was increased in infected nymphs and the speed was similar between infected and uninfected nymphs, the total distance walked over 10 days was increased in infected nymphs (median = 180.1 cm) as compared to uninfected nymphs (median = 67.7cm, Wilcoxon test: $p = 0.011$).

At 3.5mm Hg infected nymphs walked longer but not more often than uninfected nymphs.

1.9mm Hg Saturation deficit: (15°C and 85% RH) 21 nymphs were examined for their behaviour and their infection status. Five of them were infected with *B. burgdorferi*. The number of walking events over 10 days was similar in infected nymphs (median = 5) than in non infected nymphs (median = 5, Wicoxon test: $p = 0.56$). The duration of these walking events was slightly shorter in infected nymphs (median duration=31 minutes) than in uninfected nymphs (median duration=65 minutes, Wilcoxon test: $p = 0.02$). Over 10 days the total walking duration was similar in infected ticks (median duration = 9.8 hours) and in uninfected nymphs (median = 11 hours, Wilcoxon test: $p = 0.55$). The walking speed during walking events was similar between infected (median = 0.65 cm/min) and uninfected nymphs (median = 0.54 cm/min,Wilcoxon test: $p = 0.13$). Since the walking duration and the walking speed were similar between infected and uninfected nymphs, the total distance walked over 10 days was similar in infected nymphs (median = 200.9 cm) and in uninfected ticks (median = 197.9 cm), Wilcoxon test: $p = 0.97$).

At 1.9mm Hg infected nymphs walked similar distances and not more often than uninfected nymphs.

3.4.2.2 Questing

The number of questing events was similar between infected and uninfected nymphs at either 9.3mm Hg (Wilcoxon test:$p = 0.84$), 3.5mm Hg (Wilcoxon test:$p = 0.76$) and 1.9mm Hg (Wilcoxon test:$p = 0.13$). The duration of these questing events was similar between infected and uninfected nymphs at either 9.3mm Hg (Wilcoxon test:$p = 0.59$), 3.5mm Hg (Wilcoxon test:$p = 0.29$), 1.9mm Hg (Wilcoxon test:$p = 0.83$).

The infection status had no impact on the number and duration of questing events at any of the three tested climatic conditions.

3.4.2.3 Quiescence

The number of quiescence events was significantly higher in infected nymphs (median = 5.5 events) than in uninfected nymphs (median = 1 event) at 9.3mm Hg (Wilcoxon test: $p = 0.011$). This result is a consequence of the increased number of walking events interrupting quiescence in infected nymphs. The number of quiescence events was similar in infected and uninfected nymphs at 3.5mm Hg (median = 1 event, Wilcoxon test: $p = 0.30$) and 1.9mm Hg (median = 2 events, Wilcoxon test: $p = 0.80$). The duration of these events were similar between infected and

uninfected nymphs at 9.3mm Hg (median = 24h, Wilcoxon test: $p = 0.71$), 3.5mm Hg (median = 23.2h, Wilcoxon test: $p = 0.71$) and at 1.9mm Hg (median = 17.2, Wilcoxon test: $p = 0.96$).

The infection of nymphs with *B. burgdorferi* increased the number of quiescence events under the most dessicating conditions only, and did not affect the length of these events

We conclude that the behaviour of *I. ricinus* nymphs was only affected by *B. burgdorferi* under the most dessicating conditions. Under these conditions infected nymphs interrupted their quiescence more often with longer walking events than uninfected nymphs. The distance walked by infected nymphs was thus much higher than the distance walked by uninfected nymphs. The duration of questing on the other hand was unaffected by *B. burgdorferi* sl infection.

3.5 *I. ricinus* recruitment by vertebrate hosts

Questing tick density seems to be an obvious measurement of tick-bite risk. In order to quantify the relation between the density of questing ticks in the field and the infestation of a vertebrate host, we organised walks with Beagle dogs in the forest North of Neuchâtel, in collaboration with Novartis Animal Health (Saint-Aubin, FR). Two walks in the forest North of Neuchâtel were organised during April 2000 and five during May 2002 (for details see 2.2.4). The walked path was recorded with a GPS receiver. Duration, distance walked, and average speed were measured. After the walk, dogs were kept in individual cages and ticks attached on the dogs were counted immediately after the walk and after 24h. In paralell the density of questing ticks in the field was measured by flagging.

No nymphs were found attached on dogs, only females and males. The average infestation of dogs ranged from 5 to 60 females (table 3.8). The distance walked each time ranged from 3.7 to 10 Km, and the questing female density ranged from 13 to 22 females per 100m^2. Based on the distance walked (W), the questing female density (FD), and the dog surface, we estimated the number of females met by the dogs during each walk (MET, according to equation 2.2 on page 43).

Surprisingly the number of female ticks attached to the dogs was negatively related to the estimated number of female ticks met by the dogs in the field (MET) (figure 3.16, slope: -0.14045, $p = 0.02799$, $R^2 = 0.65$). We must emphasize that the measurements were not independent since we sampled ticks in successive walks at the beginning of April 2000 and during May 2002. In fact the proportion of attachment increased throughout the sampling period: starting from 1% in April (2000) and increasing from 6.4% to 20% in May 2002 (table 3.8). No correlation was found between dog infestation and saturation deficit recorded locally during sampling (p=0.44). No nymhs were found attached on the dogs although they were abundant in the field. Similarly to females, the number of males found on the dogs increased during spring.

We conclude that the number of females attached on the dogs was not proportional to the number of females potentially met by the dogs but rather increased with time from the beginning of April to the end of May

Parameter	Walk 1	Walk 2	Walk 3	Walk 4	Walk 5	Walk 6	Walk 7
Date	03.04.00	07.04.00	06.05.02	07.05.02	08.05.02	15.05.02	16.05.02
Start Time	10:11	10:20	10:00	10:00	10:00	10:00	10:00
Saturation deficit (mmHg)	5.6	1.7	3.5	4.5	6.9	3.9	
Duration	2h08	2h20	2h07	1h44	2h10	1h30	1h30
(W) Km walked (Km)	6.52	10.0	4.25	4.57	3.68	4.52	4.0
Av. speed (Km/h)	3.04	4.0	2.0	2.64	1.7	3	2.67
Number of dogs (n)	2	2	4	4	4	4	4
Av. dog infest. (fem)	**5**	**6**	**23.5**	**37.5**	**40.25**	**41.75**	**60.25**
Av. dog infest. (mal)	**4**	**4**	**7.5**	**11**	**22**	**10**	**13.5**
Flag: surface sampled (m2)	262.5	200	200	NA	425	200	NA
(FD) Flag: fem density (n/100m2)	14	13	18	NA	22	14.5	NA
Flag: mal density (n/100m2)	12	13	17	NA	20	16	NA
(MET) Estimated num. of fem. met(n) (if dog sampling area=50 cm^2)	**456**	**650**	**383**	**411***	**405**	**328**	**290***
Proportion of attachment (%)	1.1%	0.9%	6.4%	9.1%	9.9%	10.3%	20.7%

Table 3.8: **Path walked, dog infestation and questing tick density.**
NA: not available; Av: average; Fem: female; Mal: male; Nym: nymph
* based on tick density recorded the day before.

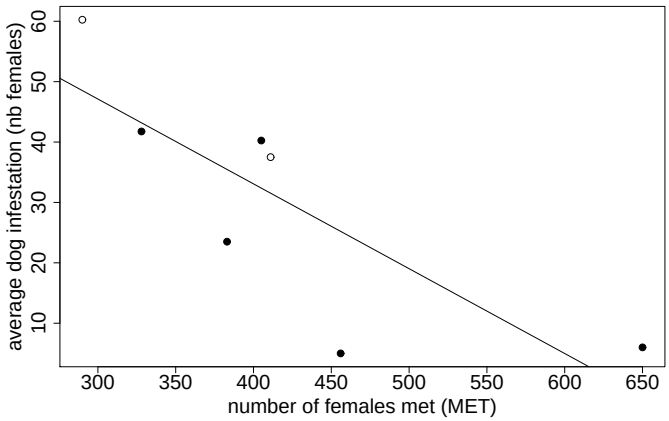


Figure 3.16: **Infestation of beagles as a function of the expected number of female *I. ricinus* met during one walk (MET).**

(○ density of ticks recorded the day before dog walks; ● density of ticks recorded during dog walks)

3.6 Microclimate in the forest North of Neuchâtel

The millimeter sized ticks experience strongly heterogeneous environmental conditions in the field. Microclimatic conditions are influenced by a lot of factors up to the shadow provided by leafs exposed to the sun, the color and nature of the substrate, the slope, the evapotranspiration of plants, the perturbations in air flows, and many other factors. It is thus very difficult or impossible to measure the exact climatic conditions experienced by ticks in the field. However, as we observed under laboratory conditions, the host-finding behaviour of ticks is under the influence of climate. So how can we study the impact of climate on the phenology of ticks in the field? Consistent, continuous and verified climatic data are available at Meteosuisse in Zürich. These data are recorded by automatic weather stations located all over Western Switzerland. Since meteorological data collection is not trivial, and since microclimatic data are not available at our sampling sites, nor would these data cover the heterogeneity met by ticks in the field, we wanted to correlate tick phenology with mesoclimatic data as provided by Meteosuisse. However these automatic weather stations are not located within forests. In Neuchâtel, the automatic weather station is located at the "Observatoire Cantonal", approximately 0.4 Km South of the forest. We therefore expect the climate within the forest to be slightly different from the climate measured outside. To estimate this difference, we recorded the hourly average ground temperature, air temperature, and air relative humidity in the forest, close to the field arenas (see 2.2.1.4 on page 25) continuously over three periods in 2000, and compared these measurements with those recorded by the automatic weather station.

The correlation between temperature recorded at the Neuchâtel observatory and the temperature recorded 60 cm above ground in the forest, was excellent ($R^2 = 0.93$, $slope = 0.932$, figure 3.17). The temperature measured 10 cm above ground in the forest tended to be lower than the temperature recorded at the observatory ($R^2 = 0.92$, $slope = 0.876$). A lower, but significant correlation between micro and mesoclimate was observed for relative humidity ($R^2 = 0.63$, $slope = 0.861$) and saturation deficit ($R^2 = 0.7$, $slope = 0.636$).

We therefore conclude that mesoclimatic parameters measured close to a forest can be used to assess temperature within that forest, and with some caution relative humidity and saturation deficit as well.

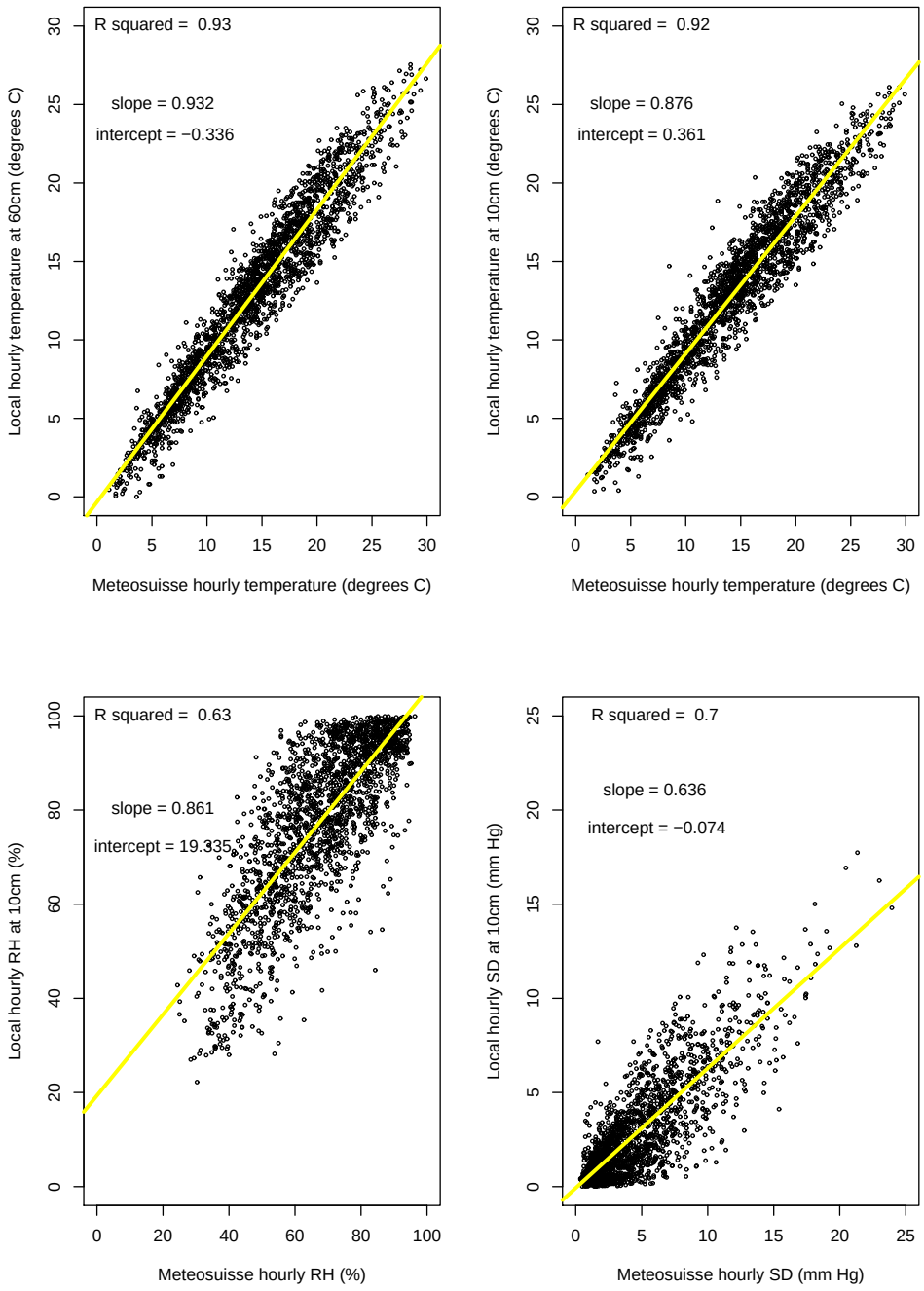


Figure 3.17: **Correlation between hourly micro and mesoclimatic variables in Neuchâtel.** Measurements of hourly averages of temperature and relative humidity measured in Neuchâtel. All graphs show the mesoclimate (measured by Meteosuisse) on the x axis, and the microclimate (in the forest North of Neuchâtel) on the y axis. Saturation deficit (bottom right graph) was calculated according to formula 2.1 in section 2.2.1.3.

3.7 *I. ricinus* phenology

Questing tick density was studied from different perspectives: First we were interested in the spatial distribution of ticks on a kilometer scale. To assess the randomness or the non-randomness of this distribution we measured questing tick density over an extended surface in Neuchâtel. We were then interested in the phenology of *I. ricinus*: the evolution of the questing tick density. To estimate this phenology we sampled ticks monthly at each sampling site. First we estimated the phenology of *I. ricinus* repeatedly over an extended surface in Neuchâtel. Second we were interested in the phenology of ticks along an altitude gradient, and thirdly we were interested in the spatio-temporal variations of tick phenology on regional scale. We therefore studied the phenology of ticks in selected locations all over Western Switzerland, including Neuchâtel, the Chaumont mountain, Vaumarcus, Bavois, Eclépens, St-Livres, Bière, Bois-de-Portes, Brochus-Creuson, Aigle, Viège and Interlaken. During these studies, *I. ricinus* was the only tick species collected by flagging.

3.7.1 Spatial distribution of ticks on a kilometer scale

In order to estimate the sampling area necessary for the estimation of questing tick density we studied the horizontal distribution of ticks. We wanted to verify the null hypothesis of random distribution. The distribution of ticks on a kilometer scale was studied from the data collected during the extended surface sampling in Neuchâtel from 1999 to 2002 (1049m^2 sampling surface over 44 sampling sessions). The number of ticks (nymphs and adults separately) collected every 18 meters along the path was recorded for each sampling session. If ticks were randomly distributed one would expect tick counts to follow a Poisson distribution. We therefore calculated the Poisson index for every capture session and found that the Poisson index for nymphs and for adults was not significantly different from 1 (see 2.2.2.8 on page 40 for statistical procedure). The null hypothesis of a Poisson distribution for the tick counts can thus not be rejected. This however is not sufficient to show that ticks are randomly distributed. Indeed it is possible that tick counts follow a Poisson distribution but are spatially non random. We therefore performed an additional analysis and calculated an autocorrelation function (ACF, see figure 3.18) for nymphs and adults along the sampling path, for each sampling session separately ($n = 44$). The maximal lag autocorrelation significant for adults as well as for nymphs was 1, as for example on the 14.3.2000 (figure 3.19). However, most of the capture sessions (86%) showed no significant autocorrelation for nymphs or for adults.

We therefore conclude that, in the Neuchâtel sampling area, over 1049m^2, nymphs and adults were mostly randomly distributed, but when a spatial autocorrelation was found it was not more than a single lag, so that a sampling size of 100 meters is sufficient for the estimation of questing tick density on a kilometer scale.

Autocorrelation function (ACF)

The autocorrelation function expresses the correlation of each observation with its neighbours in spatial data or time series. The autocorrelation of lag 1 represents the correlation of each observation with its immediate neighbour. The autocorrelation of lag 2 represents the correlation of each observation with its second neighbour and so on. Confidence intervals for the autocorrelation function can be calculated using the Fisher transformation as described in (Afifi and Azen, 1972) (pp. 103-104). In the case of multiple autocorrelation comparisons the alpha error value choosen for significance has to be divided by the number of tests performed.

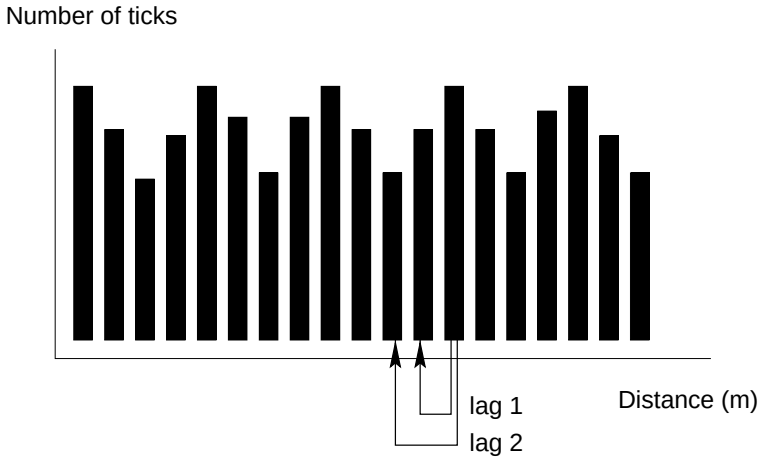


Figure 3.18: **Autocorrelation function (ACF)**

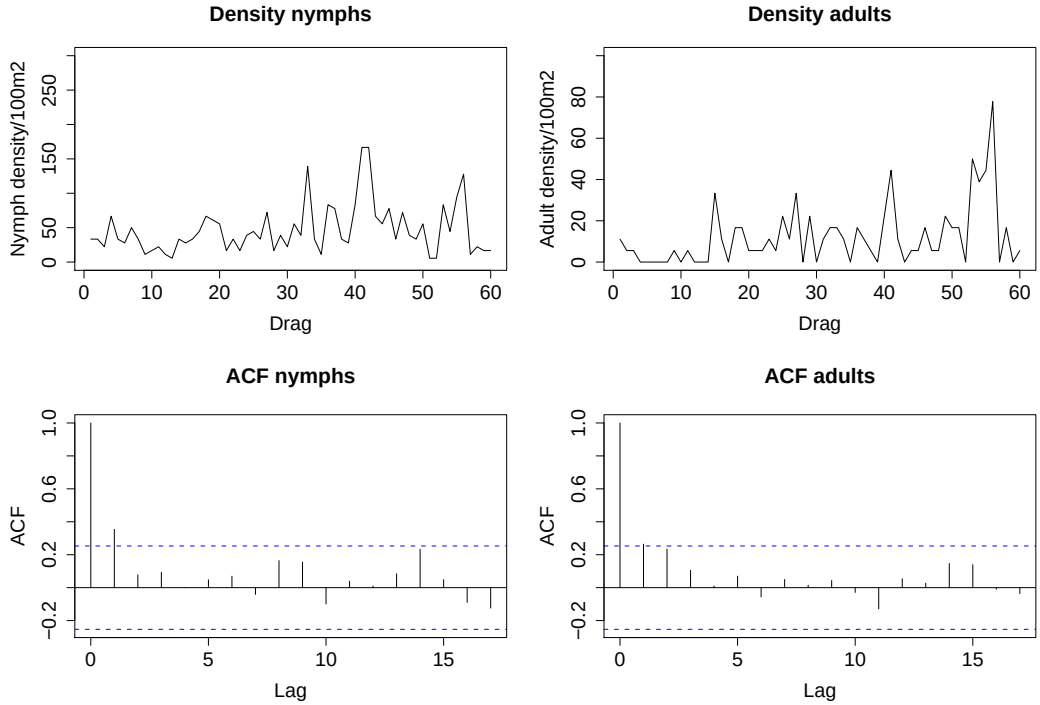


Figure 3.19: **Tick distribution along the sampling path in Neuchâtel on the 14.3.2000** Tick density as a function of the path (top graphs), autocorrelation function (ACF) (bottom graphs), nymphs (left graphs), adults (right graphs). One lag = one drag of 18 m. The dotted lines indicate the 5% significance level (without correction for multiple tests).

3.7.2 *I. ricinus* phenology at different altitudes

To study the phenology of ticks in different climatic conditions, we observed the phenology of ticks at three different altitudes within a short horizontal distance and within the same continuous forest. To do so, three locations on the slope of a single mountain (620, 740 and 900 meters above sea level, within a horizontal distance of 1.8 Km) were sampled monthly from March 1999 to November 2001 in the forest North of Neuchâtel. Tick density expressed as the cumulative tick density (CTD)[9] decreased with altitude (data in appendix C on page 217, Jonckheere test $p = 0.017$, $n = 9$ for nymphs and $p = 0.0098$, $n = 9$ for adults).

The phenology of ticks varied considerably with altitude (figure 3.20 to 3.22). The onset of tick activity (O10[10]) in spring was inversely related to altitude, especially in nymphs (Jonckheere test $p = 0.007$ for nymphs and $p = 0.0590$ for adults). The density then increased and reached a main spring peak in April-May. The time of the peak was not related to altitude (Jonckheere test: $p = 0.18$ for nymphs and $p = 0.13$ for adults). The peak tick density however decreased with altitude (Jonckheere test: $p = 0.0098$ for nymphs and $p = 0.035$ for adults). A noticeable exception occurred in 2001 when the highest density of adults was recorded at the medium

[9] the integral of the tick density curve as defined on page 28

[10] the time when tick density increased above 10% of the maximum tick density

altitude. Variance among years of the questing nymph density during the spring peak was linearly and inversely related to altitude (F-test: $p = 0.0135$). A secondary peak of questing nymphs was sometimes observed in autumn. The nymphal density during this autumn peak decreased with altitude (Jonckheere test: $p = 0.005$). During 2000, the autumn peak of nymphs at the two lowest altitudes reached 70% of the spring peak. The end of tick activity (F10) was not related with altitude (Jonckheere test: $p = 0.85$ for nymphs and $p = 0.25$ for adults).

We hypothesised that nymphs active in autumn were derived from spring fed larvae. We did not record the larval density in the field, but if we admit that nymphs provide an indication for larval activity in spring, we may compare the autumn nymph peak with the parameters recorded in spring for nymphs. By doing that for all 3 altitudes together, we observed that the maximum nymph density in autumn (PTD2N) was related to the date of nymph questing onset in spring (the earlier nymphs onset, the higher the autumn peak; F-test: O25N[11], $p = 0.003$; O10N[12], $p = 0.03196$). On the other hand, PTDN2 was not related to the maximum nymph density in spring (PTDN1, $p = 0.19$, F-test). The date of onset of the autumn peak (O2.25N) was not related to the date of onset of the spring peak (O25N, F-test: $p = 0.14$), nor was it related to the peak nymph density in spring (PTD1N, F-test: $p = 0.95$). In adults, autumn peaks were much less important than in nymphs at all altitudes.

Within the same forest and within a short horizontal distance, altitude had a very important impact on tick density and phenology: the cumulated tick density, the onset of tick activity, the spring peak density, and the autumn peak density were all related to altitude. At lower altitude ticks were more numerous, but their phenology was much more variable among years than at higher altitudes.

[11] above 25% of the peak tick density
[12] above 10% of the peak tick density

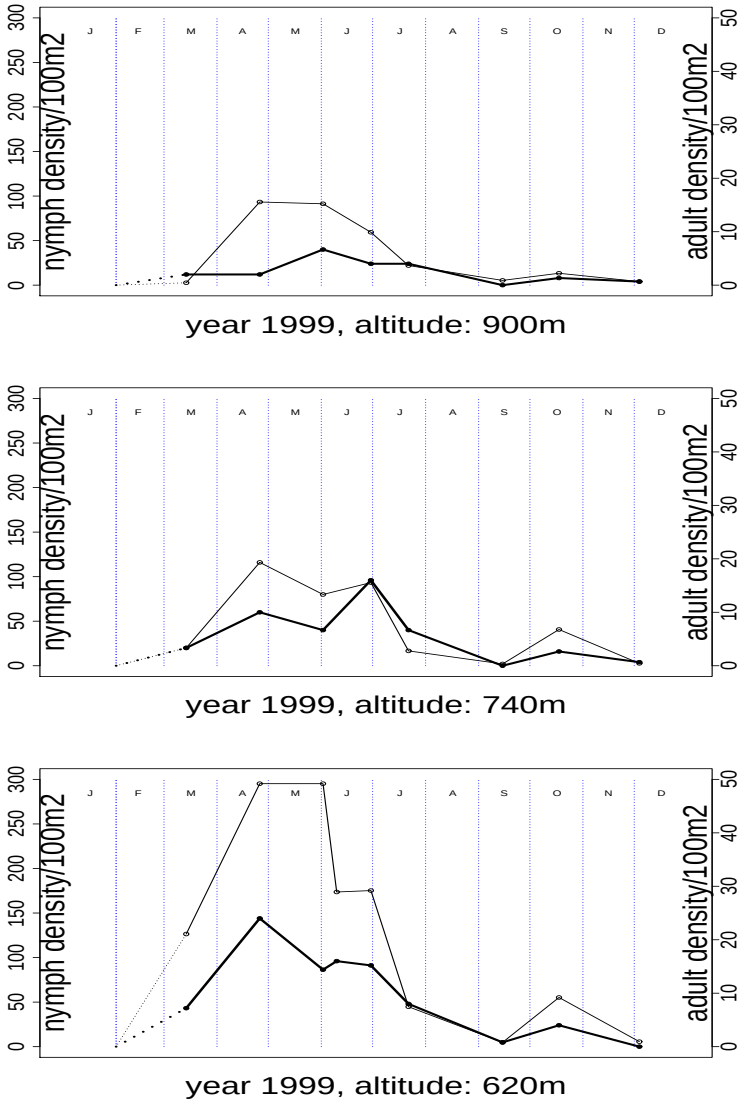


Figure 3.20: **Timeplot of the questing tick density at Chaumont in 1999;**
thick line and right scale: adults; thin line and left scale: nymphs;
high (top), middle and low altitude (bottom graph).

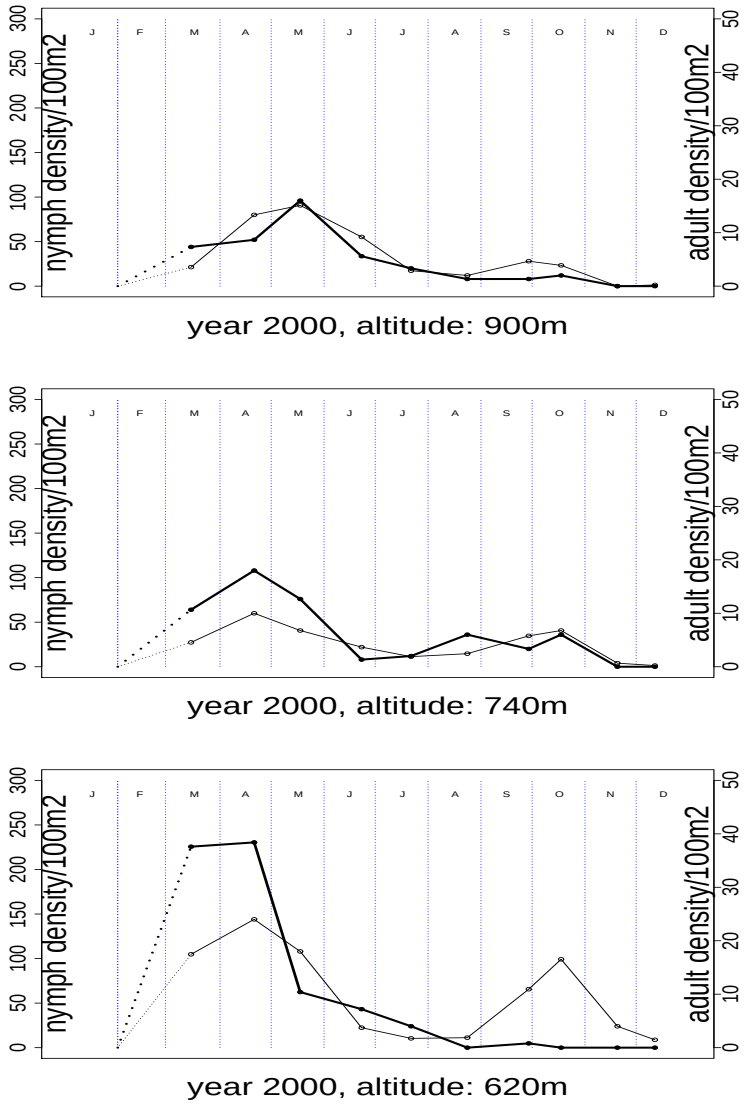


Figure 3.21: **Timeplot of the questing tick density at Chaumont in 2000;**
thick line and right scale: adults; thin line and left scale: nymphs;
high (top), middle and low altitude (bottom graph).

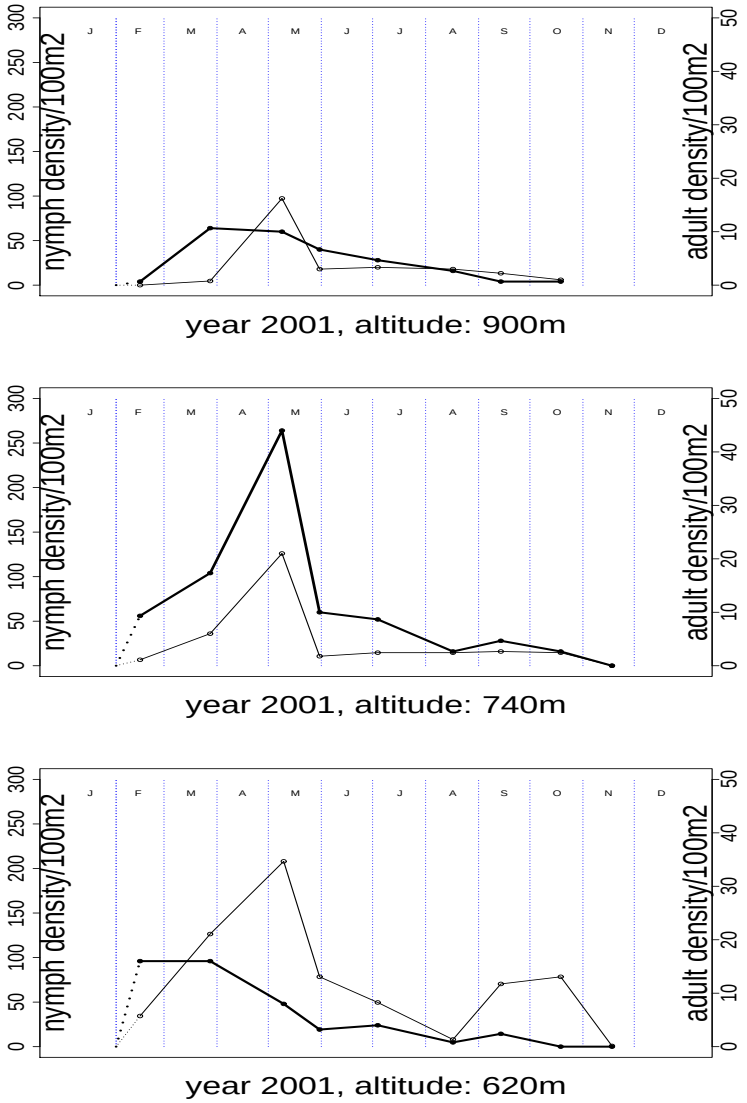


Figure 3.22: **Timeplot of the questing tick density at Chaumont in 2001;**
thick line and right scale: adults; thin line and left scale: nymphs;
high (top), middle and low altitude (bottom graph).

3.7.3 *I. ricinus* phenology on a regional scale

Previous observations on the phenology of *I. ricinus* in Neuchâtel showed that the onset of tick activity in spring was related to temperature (Perret et al., 2000). We therefore hypothesised that the same climatic constraints underly the phenology of ticks all over Western Switzerland. To test whether and how tick phenology could be related to climatic conditions, we continued the sampling in 4 zones in Neuchâtel from 1999 to July 2002 and completed these datasets with measurements of the questing nymph and adult density in 8 areas over Western Switzerland[13] sampled at monthly intervals over three years (1999-2001)

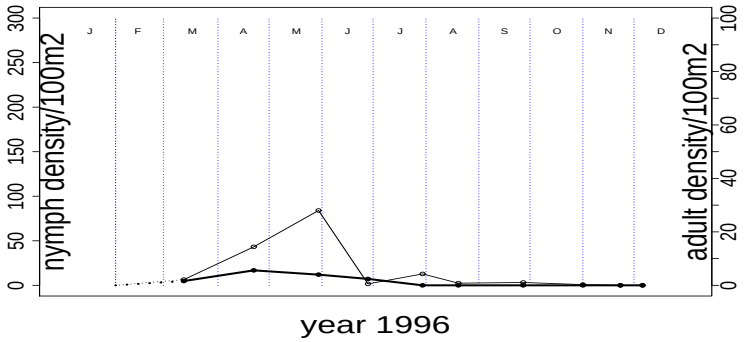
In this section we will first examine the density and the phenology of *I. ricinus* over Western Switzerland and compare sampling areas and years. We will then try to relate some of the parameters describing tick phenology with climatic parameters.

3.7.3.1 *I. ricinus* density and phenology in Western Switzerland

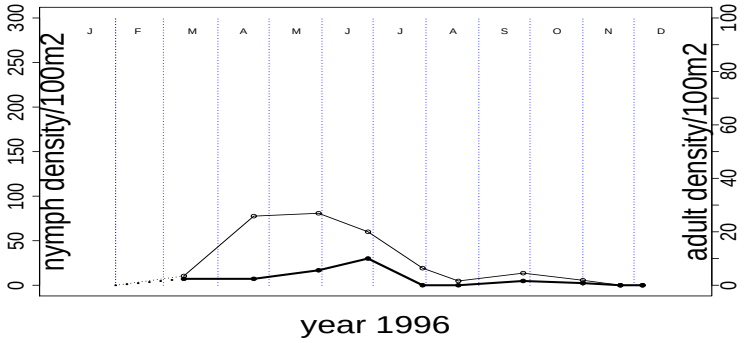
The phenologies and densities of *I. ricinus* recorded in Neuchâtel from 1996 to July 2002 (figures 3.23 to 3.29) and from 1999 to 2001 in 8 areas of Western Switzerland (figures 3.30 to 3.38) showed important variations among locations, and within locations among years. We therefore compared the density and the phenology of ticks among years and locations from 1999 to 2001. (continued on page 113)

[13] Aigle, Bavois, Bois-de-Portes, Brochus-Creuson, Eclépens, Interlaken, St-Livres, Viège. Bière was excluded because it lies higher in altitude that the other sampling areas and because no climatic data were available for Bière.

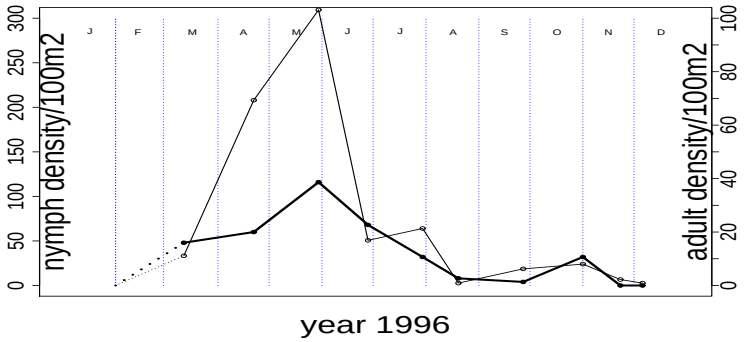
Zone 1:



Zone 2:



Zone 3:



Zone 4:

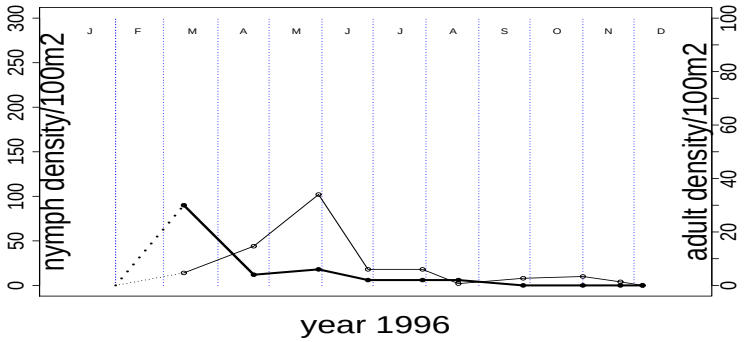
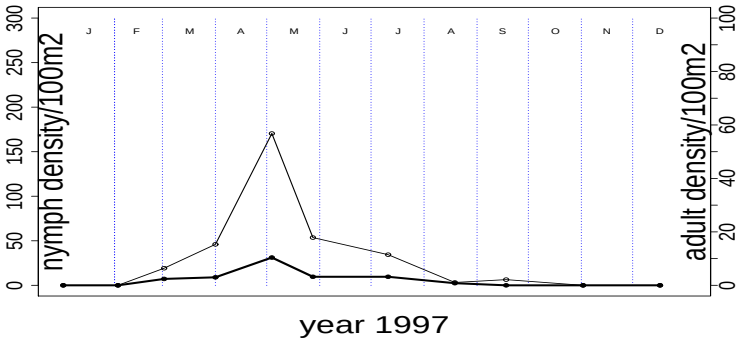
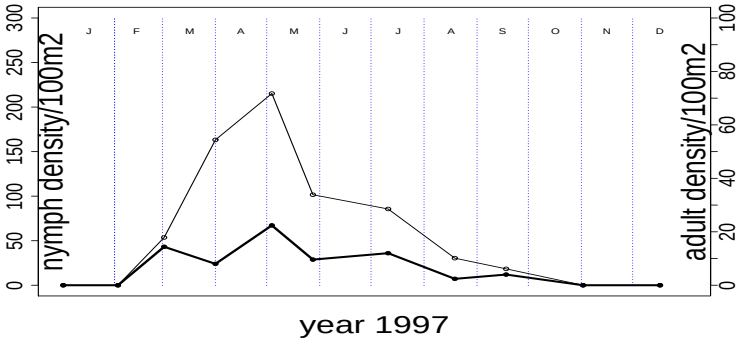


Figure 3.23: **Timeplot of the questing tick density in Neuchâtel in 1996;**
thick line and right scale: adults; thin line and left scale: nymphs.

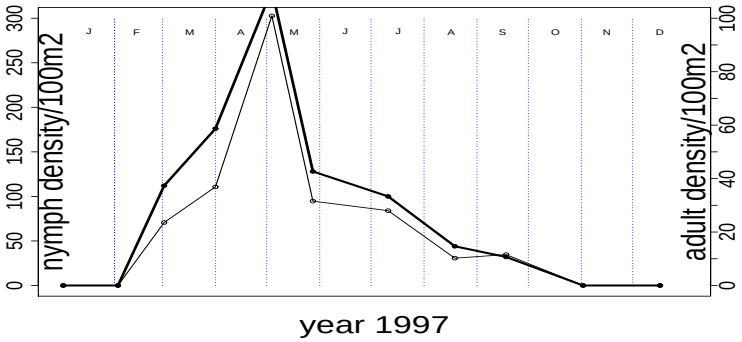
Zone 1:



Zone 2:



Zone 3:



Zone 4:

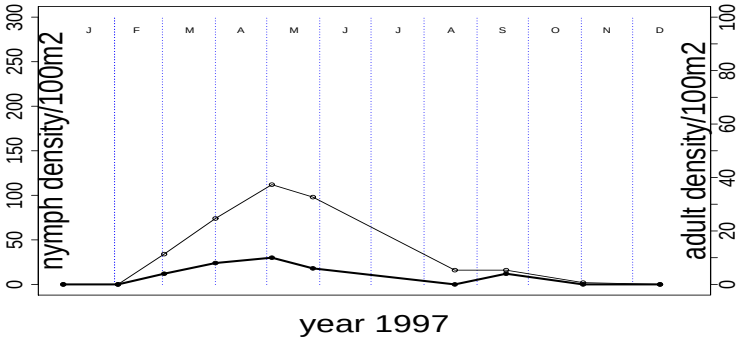
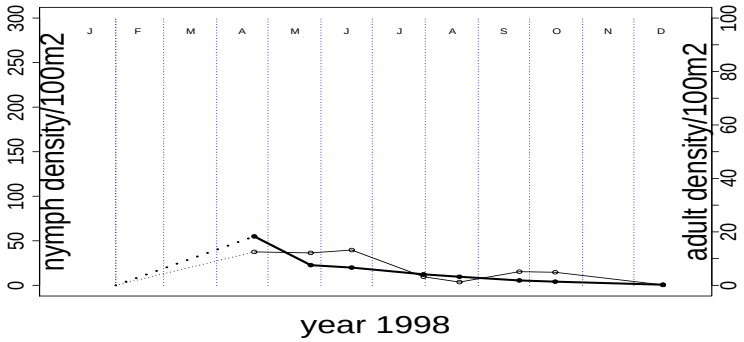
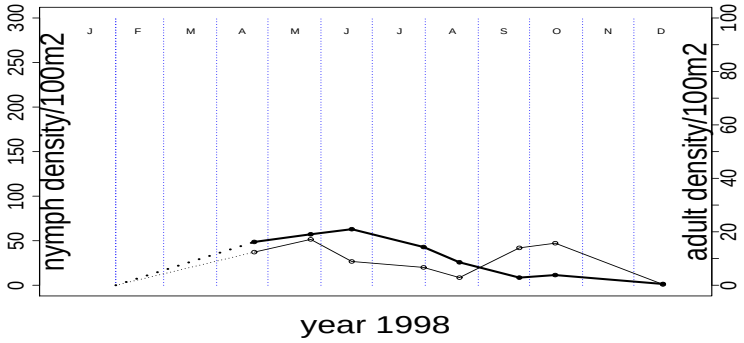


Figure 3.24: **Timeplot of the questing tick density in Neuchâtel in 1997;**
thick line and right scale: adults; thin line and left scale: nymphs.

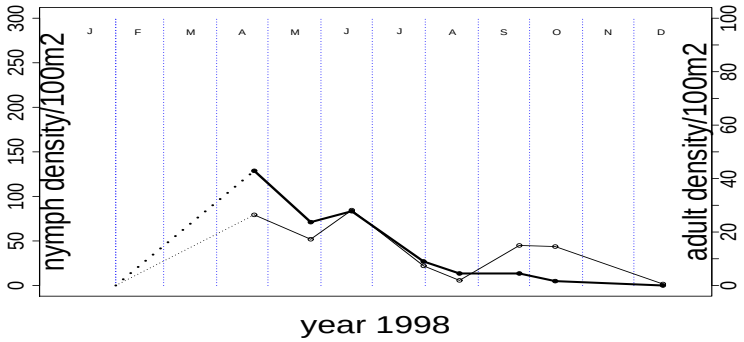
Zone 1:



Zone 2:



Zone 3:



Zone 4:

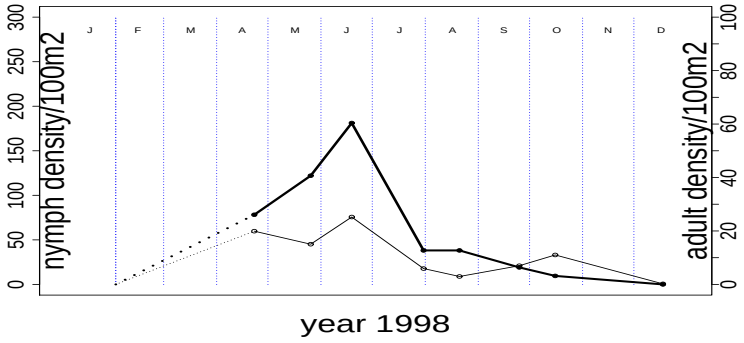
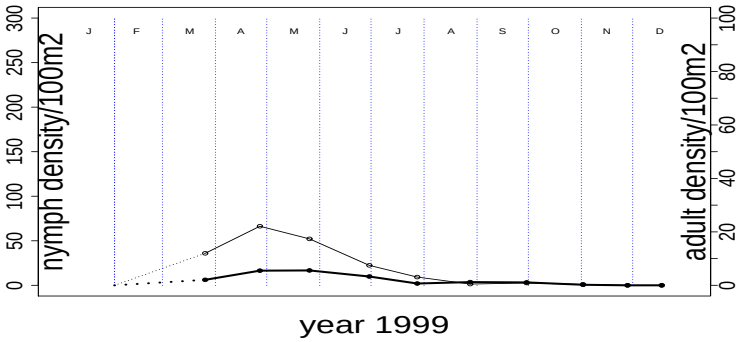
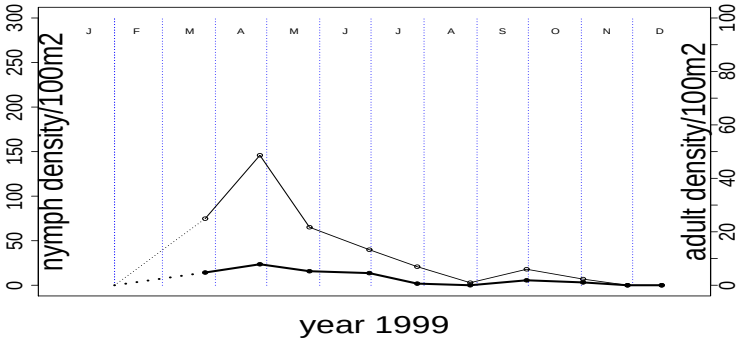


Figure 3.25: **Timeplot of the questing tick density in Neuchâtel in 1998;**
thick line and right scale: adults; thin line and left scale: nymphs.

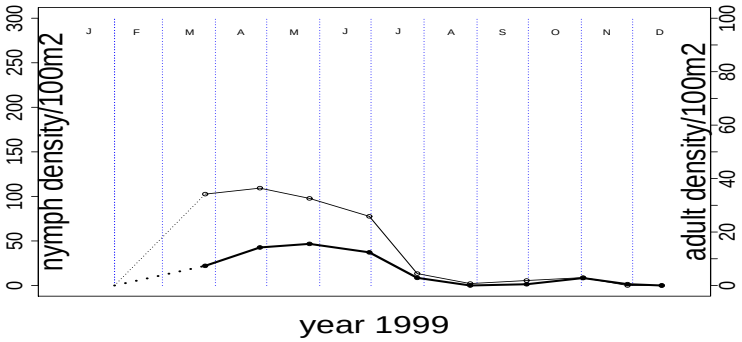
Zone 1:



Zone 2:



Zone 3:



Zone 4:

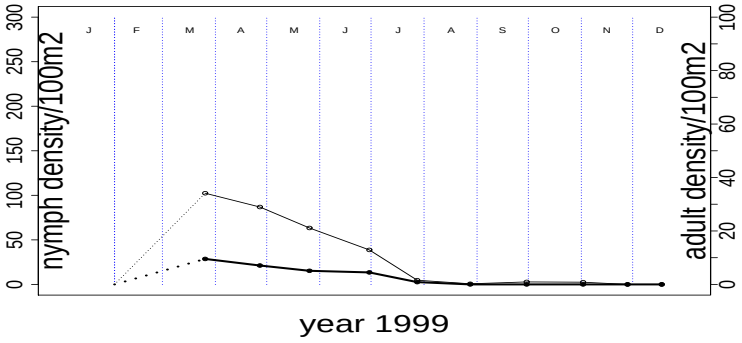
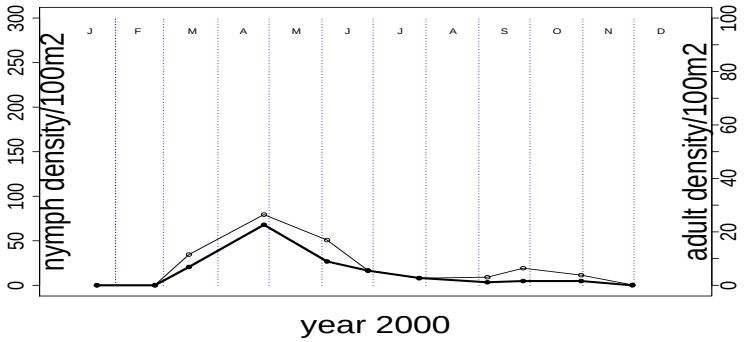
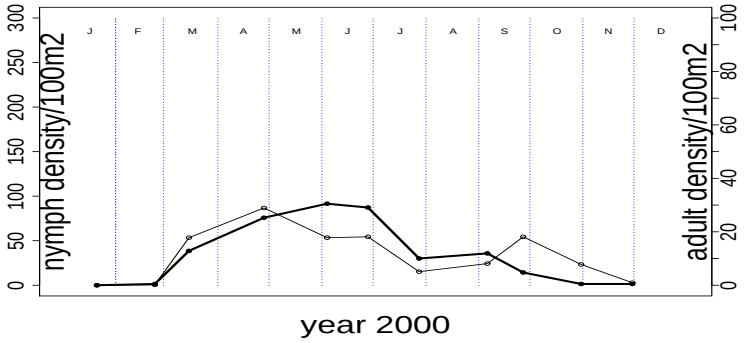


Figure 3.26: **Timeplot of the questing tick density in Neuchâtel in 1999;**
thick line and right scale: adults; thin line and left scale: nymphs.

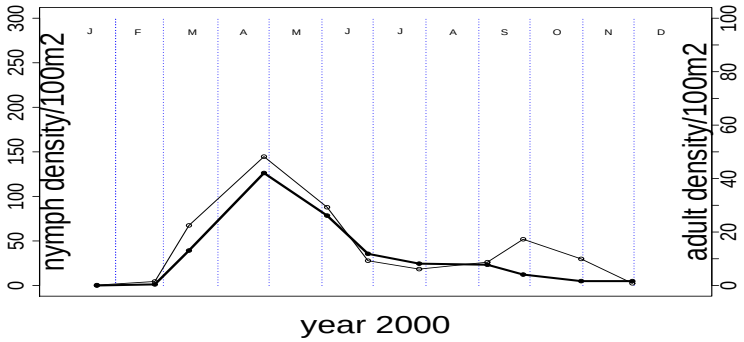
Zone 1:



Zone 2:



Zone 3:



Zone 4:

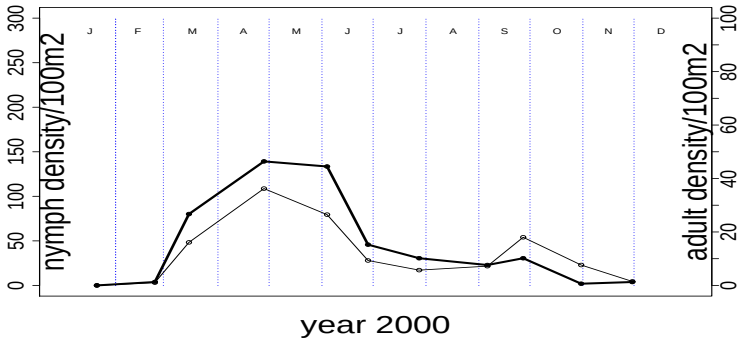
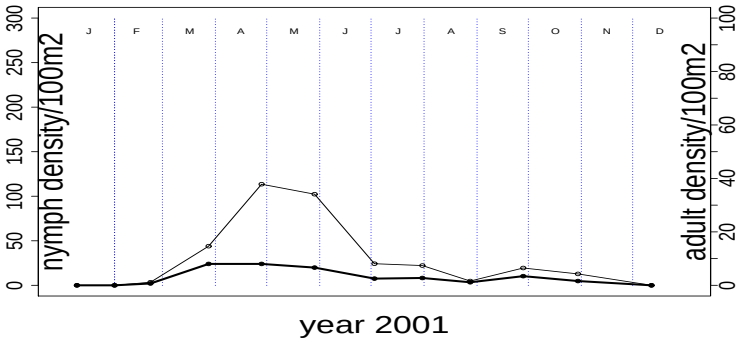
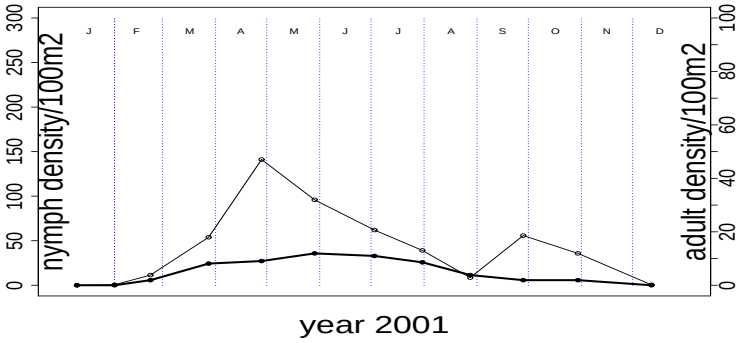


Figure 3.27: **Timeplot of the questing tick density in Neuchâtel in 2000;**
thick line and right scale: adults; thin line and left scale: nymphs.

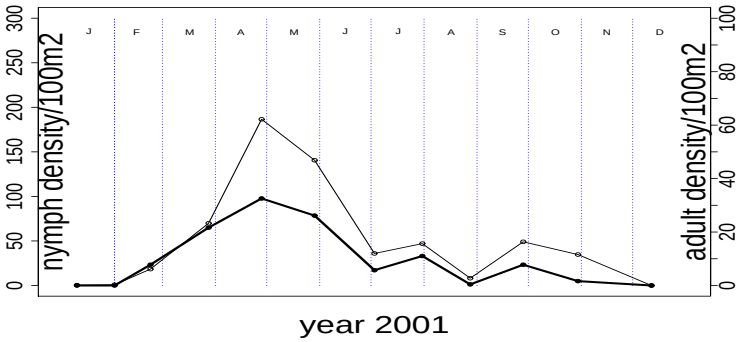
Zone 1:



Zone 2:



Zone 3:



Zone 4:

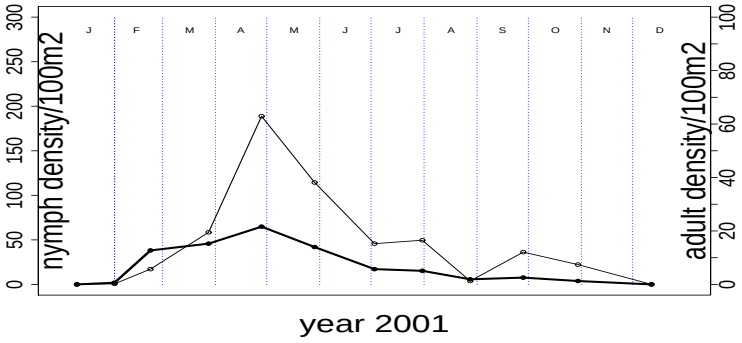
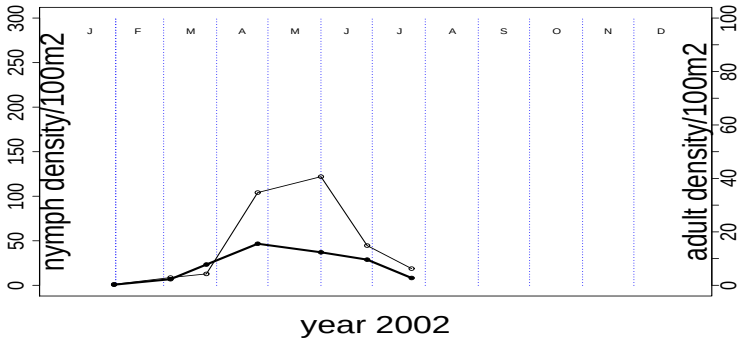
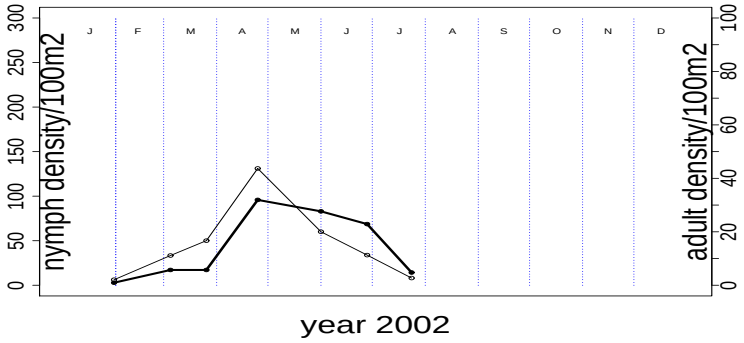


Figure 3.28: **Timeplot of the questing tick density in Neuchâtel in 2001;**
thick line and right scale: adults; thin line and left scale: nymphs.

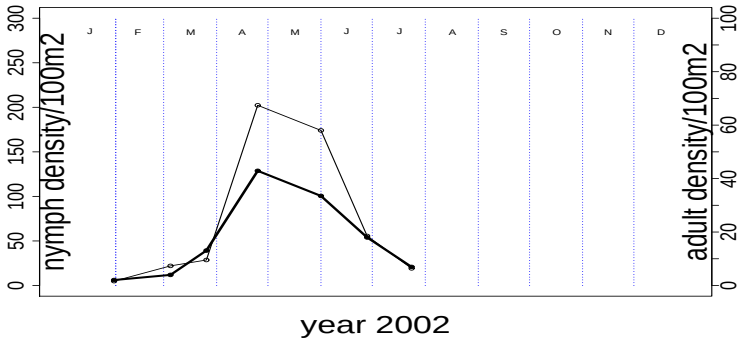
Zone 1:



Zone 2:



Zone 3:



Zone 4:

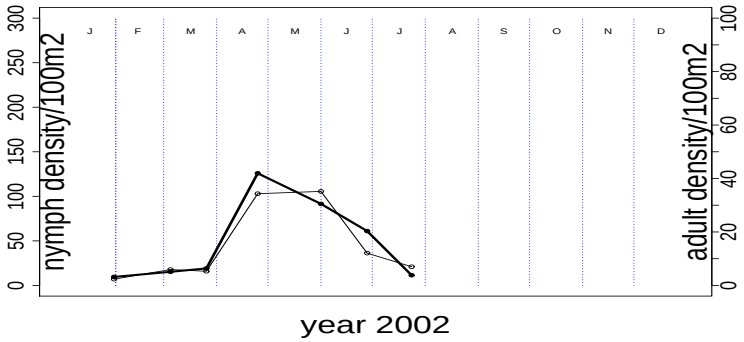


Figure 3.29: **Timeplot of the questing tick density in Neuchâtel in 2002;**
thick line and right scale: adults; thin line and left scale: nymphs.

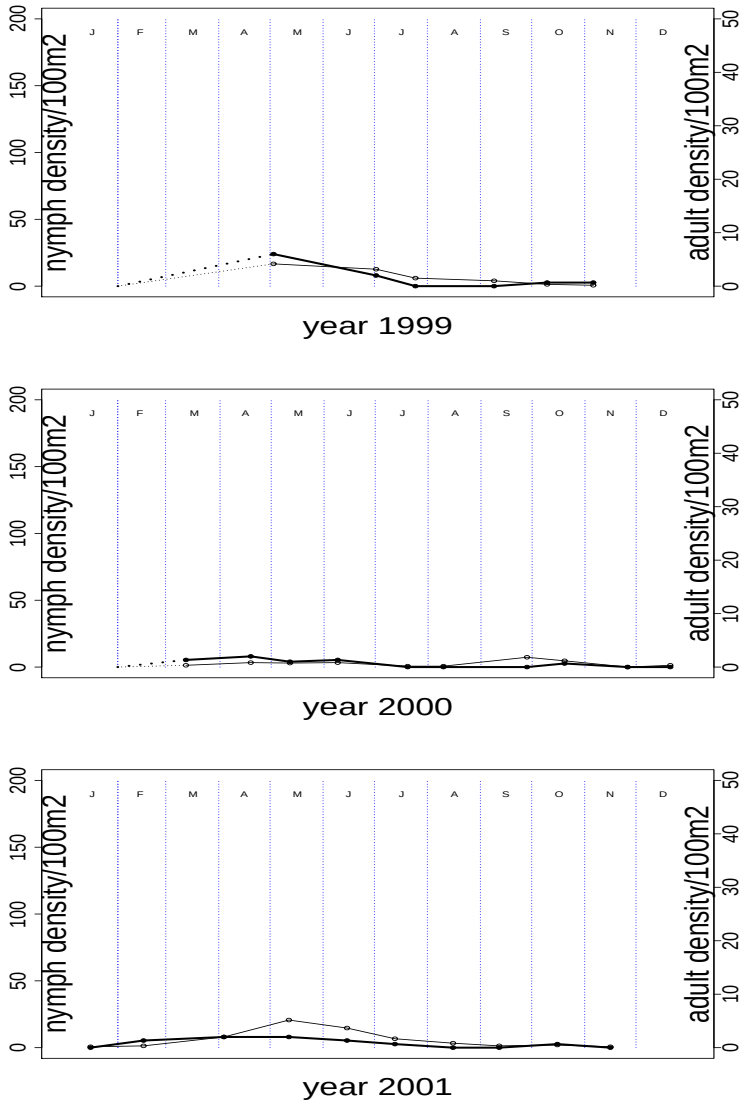


Figure 3.30: **Timeplot of the questing tick density in Aigle**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

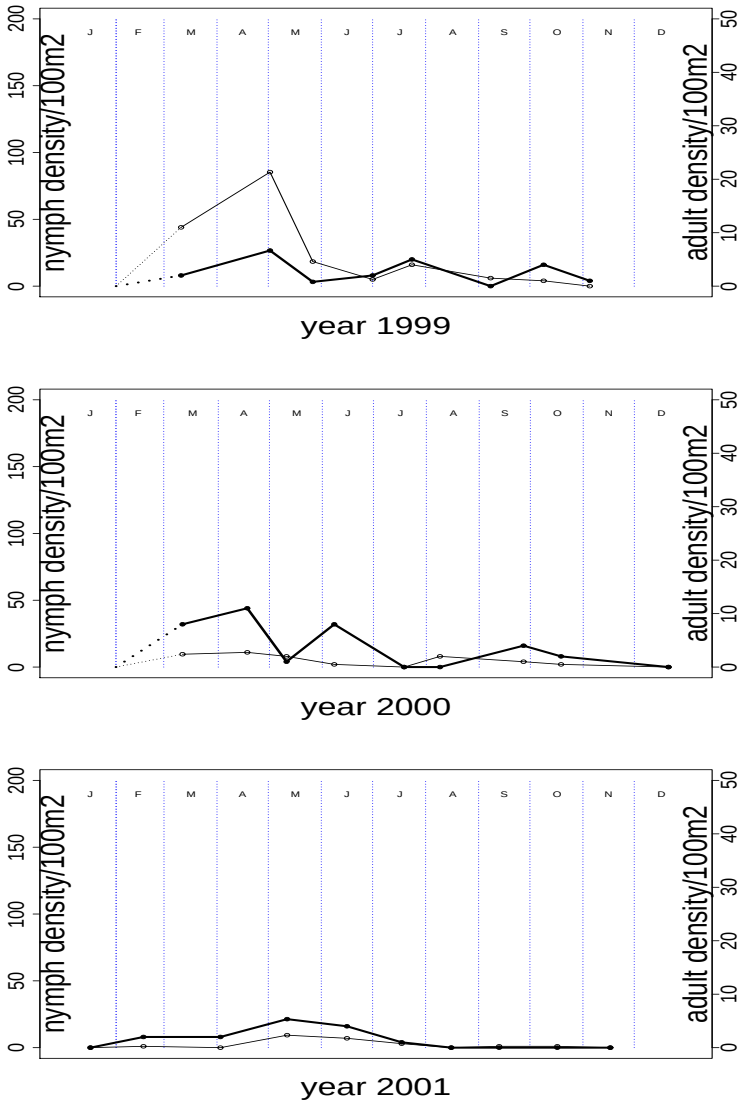


Figure 3.31: **Timeplot of the questing tick density in Bavois**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

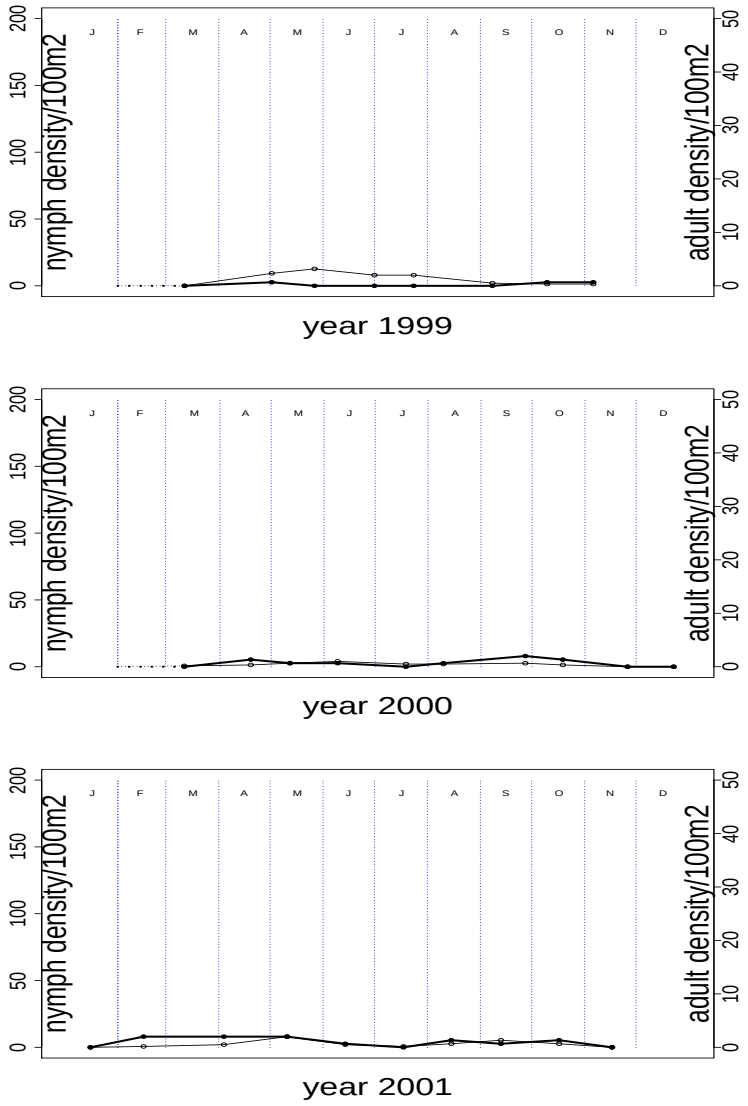


Figure 3.32: **Timeplot of the questing tick density in Bière**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

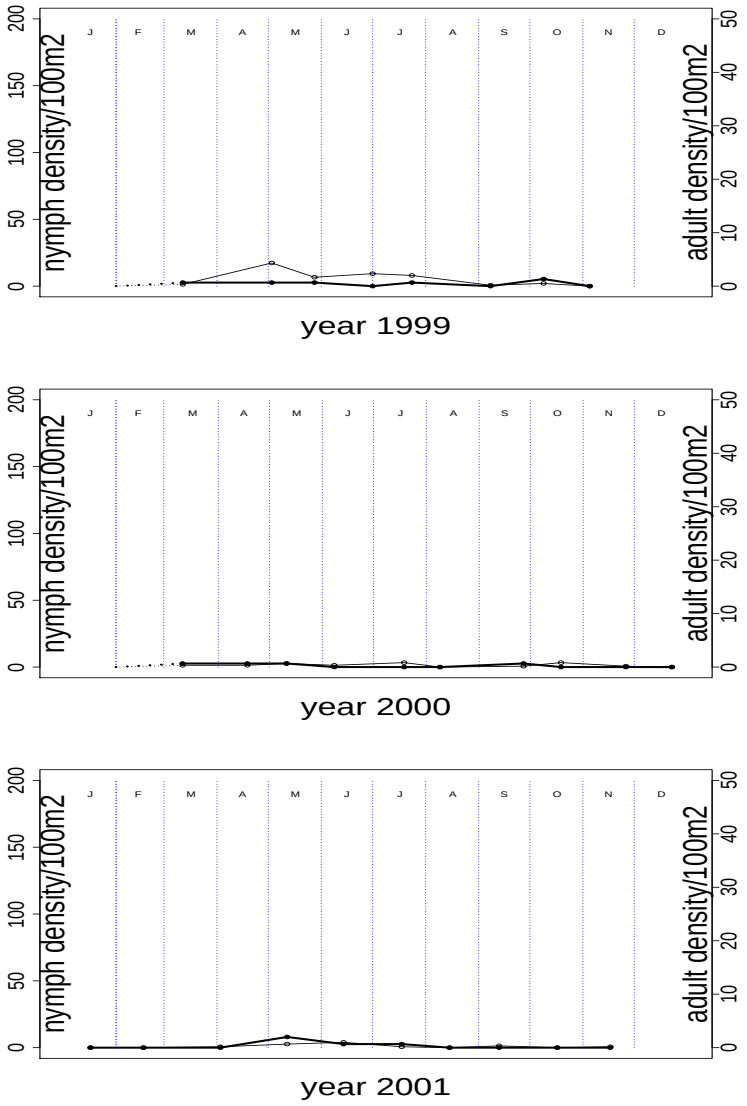


Figure 3.33: **Timeplot of the questing tick density in Bois de Portes**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

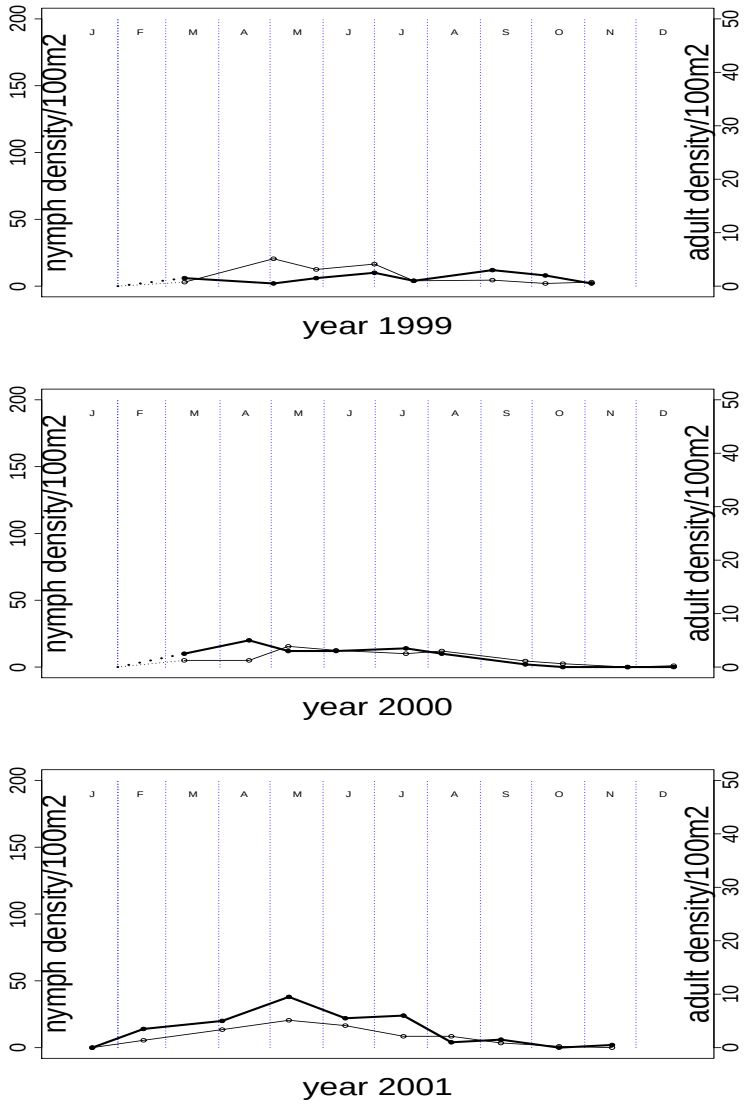


Figure 3.34: **Timeplot of the questing tick density in Brochus-Creuson**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

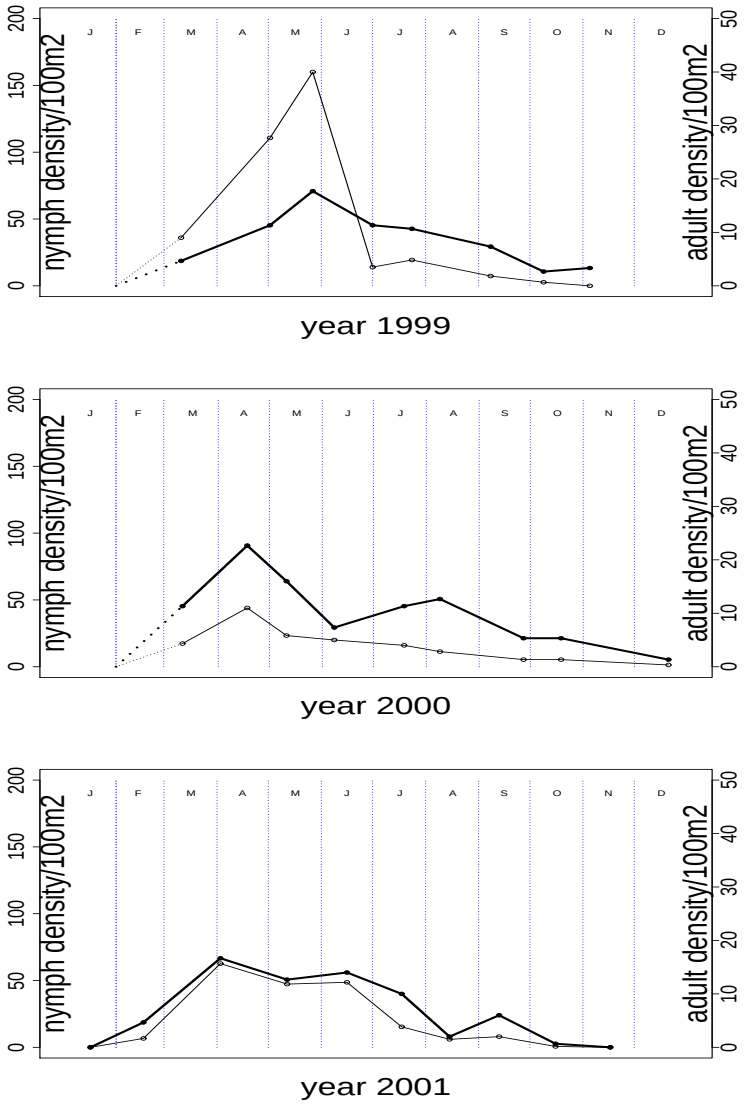


Figure 3.35: **Timeplot of the questing tick density in Eclépens**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

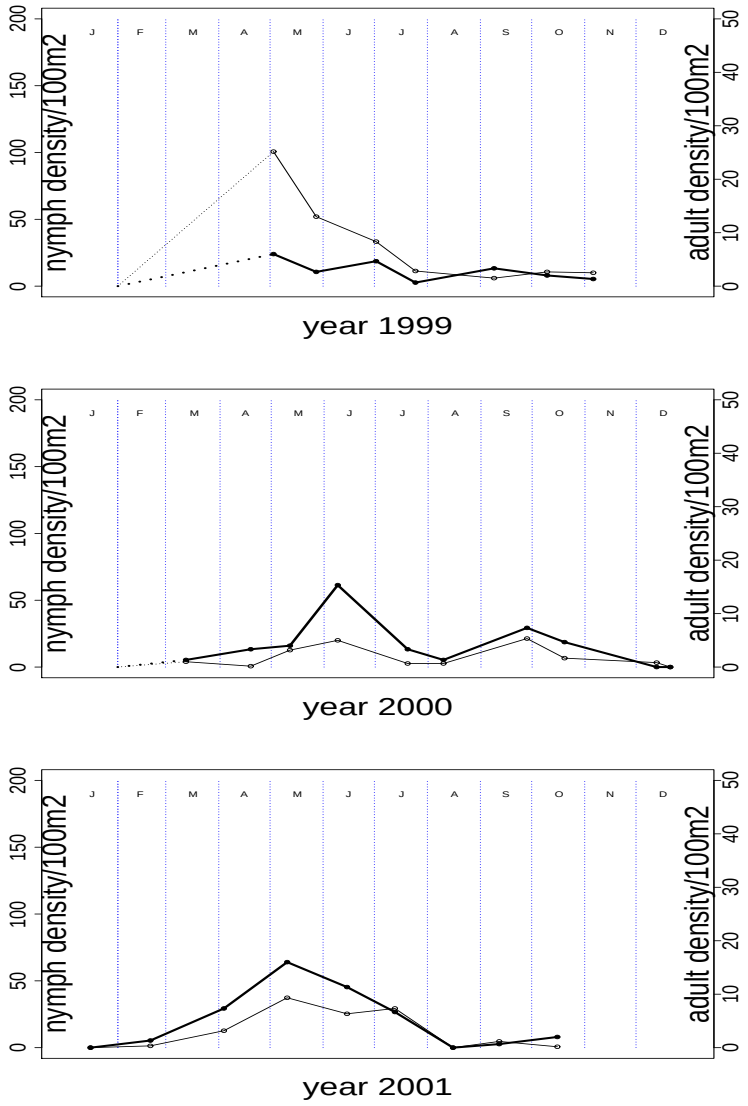


Figure 3.36: **Timeplot of the questing tick density in Interlaken**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

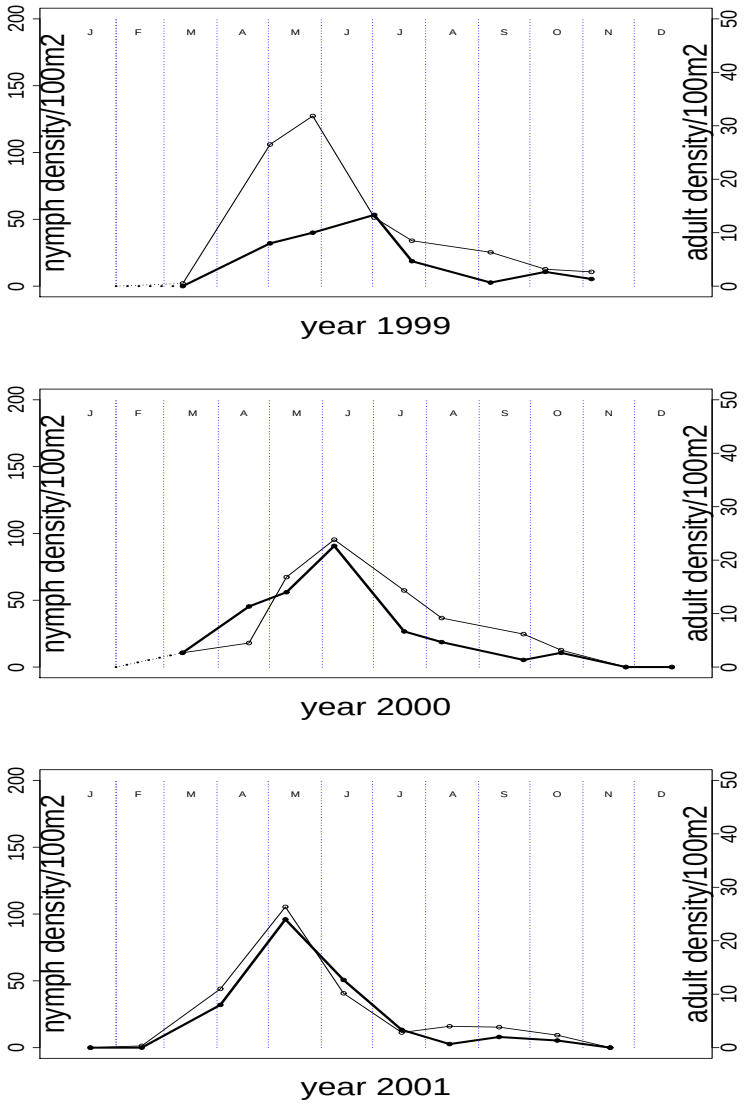


Figure 3.37: **Timeplot of the questing tick density in St-Livres**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

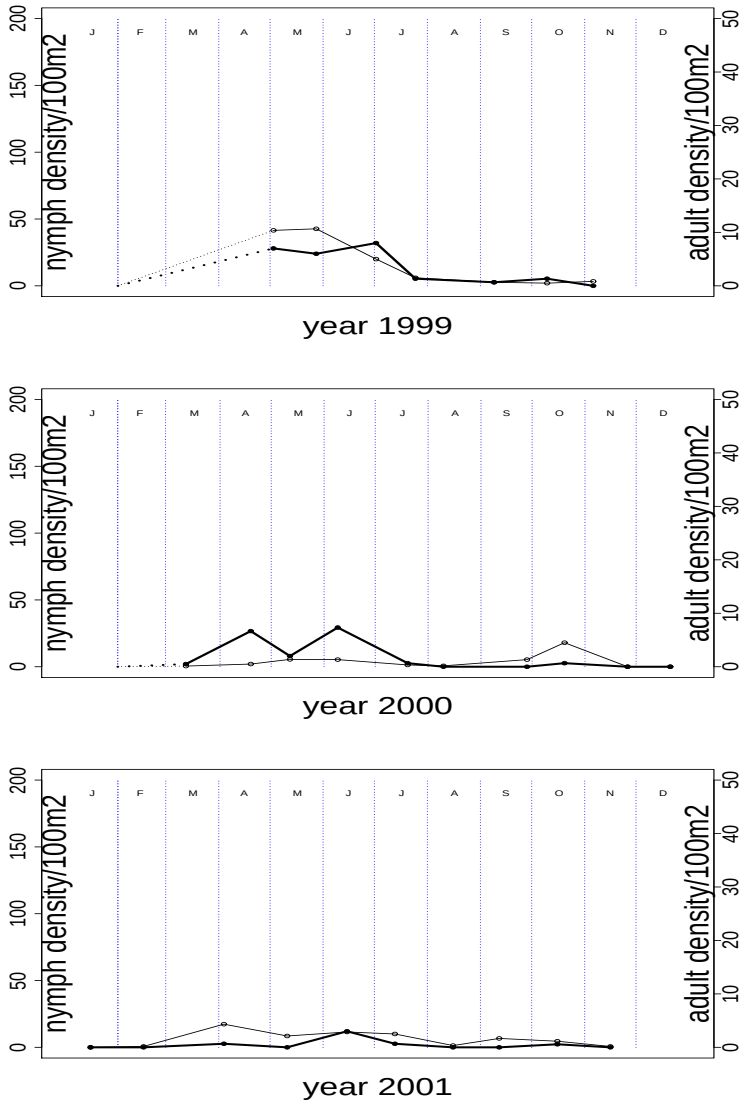


Figure 3.38: **Timeplot of the questing tick density in Viège/Visp**
thick line and right scale: adults; thin line and left scale: nymphs;
1999 (top), 2000 (middle), and 2001 (bottom).

From 1999 to 2001, over the 12 sampled locations in lowland Western Switzerland (altitude <800m), adults were approximately 5 times less abundant than nymphs (measured by the CTD, appendix C) (figure 3.39, 19736 nymphs and 4022 adults were collected over a surface of 71367m^2). Globally tick density measured by the cumulative tick density (CTD) was highly variable among locations (figure 3.40, Kruskal-Wallis test: nymphs: p=0.00098, adults: p=0.0019) but not among years (figure 3.41, Kruskal-Wallis test: nymphs: p=0.48, adults: p=0.36). Locations with high CTD in 1999 were also those with high CTD in 2000, and similarly between 2000 and 2001 (figure 3.42).

We therefore conclude that locations with different tick densities are conserved among years in Western Switzerland.

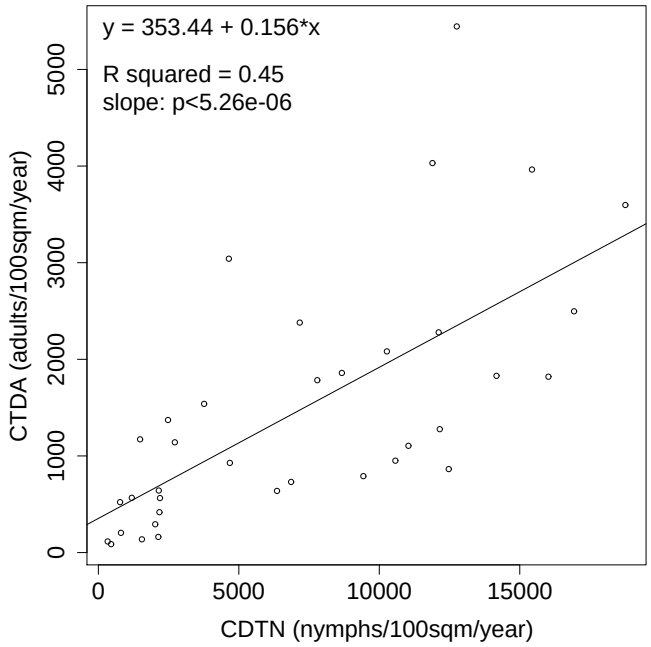
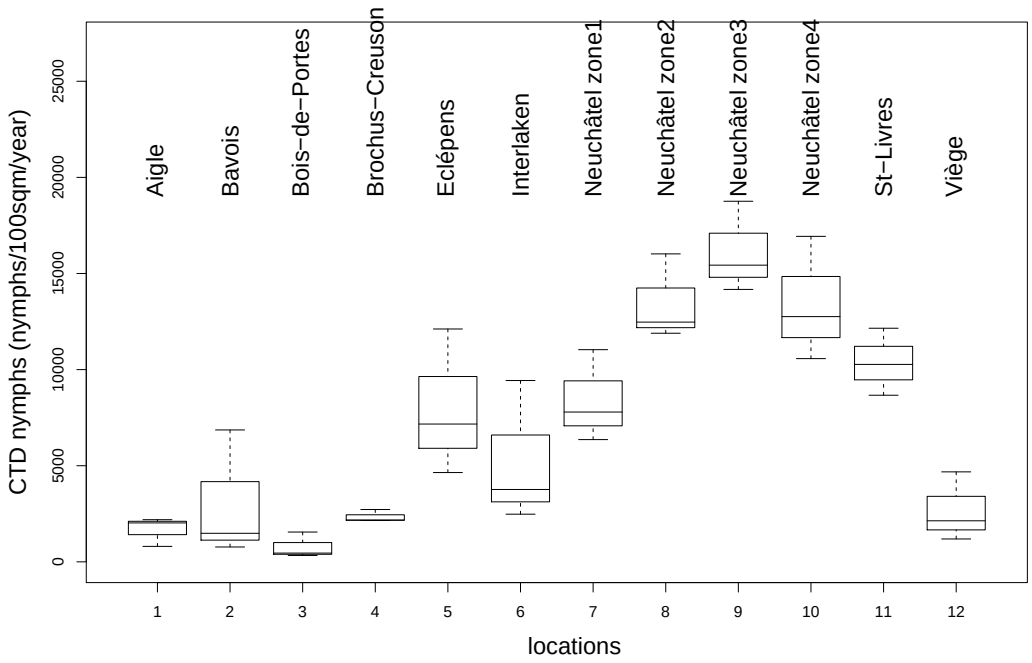


Figure 3.39: **Cumulative density (CTD) of adult *I. ricinus* as a function of the CTD in nymphs in Western Switzerland from 1999 to 2001.**
(raw data in appendix C)

Nymphs



Adults

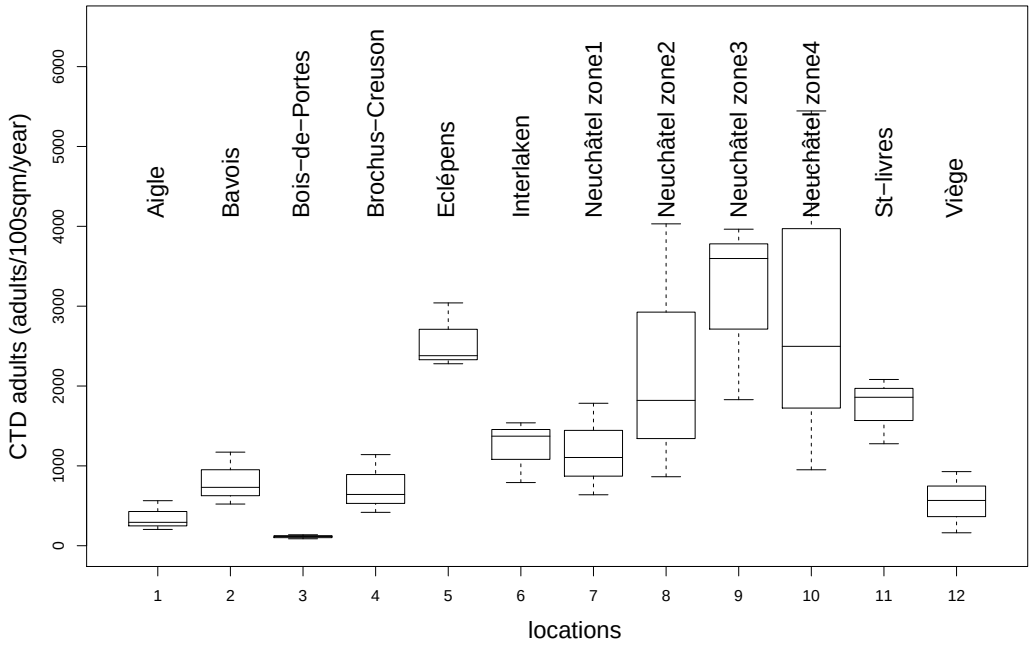


Figure 3.40: **Cumulative tick density (CTD) in Western Switzerland from 1999 to 2001.**
(raw data in appendix C)

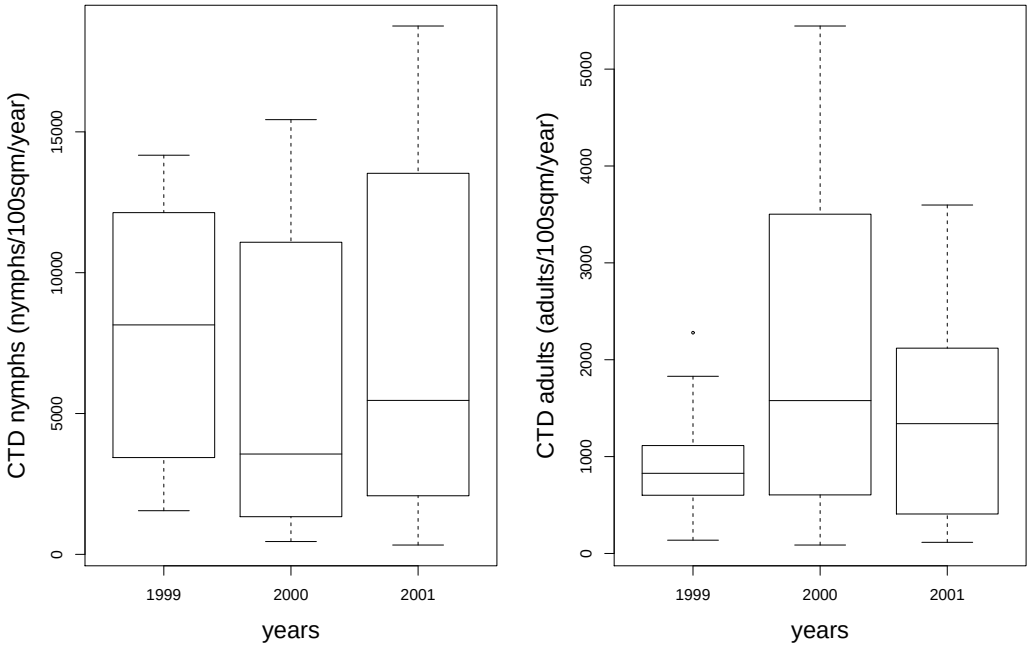


Figure 3.41: **Cumulative tick density (CTD) in Western Switzerland from 1999 to 2001.**
(raw data in appendix C)

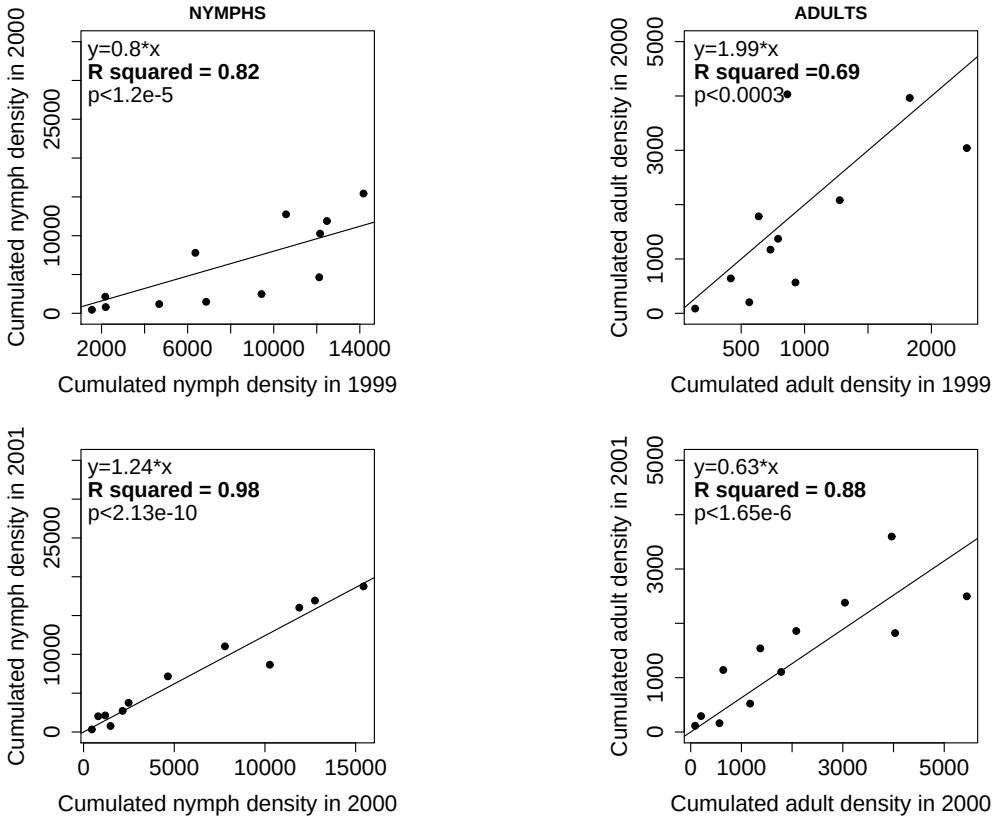


Figure 3.42: **Cumulative tick density in Western Switzerland: pairwise comparison between years**

(nymphs left, adults right, top: 1999 versus 2000, bottom: 2000 versus 2001)
(raw data in appendix C)

In addition to the density of ticks, we were also interested in their phenology. Indeed the shape of the questing tick density curve in *I. ricinus* among the studied areas varied from a single peak in spring to a double peak: one in spring and one in autumn (figures 3.26 to 3.38). To quantify this aspect of the phenology we calculated the proportion of tick activity in the first half-year (PCTD1[14]). A high value means that ticks were mainly questing in spring.

The PCTD1 of adults was linearly related to the PCTD1 of nymphs (figure 3.43). This means that when a high proportion of nymph activity occurred in spring, the same was observed for adults. We therefore conclude that the phenology of nymphs and adults evolved in paralell in Western Switzerland during 1999-2001. We observed 3 exceptions to this general rule, all occurring in 2000: Aigle, Bois-de-Porte and Viege. In Viege and Bois-de-Porte PCTDN1 was very low in 2000. This decrease in spring activity of nymphs was not observed in adults (figure 3.44). The reasons for that might be the same as in Neuchâtel during 1998 when the questing activity of nymphs was strongly disturbed by dessicating conditions (Perret et al., 2000) while adult questing was much less disturbed.

Globally from 1999 to 2001 the PCTD1 for nymphs and adults was not different among locations (Kruskal-Wallis: nymphs: p=0.19, adults: p=0.33) or among years (Kruskal-Wallis: nymphs: p=0.14, adults: p=0.28). However the differences in PCTD1 among locations were important in 1999 and 2000 (figure 3.45). We therefore verified whether the locations where PCTD1 was high during one year were also those where PCTD1 was high the year after (figure 3.46). Indeed between 1999 and 2000 this correlation was observed. However between 2000 and 2001 this was not the case. In fact there were little variations in the PCTD1 among locations in 2001 (figure 3.45).

This means that over Western Switzerland there was no systematic occurrence of an autumn peak of tick activity at particular locations or during particular years. In other words, characteristic phenologies were not conserved among years and among locations in Western Switzerland. However the phenology of nymphs and adults evolve in parallel.

[14] the proportion in % of the cumulated tick density until the 30 June

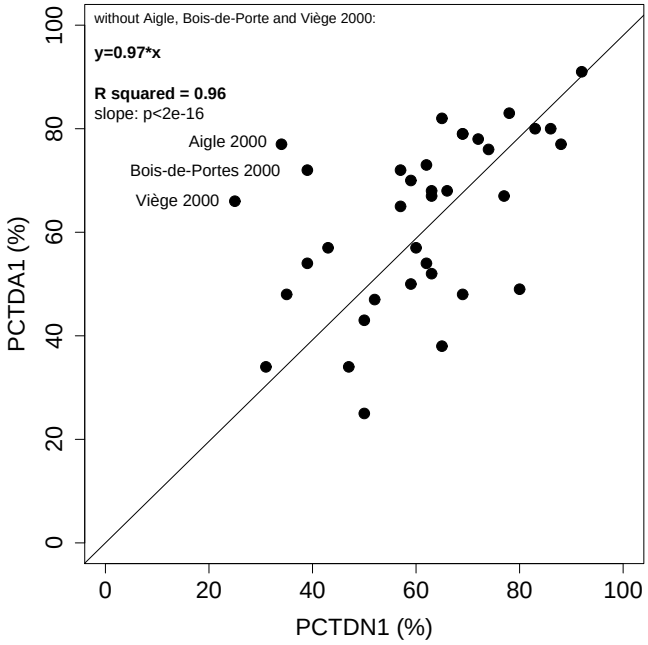
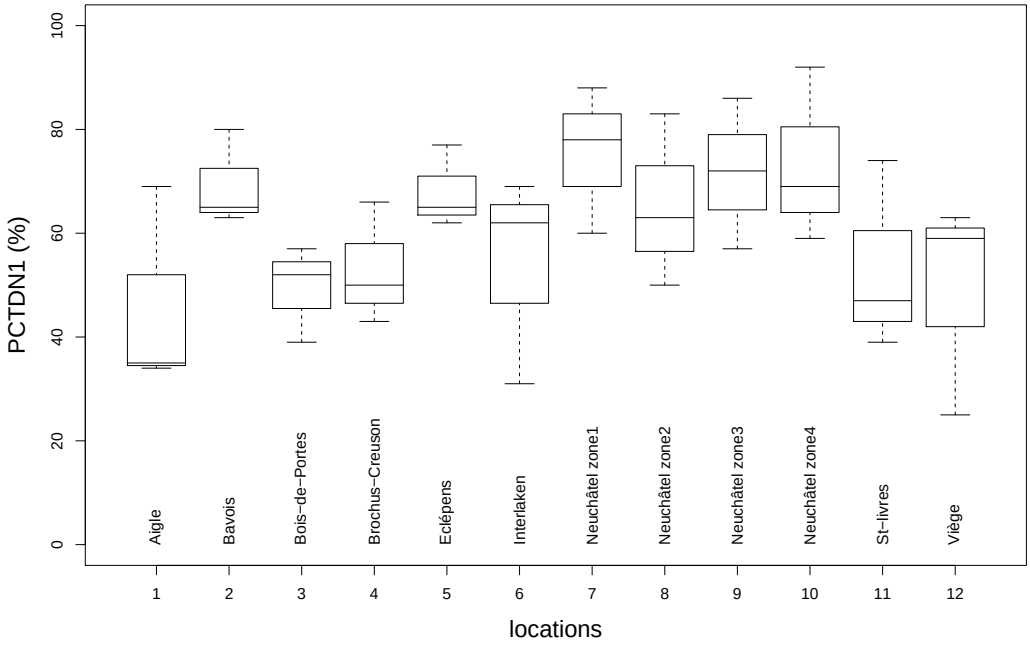


Figure 3.43: **Proportion of the cumulative tick density in spring at 12 locations over Western Switzerland from 1999 to 2001: comparison between nymphs and adults.**
(raw data in appendix C)

NYMPHS:



ADULTS:

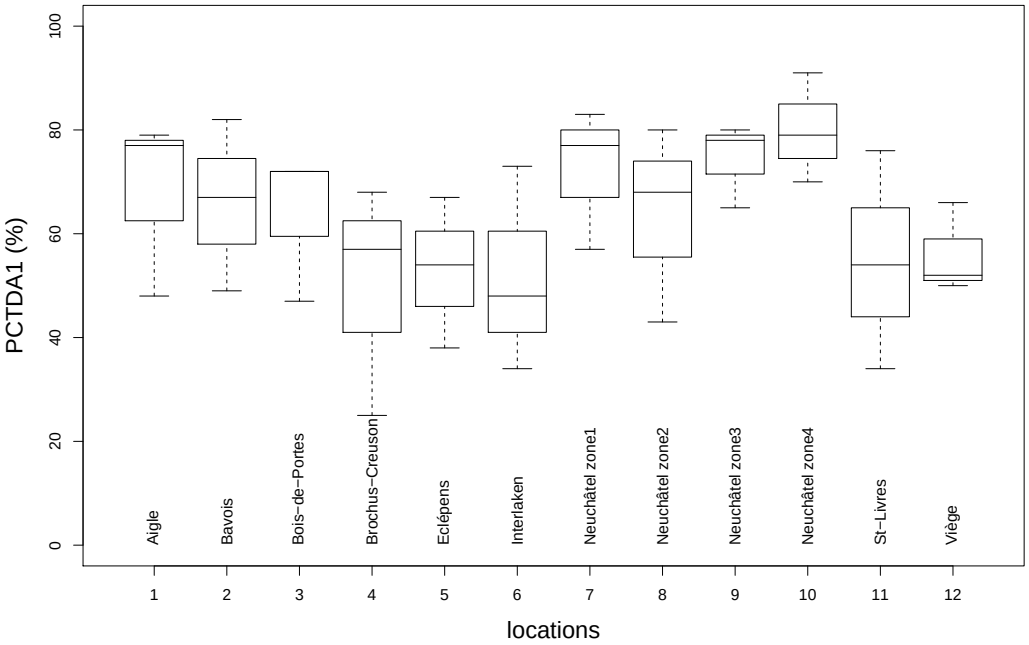


Figure 3.44: **Proportion of ticks collected up to the 30 June in Western Switzerland from 1999 to 2001.**

(raw data in appendix C)

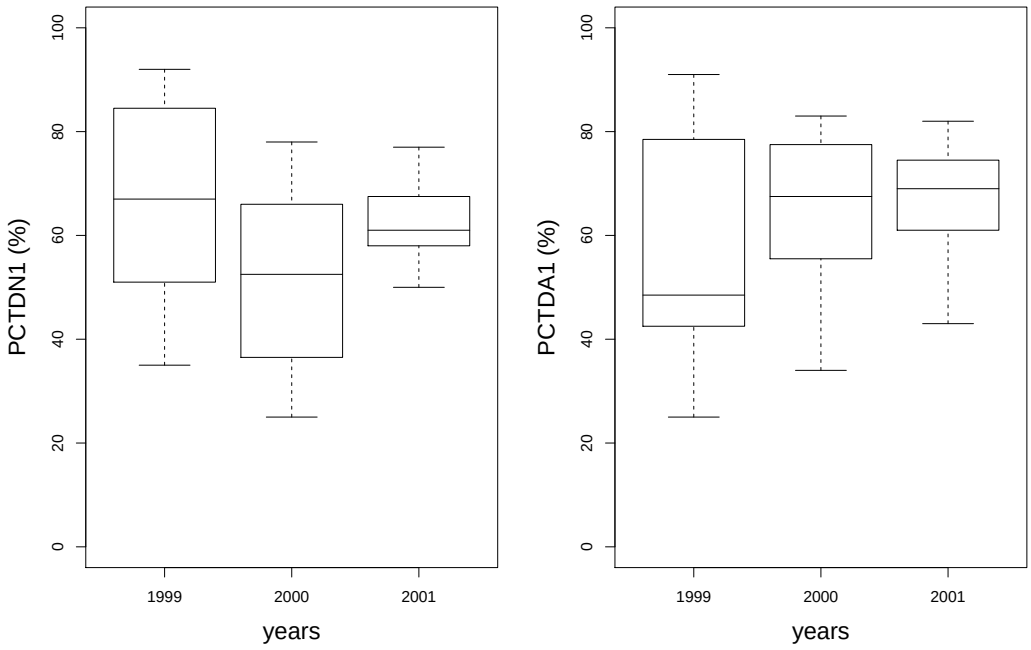


Figure 3.45: **Proportion of ticks collected up to the 30 June in Western Switzerland from 1999 to 2001. (left: nymphs, right: adults)**

(raw data in appendix C)

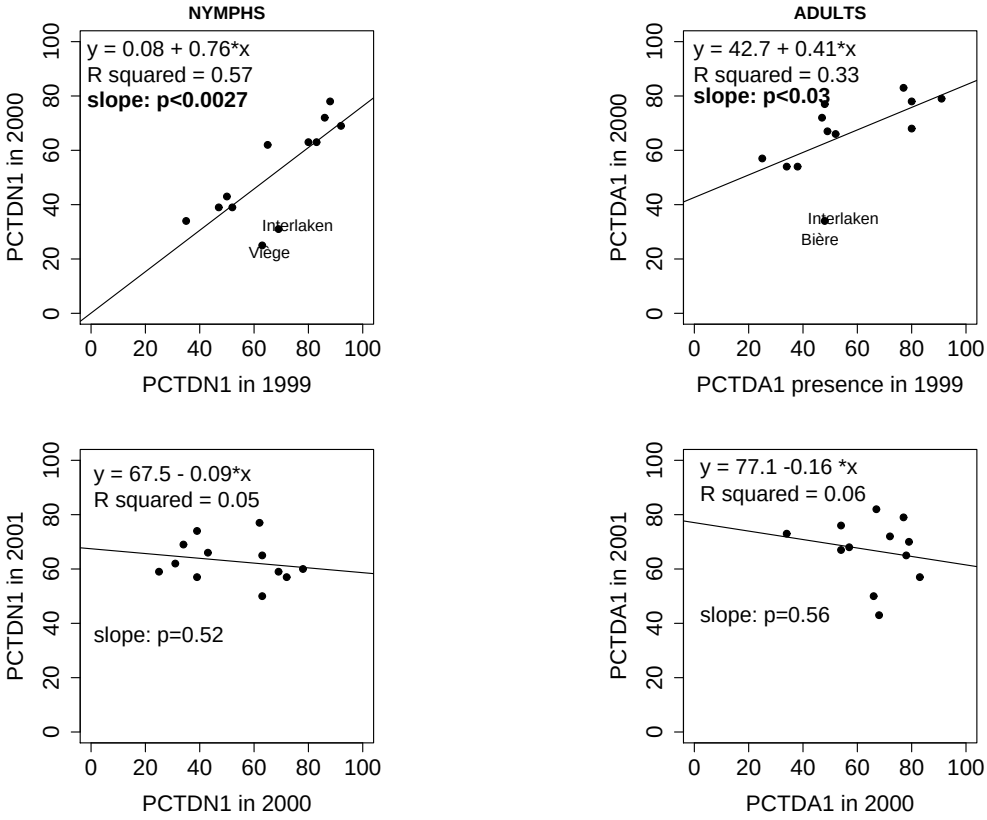


Figure 3.46: **PCTD1 in Western Switzerland: pairwise comparison between years**
(nymphs left, adults right, top: 1999 versus 2000, bottom: 2000 versus 2001)
(raw data in appendix C)

These results show that the cumulated tick density (CTD) varied among years at each location, but was rather conserved among locations troughout this study. Areas of high, medium or low tick density persistently coexist between forests at low and middle altitudes in Western Switzerland. In contrast, the proportion of ticks collected in the first half-year (PCTD1) was not conserved between years locations in Western Switzerland.

Therefore we conclude that the abundance of ticks is highly variable among locations and that this difference is conserved between years. In contrast, the phenology of *I. ricinus* at the different sampling areas varied considerably between years.

3.7.3.2 *I. ricinus* phenology in relation with climate

Variations in the phenology of ticks in Neuchâtel were related to climatic conditions between 1996 and 1998 (Perret et al., 2000), and in particular the onset of tick activity was related to temperature. In addition Randolph et al. (2002) observed that the time of emergence of ticks in autumn is correlated with the cumulated summer temperature in Great Britain. To verify whether these observations are generally valid in Western Switzerland we examined tick phenology in relation to climate at the locations where climatic data were available from automatic weather stations.

No climatic data were available for the sampling area in Bière, we therefore did not take this location into consideration for this analysis. In addition, Bavois and Bois-de-Portes were repeatedly completely flooded and were also removed from the dataset. At these two locations ticks showed a very low tick density with an average CTD from 1999 to 2001 of 3040 nymphs and 808 adults/100m^2/year in Bavois and 779 nymphs and 113 adults/100m^2/year in Bois-de-Portes (considering only the dates when sampling was possible) .

The analysis of the relations between climate and tick phenology was therefore based on 6 locations in Western Switzerland[15] sampled from 1999 to 2001 (figures 3.30 and 3.34 to 3.38) and on the 4 zones sampled in Neuchâtel from 1996 to 2001 (figures 3.23 to 3.28). The corresponding Meteosuisse weather stations are listed in table 2.2 on page 31.

Between 1996 and 1998 we observed a minimum temperature threshold of 7°C for tick activity in Neuchâtel (Perret et al., 2000). To verify that this was also true in 1999, 2000 and 2001 and at other locations, we plotted the questing tick density recorded in Western Switzerland (including Neuchâtel until August 2002) for all samplings as a function of temperature recorded in the field during sampling (figure 3.47). The lowest temperature threshold for tick activity was again observed at 7°C. Below this temperature ticks were only occasionally encountered (maximum tick density was 5.5 nymphs per 100m^2 in Brochus-Creuson on the 15.2.2002 and 6.6 adults per 100m^{2}in Viège on the 18.4.2000).

Saturation deficit plays an important role in tick questing behaviour. This was shown in nature, (Perret et al., 2000), in semi-natural arenas (Randolph and Storey, 1999), in arenas submitted to natural climatic conditions (paragraph 3.3.1) and by continuous tracking of ticks in laboratory conditions (paragraph 3.3.2). We therefore also plotted the questing tick density observed in Western Switzerland as a function of saturation deficit measured in the field during

[15] Aigle, Brochus-Creuson, Eclépens, Interlaken, St-Livres, Viège

sampling (figure 3.48). A maximum threshold for tick activity was found at a saturation deficit of 15mmHg. Above this value the maximum questing tick density recorded was 8.6 nymphs/100m^2 and 3.8 adults/100m^2 in Neuchâtel on the 27.8.2001.

Most *I. ricinus* activity in Western Switzerland was limited to temperatures above 7°C and to saturation deficits below 15mm Hg.

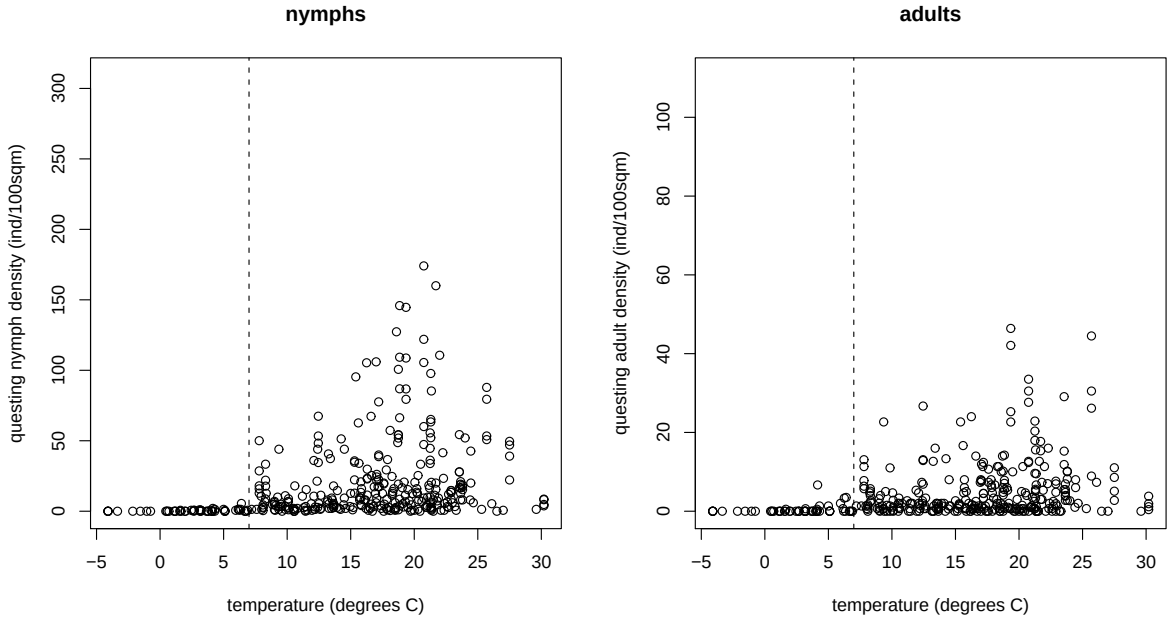


Figure 3.47: **Questing tick density in Western Switzerland as a function of air temperature**
364 flag samplings in Western Switzerland in 1999, 2000, 2001 and up to August 2002 in Neuchâtel. Temperature was measured at sampling location during tick sampling 60cm above ground. Dotted line indicates 7°C.

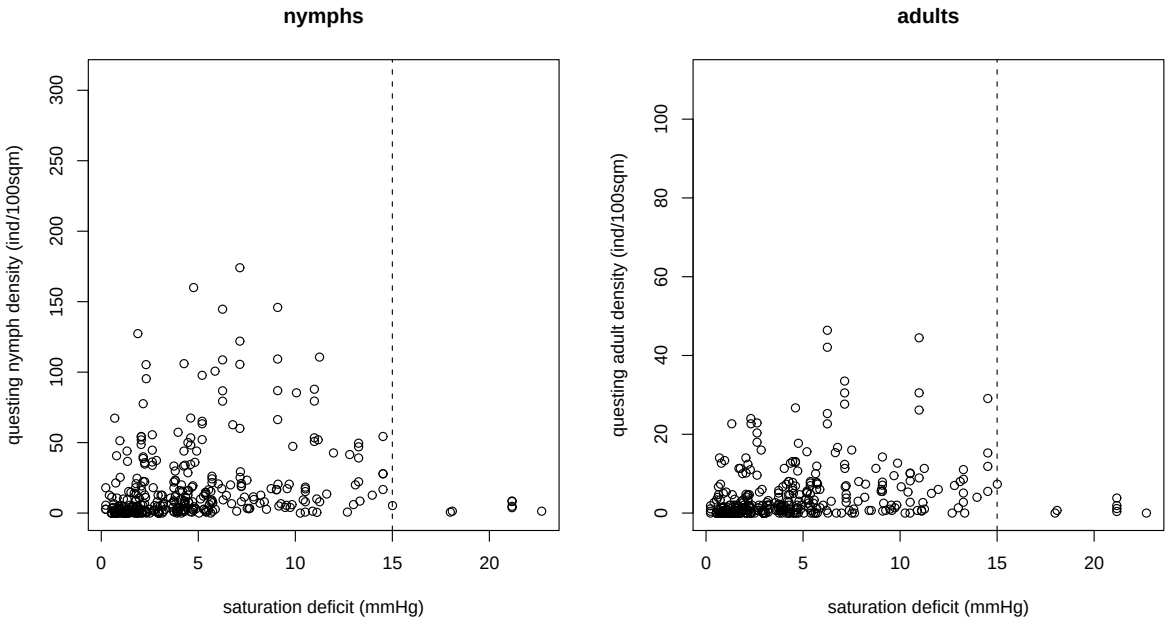


Figure 3.48: **Questing tick density in Western Switzerland as a function of saturation deficit**
364 flag samplings in Western Switzerland in 1999, 2000, 2001 and up to August 2002 in Neuchâtel. Temperature and relative humidity were measured at sampling location during tick sampling 60cm above ground. Dotted line indicates 15mm Hg.

We will now relate descriptive statistics of tick phenology, particularly the activity in spring and autumn with climatic parameters measured by Meteosuisse weather stations.

***I. ricinus* activity in spring:** The date of onset of nymph and adult activity (O10[16]) was negatively correlated with the cumulated hourly average temperature calculated from the 1st of January to the 1st of February (figure 3.50, $p = 0.01$ for nymphs and for adults), and not with the cumulated hourly average temperature calculated from the 1st of January to the 1st of March, April and May (data not shown). Similarly, O25N[17] was also negatively correlated with the cumulated hourly average temperature from the 1st of January to the 1st of February ($R^2 = 0.2311$, $p = 0.0008$, $slope = -0.00825$, $intercept = 76.5$), and not with the cumulated hourly average temperature to the 1st of March, April and May (data not shown). Finally the time of peak of nymphs (TPN) was also negatively correlated with the cumulated hourly average temperature on the 1st of February ($R^2 = 0.2686$, $p = 0.0003$, $slope = -0.021$, $intercept = 159.05$), but was also correlated with the cumulated hourly average temperature calculated from the 1st of January to the 1st of March and April with a lower coefficient of correlation (R^2 of 0.08349 and 0.08617 respectively; $p = 0.03552$ and $p = 0.0332$ respectively). The recorded average temperatures recorded by Meteosuisse weather stations varied from -3.7 in Viège in 2000 to 3.94 in Aigle in 2001 (figure 3.49).

In brief we observed that in Western Switzerland from 1999 to 2001[18], the onset of tick activity, the increase in tick activity and the time of peak tick density in spring occurred faster when and where the cumulated temperature during January was higher.

[16] the date when tick questing density reached 10% of the peak tick density

[17] the date when nymph questing density reached 25% of the peak tick density

[18] **Neuchâtel from 1996 to 2002**

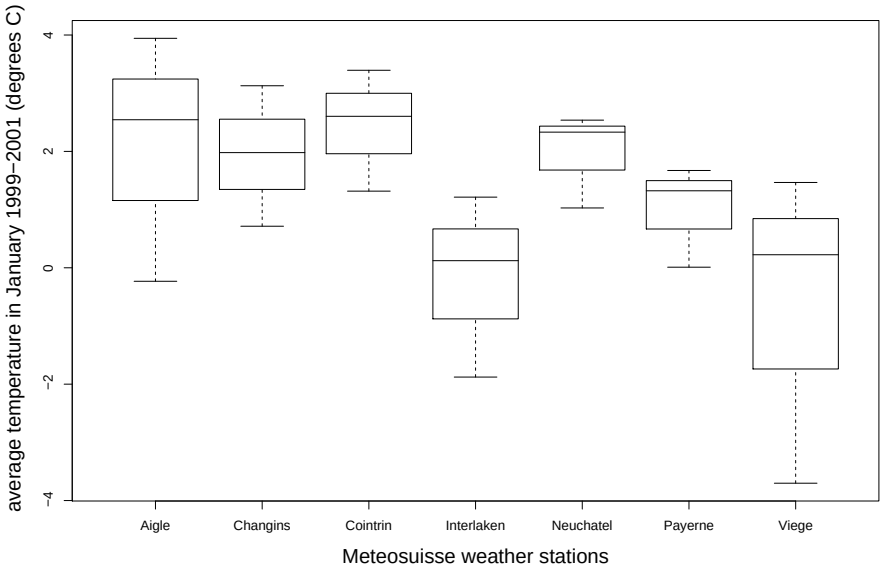


Figure 3.49: **Average temperature in January at 7 weather stations in Western Switzerland**
Minimum value is -3.7 in Viège in 2000 and maximum value is 3.94 in Aigle in 2001.

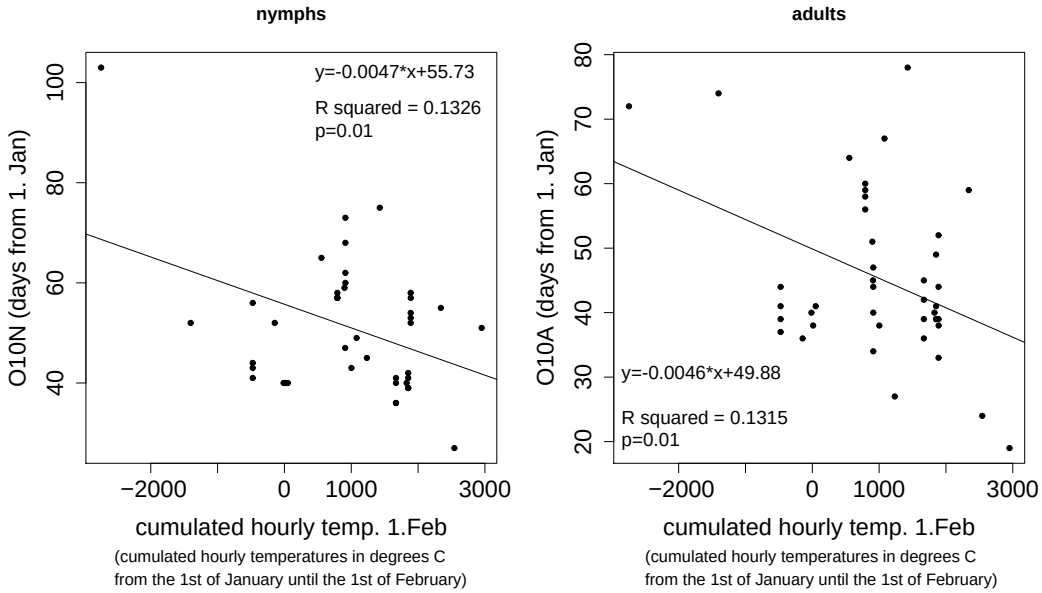


Figure 3.50: **Onset of tick activity (O10) in Western Switzerland as a function of the hourly cumulated temperature on the 1st of February (°C)**
based on the descriptive statistics obtained from 42 questing timeplots of ticks at 6 locations in Western Switzerland between 1999 and 2001 and at Neuchâtel from 1996 to 2002.

***I. ricinus* activity in autumn:** The proportion of nymphs active in the second half-year[19] (100-PCTDN1) was related to the cumulated temperature calculated from the 1st of March to the 1st of July ($p = 0.00084$, $R^2 = 0.23$, figure 3.51). This was not the case for adults ($p = 0.96$, $R^2 = 0.02$).

This shows that in Switzerland, the activity of nymphs in autumn is proportional to the temperature occurring from March to July

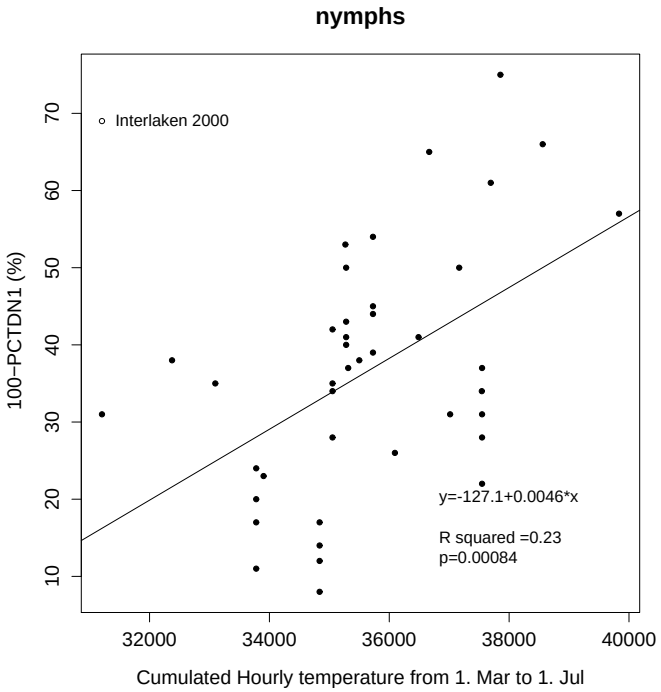


Figure 3.51: **Proportion of nymphs collected in the second half-year (100-PCTDN1) at 6 locations in Western Switzerland from 1999 to 2001 and at Neuchâtel from 1996-2001 as a function of the cumulated hourly average temperature from March 1st to July 1st (°C).**
The point measured at Interlaken in 2000 was considered as an outlier due to the damages made to the forest by the hurricane Lothar and was therefore removed from the regression analysis.

In the correlations between climatic variables and phenology descriptors presented above the proportion of the variance explained by climatic variables (R^2) is relatively small. However significant correlations were found between the onset of tick activity in spring and the temperature in January and between the occurrence of an autumn peak of activity in nymphs and the temperature between the 1st of March and the 1st of July. From 1999 to 2001 these parameters varied (figure 3.52): 2000 being the coldest year in January and the warmest year from March to the end of June. Simultaneously 2000 was characterised by marked autumn peaks in nymphs (figure 3.53). On the other hand 2000 was the coldest year in January and tick onset (O10) was delayed in nymphs and in adults (figure 3.53). Similarly the time of peak nymph density was

[19] after the 30 June

also delayed in 2000 (data not shown). The peak nymph density was even reached only during autumn at Interlaken and at Viège during 2000 (figures 3.36 on page 110 and 3.38 on page 112).

In summary, the onset of tick activity in spring was correlated with the temperature in January, while the occurrence of an autumn peak of activity was related to the cumulated temperature between the 1st of March and the 1st of July.

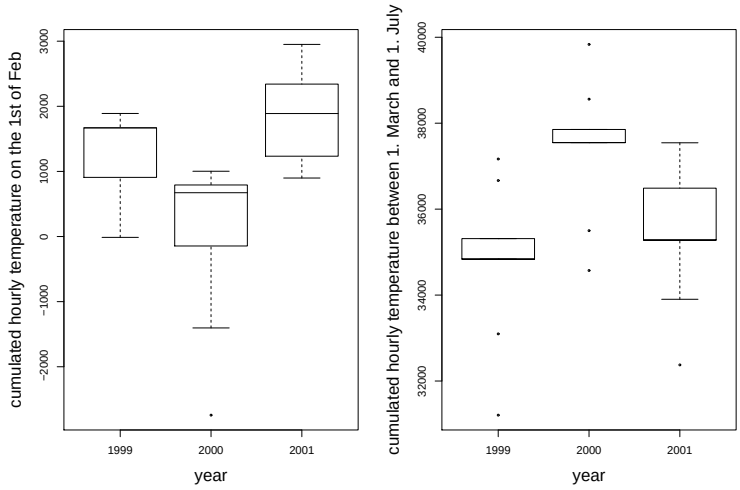


Figure 3.52: **Cumulated hourly temperature at the tick sampling locations in Western Switzerland from 1999 to 2001. From the January 1st to the February 1st (left) and from March 1st to July 1st (right).**

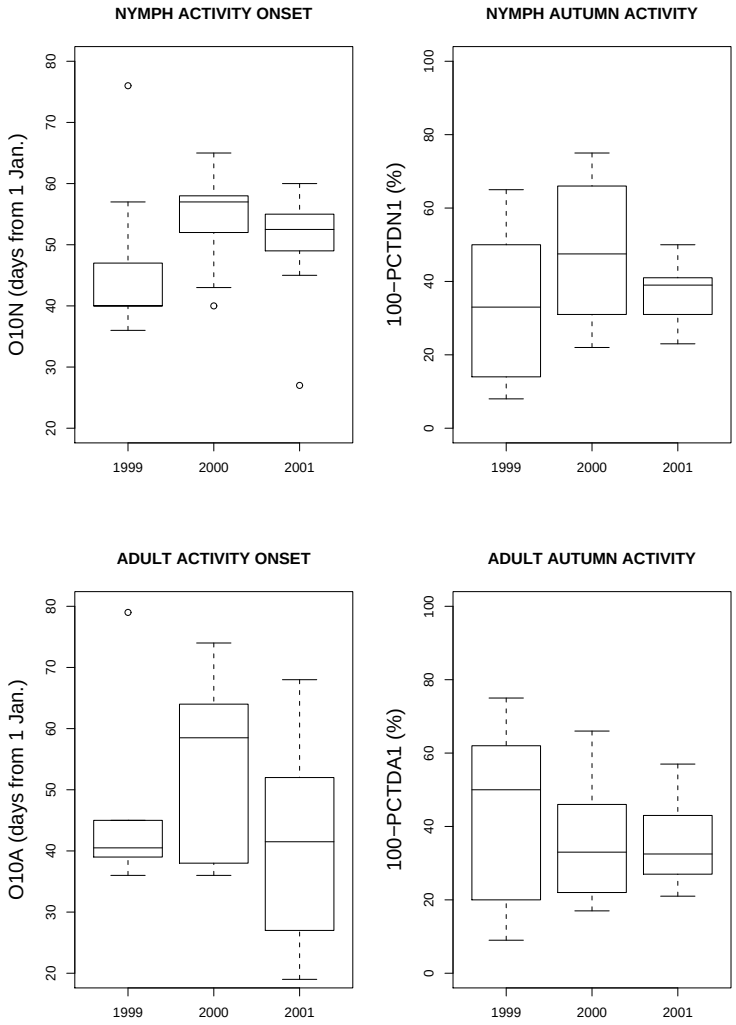


Figure 3.53: **Onset of tick activity (O10) (left column) and proportion of activity in the second half-year (100-PCTD1, right column) for nymphs (top row) and adults (bottom row) *I. ricinus***

3.7.3.3 Exceptional environmental conditions: impact on *I. ricinus* phenology

During this study, two sites were subjected to exceptional environmental conditions. In Vaumarcus the forest at our sampling site was completely destroyed by the construction of the N5 Highway, while during winter 1999-2000 the forest in Interlaken was partly destroyed by hurricane Lothar.

Impact of total forest destruction on the tick population: Vaumarcus was initially sampled within our survey of ticks in Western Switzerland. This point was removed from the analysis since the forest at the exact location of our sampling site was completely destroyed during winter 1999-2000 to build the N5 highway. Ticks were still collected until the 19th of October 2000 to study the impact of total forest destruction on the phenology of ticks at a location where ticks were present at a relatively high density in 1999 (CTDN of **17880** nymphs/100m^2/year and CTDA of **1943** adults/100m^2/year for 1999). The sampled area was part of a big forest ranging from the shore of the Neuchâtel lake up to the top of the first Jura Mountain reaching an altitude of approximately 1000 meters (figure 3.54). The destroyed portion covered a surface of approximately 4.5 ha adjacent to open grassland to the South and East and adjacent to the forest to the North and West. Trees were cut and removed and the remaining vegetation was mechanically chopped into pieces of 1-30cm in length and left in place to protect the ground from the falling rain.

During the year following the forest destruction the density of nymphs was tremendously reduced (CTDN of **531** nymphs/100m^2/year representing a 33x reduction in tick density, figure 3.54). In contrast, the density of adult *I. ricinus* remained similar during 2000 as it was during 1999 (CTDA of **1224** in 2000). After forest destruction ticks experienced very severe microclimatic conditions as on the 10th of May 2000 when temperature reached 30.6°C and saturation deficit reached 22 mm Hg with 2 Beaufort of wind. However, in this desertic landscape, patches of surviving grass existed (containing *Mercurialis perennis* around 1m^2 in size and separated by an average distance of 10-15meters). Ticks were mostly flagged in these patches up to 10 months after forest destruction.

Effect of the Lothar storm: On the 26th of December 1999 Switzerland, as well as other European countries experienced a major storm called Lothar. This storm partly destroyed the forest in Interlaken. The data collected in Interlaken during 2000 were kept for analysis, but special attention was given to this point. While tick phenology in Interlaken was unimodal with a main spring peak during 1999 and 2001, the phenology of *I. ricinus* nymphs and adults was changed to a bimodal pattern with a distinct autumn peak after the storm in 2000 (figure 3.36 on page 110).

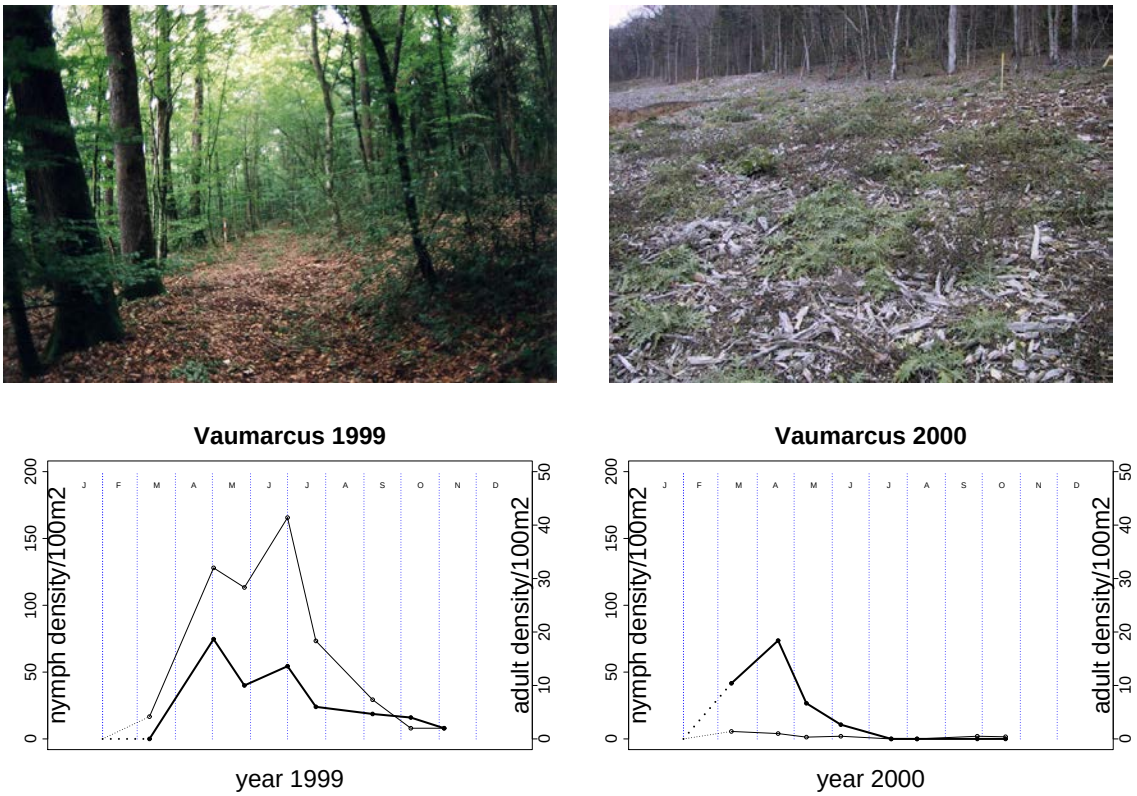


Figure 3.54: **Timeplot of the questing tick density in Vaumarcus before and after forest destruction.**
thick line and right scale: adults; thin line and left scale: nymphs;

(photos: Jean-Luc Perret 2002)

Chapter 4

Discussion

Tick survival: The life-span of free living stages of ticks is limited to the fixed energy reserves they possess at emergence (Smith and Grenfell, 1984) cited by (Vail and Smith, 1997). While this statement is generally true, it is subject to a condition: ticks need to maintain their water-balance at any time.

In ticks, water balance is a dynamic process involving on the one hand water loss and on the other hand water intake by active water sorption when relative humidity exceeds the pump threshold (Needham and Teel, 1991).

Survival to dessication is the first limiting condition for the free-living stage of ticks, we therefore studied *I. ricinus* unengorged male and female survival under different relative humidities and temperatures.

Female survival at 97% RH during 10 days was excellent, except at the highest temperature (34°C) and at the lowest temperature (15°C). Since 97% is above the CEH for *I. ricinus* (Lees, 1946), these ticks could absorb water from the atmosphere to survive. Reduced water sorbtion capacity at low temperature was already described by Lees (Lees, 1946). At high temperatures, the inability to maintain water balance could be due to increased water loss through the cuticule, naturally occurring as temperature increases and the kinetic energy of water molecules increases, or to changes in the cuticule structure above a critical transition temperature (CTT) (Needham and Teel, 1986). Finally, increased mortality at high temperatures may be due to the exhaustion of energy reserves through increased metabolism and possibly through tick movements. The ticks used in the survival experiment were collected in spring at the period of the year when energy variations among individuals is maximum (Randolph et al., 2002). This could explain the relatively high variations in survival observed among individual ticks.

Below the pump threshold, we found that *I. ricinus* female survival was inversely proportional to saturation deficit. Similarly, Macleod found that survival in *I. ricinus* nymphs was also inversely proportional to saturation deficit (MacLeod, 1935).

Males were much less resistant to dessication than females under all tested climatic conditions. This may be due either to a higher water permeability of the cuticule in males and/or a reduced capacity to take-up water from the atmosphere. It is also possible that increased mortality in males is due to their behaviour. In contrast to females, males were not able to survive during the whole experiment at 97% RH. This suggests that the survival time of the tested males

relied solely upon the water-loss barrier represented by the cuticule, and did not involve an active water sorption mechanism. Lees (1946) reported that males, like females, are capable of water gain at 95% RH (Lees, 1946). In his experiments males survived like females. This discrepancy between his and our results may be due to the origin of the tested ticks. Lees used laboratory reared batches of ticks of more or less similar ages while we used field collected ticks of potentially mixed ages. Water sorption capability in males or their metabolism and behaviour may thus depend upon their age. It was shown for example that virgin males survived much longer than males who have already mated (Graf, 1978a). Since our population was collected in the field in May it is highly probable that most of these males had already copulated (Graf, 1978a), thus explaining the much higher mortality of males compared to females.

In nature, climatic conditions do not remain constant, but vary continuously. Therefore the duration of tick survival in nature may not be directly related to the CEH. Indeed, ticks might be able to use brief daily periods of high relative humidity during the morning to rehydrate. Under these conditions tick survival time might rather be dependent upon the amount of available energy, cuticule permeability, and upon the water content of their body (Needham and Teel, 1991) rather than upon average saturation deficit. High water content and low cuticule permeability enable ticks to survive during the driest periods of the day until they can rehydrate during the most humid periods, without having to undertake movements to reach water-saturated areas. Indeed movements are costly in energy and in water loss due to increased spiracle opening during mobility (Rudolph and Knülle, 1979) (Knülle and Rudolph, 1982) (Needham and Teel, 1986) (Lighton et al., 1993).

Tick questing behaviour (appetitive search): In nature ticks have the choice of leaving their questing place to find humid micro-climatic conditions to rehydrate. Do they do so? To answer this question we followed tick movements continuously under changing light conditions using a constant infrared source [1]. We showed that indeed *I. ricinus* nymphs almost always interrupt questing to start quiescence spontaneously, in the absence of any host stimuli (section 3.3.2). During quiescence most nymphs prefer to stay in a humid place rather than in a dry place if they are given a choice (3.2 on page 62). This clearly demonstrates that ticks need to rehydrate after questing. To reach the humid quiescence loci *I. ricinus* nymphs walked an average distance of 0.4m (max 1.1m) at each questing interruption (at 9.3mmHg saturation deficit). Our experimental setup enabled us to observe movements on a vertical plane. In nature ticks leaving questing or quiescence move in a very hetergeneous environment which will imply horizontal as well as vertical movements to reach either a questing or quiescence place. We therefore assume that at least part of the movements recorded in our experiments would be horizontal in natural environment. This also means that *I. ricinus* nymphs move horizontally repeatedly after each questing event to find favourable rehydration places potentially resulting in significant horizontal movements.

We demonstrated that the duration of questing depends upon dessicating conditions (saturation deficit). When conditions are less dessicating ticks quest for longer periods before becoming

[1] Previous experiments failed to show any impact of an infrared source on tick behaviour (Pauchard, 1989)

quiescent so that the proportion of questing individuals in the tick population is influenced by the prevailing saturation deficit. This explains the reductions in questing tick density under high saturation deficit we observed in the field arena experiment (3.3.1 on page 64) and which was reported in recent studies both in quasi-natural arenas (Randolph and Storey, 1999) and in the field (Perret et al., 2000); (Randolph et al., 2002). Longer periods of questing may increase the host-finding probability and thus influence tick population dynamics (Randolph et al., 2002). This demonstrates the important role of saturation deficit on tick populations in different ecozones occupied by *I. ricinus*.

While dessication dictates when ticks should interrupt questing to move down the vegetation to rehydrate, the factors driving quiescence interruption remain unknown. Using the continuous video-tracking system we were able to follow ticks that left their questing place, i.e. when they are not visible in nature. We were thus able to observe quiescence interruption.

We discovered that in the absence of any host stimuli, quiescence was often interrupted by walking events that did not necessarily lead to questing. In fact most of tick walks occurred during darkness under all our climatic conditions. Preference for questing interruption during darkness was described in the field by Lees and Milne (Lees and Milne, 1951) who suggested it to be due to an immediate response to temperature drops at night. Our data show that a drop in light intensity is sufficient to trigger mobility in *I. ricinus*, and need not necessarily be accompanied by T°C or RH shifts. Similarly Carroll et al. (Carroll et al., 1998) observed over 2 nights that *I. scapularis* moves preferentially during darkness. In our experiments we showed that even when the light cycle was changed from a 14:10 L:D to a 8:4 L:D cycle, ticks continued to move during darkness. In addition, we showed that irrespective of the climatic conditions applied, not only questing interruption, but most tick walking occurred during darkness. Since these movements occurred spontaneously without any host stimuli we believe that they correspond to an appetitive search behaviour (Hamilton and Hurd, 2002) resulting in an increase of the host encouter probability. Such an increase may be realised by finding favourable questing sites in nature. We therefore suggest that part of these movements represent activities that enable ticks to find a favourable questing site in nature. Good questing sites are those where the host-encounter probability is high during the questing permitted by the prevailing climatic conditions. We observed that the distances walked after quiescence interruption increased with dessicating conditions (high saturation deficit), suggesting that *I. ricinus* nymphs are ready to walk long distances (mean 1.10 meter and up to 9.65 meters) repeatedly during the night to find microclimatic conditions suitable for questing. This result strengthens the conclusions made by Randolph and Storey (Randolph and Storey, 1999) who concluded that increased fat use under high saturation deficit was probably due to an increase in tick mobility under such conditions. This is true not only because nymphs have to interrupt questing more often under drier conditions, but also, as we now know, because they walk for longer distances before starting to quest under high saturation deficit.

While previous observations concluded that *Ixodes* ticks are not moving much horizontally (Lees and Milne, 1951), others concluded differently. In fact Gigon (Gigon, 1985), Gray (Gray, 1985), Falco (Falco and Fish, 1991) and Schulze (Schulze et al., 1997) all observed that *I. ricinus* and *I. scapularis* move horizontally more than one meter over a week. In addition, it has been

shown that *I. scapularis* adults choose their questing sites according to chemostimuli left by hosts (Carroll et al., 1998). These discrepancies might in fact be due to the human observer himself. Indeed appetitive search behaviour tends to increase the probability of encounter with host-stimuli. If these stimuli are encountered no appetitive search needs to occur and the host-finding behaviour will turn to an orientation response towards the host at this favourable site, so that no or shorter horizontal movements may be observed in the presence of hosts, or in the presence of an observer. Since we used a completely automated observation system, and since we discarded the hours following tick manipulation from the analysis, we were able to study the appetitive search of ticks, occurring in the absence of any host stimuli, and we observed that *I. ricinus* nymphs move during darkess and spontaneously alternate between questing and quiescence. The factors leading walking ticks to start questing remain unknown but could be endogenous since ticks started to quest in our experiments despite constant climatic conditions and in the absence of host stimuli.

Why do nymphs preferentially move during darkness? During mobility, respiration and thus spiracle opening increases in ticks (Lighton et al., 1993), resulting in increased water-loss (Rudolph and Knülle, 1979), (Knülle and Rudolph, 1982). The time needed to search for suitable questing or quiescence loci is unpredictable for a tick in nature. By undertaking movements during less dessicating conditions *I. ricinus* minimizes water loss and the energy costs to reabsorb it. Since darkness coincides with the less dessicating conditions in the field, *I. ricinus* uses darkness to trigger mobility when dessication risk is lowest. Another reason for mobility during darkness might be associated with hosts. As mentionned above, appetitive search enables ticks to increase host-encounter probability. As a wide range of tick hosts show peak activity just after dark (Hausser, 1995), tick mobility during this period may also increase the host-finding probability either by direct encounter, or by orientation to stimuli of passing hosts.

Given these results, the existence of a difference in tick-bite risk between night and day can legitimately be asked. Some authors have tried to measure such differences by flagging. More ticks (*I. ricinus* or *I. scapularis*) were collected during the night at some locations (Durden et al., 1996) (Mejlon, 1997), while no difference between night and day was found in others (Durden et al., 1996), (Mejlon, 1997), (Klaus-Hügli et al., 2002), or less ticks were collected during the night than during the day (Milne, 1947) Ticks of the *I. ricinus* species complex undertake questing to maximize host encounters with a reasonable energy cost. Questing at the tip of grass stems corresponds to an optimal place to grab a host. We therefore estimate that flag sampling most efficiently samples questing ticks. Walking ticks might also be able to grab the flag with a lower probability though, but quiescent ticks have very little chances, if any, to catch the flag. The differences between night and day in the number of flag collected ticks might be related to the proportion of ticks questing during the day. Indeed, during the day few ticks are walking, therefore the flag only samples among questing ticks. During the night, on the other hand, the flag samples among questing and, possibly with a lower efficiency, among walking ticks since quiescent ticks start to walk. If a small proportion of ticks are questing during the day, the number of ticks collected during the night should increase as ticks start to walk. On the other hand, if a larger proportion of ticks are questing during the day, those starting to walk during the night reduce their probability to grab the flag and the number of collected ticks should decrease. Circadian

variations in tick bite risk may therefore be related to the proportion of the ticks questing during the day. Since questing duration and therefore the proportion of questing ticks is dependent upon dessicating conditions (see 3.3.2), we expect that the difference found in nocturnal tick density also depends upon the dessicating conditions. This was observed in Sweden (Mejlon, 1997) where no difference in the nocturnal density of ticks was found within the forest, while in an open grassland (with a higher saturation deficit), significantly more larvae and adults were collected during the night (Mejlon, 1997). This phenomenon might also be accentuated by the fact that, as described in our study (section 3.3.2), in dessicating conditions ticks walk for longer periods than in more humid conditions (3.3.2).

Light perception in *I. ricinus* Unlike other ticks (Philis and Cromroy, 1977) (Kaltenrieder et al., 1989), *I. ricinus* does not have eyes with corneas. So how can this tick perceive the changes in light intensity that trigger its movements after dark? Although the existence of photosensitive cells in a variety of species, including *Ixodes holocyclus*, was suspected by Binnington (Binnington, 1972), none was described for *I. ricinus*. Diehl and Vlimant (Perret et al. submitted) described 20-21 cells containing a rhabdomere, located dorsolaterally behind coxa 2 on each side of larval, nymphal and adult *I. ricinus* (figure 4.1). These cells most probably play a role in the perception of shifts in light intensity that trigger tick movements during darkness. They may also be implicated in the perception of changes in the photoperiod and trigger morphogenetic diapause (Belozerov, 1982). Finally, these photosensitive cells may also be implicated in host detection.

Since photosensitive cells were found in all three life stages of *I. ricinus*, we expect nocturnal preference for movements in larvae and adults as well. Synchronised movements during darkness would carry a further advantage for adult ticks beyond avoidance of water loss. Mating in *I. ricinus* occurs mostly off the host (Graf, 1978a), and synchronised mobility of adults with sundown could serve to increase encounters between sexes.

Impact of *B. burgdorferi* sl on tick behaviour By measuring the walking behaviour of hydrated *I. ricinus* females on the locomotion compensator over 6 minutes we did not observe any difference between infected and uninfected ticks. Since these ticks are placed on the locomotion compensator by a human and have thereby experienced host stimuli prior to the measurements, the behaviour observed on the locomotion compensator corresponds to an activation/orientation behaviour and possibly to an attraction behaviour (Hamilton and Hurd, 2002). Previous observations by Alekseev (Alekseev, 1999) (Alekseev et al., 2000) showed that *B. burgdorferi* infected *I. persulcatus* and *I. ricinus* ticks had a "lower activity" than uninfected ticks. In these experiments tick behaviour was measured by a single number: an "activity index" calculated on observations of nymphs walking on a tilted surface during 3 minutes (A. N. Alekseev personal communication). With this index it is difficult to assess the impact of *B. burgdorferi* on the reproduction rate of both ticks and the transmitted pathogen. Another series of experiments realised with *I. scapularis* in the US (Lefcort and Durden, 1996) showed that *B. burgdorferi* infection influences nymphal and adult behaviour by increasing their phototaxy and preferrence for vertical surfaces. Their observations spanned over 5 hours with a human presence every hour and may therefore also

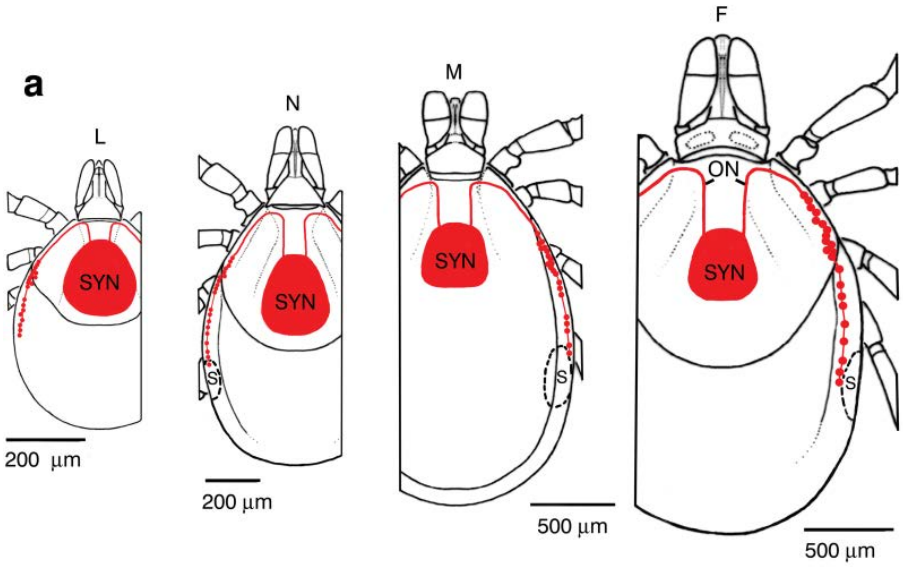


Figure 4.1: **Photoreceptor cells of the tick *I. ricinus***
Courtesy of Pr. P.-A. Diehl and M. Vlimant, tick drawing: Y. Borcard

be associated with activation and orientation after a host stimulus. In this experiment Lefcort and Durden (Lefcort and Durden, 1996) used ticks without mention of age and hydration status, from a laboratory colony. In addition these laboratory reared ticks may have been selected during successive generations in the laboratory so that they probably behave differently from field-collected ticks.

By observing ticks continuously during 10 days without taking the first hours after tick manipulation into account, we were able to observe the "appetitive search" (Hamilton and Hurd, 2002) of ticks. This means the host-seeking behaviour in the absence of hosts. We found that walks during darkness occurred more often in *B. burgdorferi* infected nymphs than in uninfected nymphs. The duration of these walks and the distances covered were more important in infected nymphs than in uninfected ones. This phenomenon was only observed under moderately to highly dessicating conditions. We suggest that these observations are due to vector behaviour manipulation by the pathogen (Hamilton and Hurd, 2002). Vector manipulation implies that the parasite (*B. burgdorferi*) is manipulating the vector (*I. ricinus*) to increase its own fitness by increasing its reproduction rate (r). So how can *B. burgdorferi* increase its own fitness by increasing the walking of ticks during darkness in highly dessicating conditions, and not in humid conditions? It is not unusual that the longevity of ticks exceeds that of their hosts (Needham and Teel, 1986). Juvenile uninfected hosts must therefore regularly be reinfected by *B. burgdorferi*. These newly infected hosts may then amplify the *B. burgdorferi* population by infecting ticks feeding upon them. *B. burgdorferi* r may therefore be strongly dependent upon the ability of spirochetes to infect new uninfected hosts as early as possible. On the other hand the reproduction rate of ticks is increased by maximising the host-finding probability over its entire life. Under dessicating conditions the duration of questing is reduced, thus momentarily reducing the host-finding probability of ticks but increasing the tick life duration by avoiding water-loss (and the associated

energy costs to reabsorb it) during very short questing events. Such delays in the host-finding process conflict with the interest of the spirochete to reach uninfected hosts as early as possible. While spirochetes do not increase tick resistance to dessicating conditions (the questing duration was not different between infected and uninfected nymphs), they might influence the appetitive search by increasing tick walking to find suitable questing sites during darkness, thereby momentarily increasing the host-finding probability. In contrast, under less dessicating conditions, the questing activity of ticks are adequate for both the tick and the spirochete thus explaining why no difference in questing behaviour were found in humid conditions. Different genospecies of *B. burgdorferi* sl have been shown to infect preferentially some species of hosts (reviewed in (Humair and Gern, 1998)). It would be interesting to verify if these genospecies influence the behaviour of ticks to increase the host-finding probability with the most appropriate hosts.

A classic example for vector manipulation by a parasite is the lancet fluke/ant system (Cheng, 1986). In this parasitic relation an individual metacercariae of a trematode (*Dicrocoelium dendriticum*) present in the brain of an ant changes the ant behaviour so that it remains at the top of grass stems during nighttime, thus increasing the probability of the ant being eaten by a vertebrate host, and thereby increasing the trematode's r. In order not to kill the ant under the dessicating condition of daytime the parasite only acts during the night. Similarly, *B. burgdorferi* changed the tick behaviour by affecting the walking of *I. ricinus* during the night only, when dessicating conditions are low, and not by affecting the questing duration.

The mechanism responsible for this manipulation is unknown. Indeed parasites may use different means to achieve host manipulation. *D. dendriticum* for example probably directly interfers with the brain functions of the ant (Romig et al., 1980). Indirect control of host behaviour may also occur in reaction to the physiological impact of a parasite. For example, tsetse flies infected with trypanosomes feed more readily and probe more often than uninfected flies (Lehane, 1991). It is believed that trypanosome infection reduces the cross sectional area of the food canal, increases pressure in the hypopharynx to inject the saliva, and alters the function of mechanoreceptors detecting the blood flow rate in the food canal of tsetse flies thereby making feeding more difficult so that feeding time is increased and the number of bites is increased as well (Lehane, 1991). This all leads to a unique benefit for the pathogen which has more time during feeding to be transmitted to a host, and which meets more hosts. Similar example exist for the transmission of parasites by sandlies, fleas and mosquitoes (Lehane, 1991).

Some insect-borne parasites also reduce the reproduction rate of their vectors (Lehane, 1991), potentially reducing the energy costs of reproduction in favour of the host-seeking activity. In contrast to insects, no difference in reproduction rate between infected and uninfected ticks was found for *I. persulcatus* (Nakao, 1995). In *I. ricinus*, females may transmit *B. burgdorferi* transovarially to larvae (Bellet-Edimo, 1997). As this transovarial transmission is important for the r of *B. burgdorferi* (Randolph and Craine, 1995), we do not expect *B. burgdorferi* to interfere with the fertility of female *I. ricinus*. In addition males are probably able to transmit *B. burgdorferi* sexually to female ticks (Alekseev and Dubinina, 1996). Therefore *I. ricinus* males do not represent an evolutionary dead-end for *B. burgdorferi*. For these reasons we neither expect *B. burgdorferi* to influence the sex ratio in ticks.

In contrast to insects, ticks feed for days, leaving a lot of time for parasite transmission, and

ticks do not repeatedly feed at each instar, but moult after each blood meal. A parasite can therefore not interfere with the feeding process of ticks to increase the number of host encountered without enabling the tick to proceed in its life-cycle. It was even shown that *Babesia microti* enhances the feeding and the moulting success of *Ixodes trianguliceps* larvae (Randolph, 1991). Similarly, the increased activity in the host-finding behaviour of *I. ricinus* nymphs that was observed in our studies could be the result of a dual benefit to *B. burgdorferi* and to the tick. In *I. ricinus* spirochetes were observed in the hypodermis, muscles, salivary glands and in high numbers in the synganglion of unfed *I. ricinus* (Burgdorfer et al., 1989), (Lebet and Gern, 1994) (Leuba-Garcia et al., 1994) (Leuba-Garcia, 1997). In Switzerland up to 11% of *I. ricinus* nymphs have a systemic infection (Lebet and Gern, 1994). These spirochetes may therefore be able to influence the physiology of all the organs which are involved in processes like blood meal digestion, water sorption, motion control or host detection.

This emphasizes the different evolutions of pathogens in insects and in ticks and opens a new field of investigation for the study of the relations between ticks and *B. burgdorferi* sl, and more generally of vector manipulation by their "parasites".

Tick bite risk: In tick-bite risk assessment studies, questing tick density is considered to be a direct measurement of tick-bite risk (Falco et al., 1999). However, we found a negative relation between questing tick density and the number of *I. ricinus* females attached on dogs. It must be noted though that in this study the walks with beagles were successive and spanned from the beginning of April to the middle of May. It was shown by Graf (Graf, 1978a) that during this period, the proportion of mated females in the field increases, suggesting that most matings in *I. ricinus* occur away from the host, in the vegetation. We suggest that the higher attachment rate of female *I. ricinus* on dogs later in spring observed in our study may be a consequence of the fertilization of females in the tick population. It is known that unmated females may sometimes attach when confined within a net on a rabbit ear, but mating is necessary to complete feeding (Graf, 1978a). It is however unknown if given the choice to leave the host, the attachment rate of ticks is influenced by the mating status. Another possibility to explain the differences in the attachment rate of *I. ricinus* females on beagles might be related to dogs. The attachment rate of laboratory reared *I. ricinus* females on beagles was shown to reach 80% (T. Cavaliero, Novartis, personal communication) without much variations between individual dogs. No significant variations in tick attachment rate of *I. ricinus* females was observed between male and female beagle, nor between normal females and females in heat.

Whether increased attachment by female *I. ricinus* on dogs in late spring is really related to the mating behaviour of *I. ricinus* remains to be proven. However, these results show that tick bite risk of females *I. ricinus* is not directly related to the questing tick density measured by flag sampling and sets limitations to the use of questing tick density for the assessment of tick-bite risk, at least for beagles and adult *I. ricinus*.

Tick phenology: The density of questing ticks (CTD) varied greatly between locations during our study. Indeed, the CTD not only varied between distant sites, as between Neuchâtel and Brochus-Creuson (Geneva), but also within the same forest, as among the 4 adjacent zones

studied in Neuchâtel. Explanations for variations in tick density among sites have been given by others and implied the vegetation type (Doche et al., 1993)(Rizzoli et al., 2002), altitude (Rizzoli et al., 2002), the water capacity of the soil (Milne, 1944) (Jensen et al., 2000), the soil microclimate (Schulze and Jordan, 1996), the litter thickness (Milne, 1944), and biotic factors such as the density of hosts (Schulze and Jordan, 1996)(Jensen et al., 2000)(Rizzoli et al., 2002), and even acorn production (Ostfeld et al., 2001). By studying questing ticks at different locations our goal was not to add to this knowledge. We were interested in the phenology of questing ticks. This phenology varies throughout the large distribution area of *I. ricinus* (Korenberg, 2000). We wanted to verify if such variations occur among locations in Western Switzerland, if the phenology of ticks is conserved between years, and if a relation could be made between the phenology of ticks at the different locations and the climate at these locations.

First of all we verified that sampling of a surface of 150m^2 was sufficient to study the phenology of ticks at a given site. We therefore examined the horizontal distribution of ticks in the forest North of Neuchâtel, over a surface of 1049m^2. Nymph and adult distribution was random during most sampling sessions (see 3.7.1 on page 89). Random or slightly aggregated distribution of nymphs were also described in France (Vassallo et al., 2000), in the Czech Republic (Zeman and Daniel, 1999) and in another study in Switzerland (Klaus-Hügli et al., 2002). Based on these results in different areas over *I. ricinus* distribution, the minimal surface of 150m^2 sampled in most of our study sites, was appropriate to estimate tick density. This relatively small sampling surface minimized the time between successive samplings, thus enabling a better comparison of tick densities over a very short period of time (2 days) across the different locations in Western Switzerland.

Different densities of *I. ricinus* ticks were observed between our sampling sites. While these variations were not the object of our study some interesting observations were made. *I. ricinus* was present at a stable peak tick density to as high as 900m (Chaumont). This observation differs considerably from a previous study on tick distribution in Western Switzerland made during 1983 (Kaltenrieder et al., 1985) in which the authors showed that over 76 locations in Western Switzerland[2] ticks were only present in very low densities at 900m. This discrepancy between their observation in 1983 and our observation in 1999-2001 suggests either that Chaumont High is an exceptional site where *I. ricinus* was already present in high density in 1983, or that conditions affecting tick density in general in Switzerland have changed at 900m between 1983 and 1999. In Viège, at 780 meters, *I. ricinus* was also present at relatively high densities from 1999-2001. In contrast to the picture observed on the Chaumont Mountain and in Viège, ticks in Bière (at 800 meters) were only found in low densities throughout the three years studied, showing that the differences in tick density between locations at low altitude also exist among locations at higher altitudes. Comparative quantitative measurements on tick phenology are missing at different altitudes to conclude that *I. ricinus* distribution has increased towards higher altitudes during the last decade. Aeschlimann in 1972 (Aeschlimann, 1972) mentionned that *I. ricinus* was absent over 1500 meters. However during this study an infestation of cows was reported

[2] sampled once from April to June 1983

as high as 1650 meters in a region called "Pays d'Enhaut (VD)" (data not shown)[3]. It is however unclear whether this observation relates to an extension of *I. ricinus* distribution towards higher altitudes with favourable microclimatic conditions, or if it corresponds to a particular biocenose with abundant hosts for ticks.

Bavois and Bois-de-Portes are two forests at low altitude which were regularly flooded during our study. Despite flooding, ticks could be collected throughout the 3 years studied. Bavois is a very small forest patch (6.4ha) in the middle of a flat cultivated landscape (the closest forest is located 2km away). Such vegetation patches may serve as a refuge for ticks (Aeschlimann, 1972). Indeed, tick density into this patch was low, but ticks were present troughout the three years studied, showing that *I. ricinus* could withstand flooding and/or that *I. ricinus* were regularly imported in this small patch of forest in the middle of open landscape.

Flag sampling in the field only samples questing ticks. As shown above, the questing behaviour of *I. ricinus* is under the influence of climate. To assess the evolution of the proportion of questing ticks in the field we observed a known number of nymph and adult *I. ricinus* in mesh delimited arenas from March to December 2000 in the forest North of Neuchâtel and correlated these observations to climatic conditions. The proportion of questing ticks decreased irregularly from April to September. Ticks in the arenas were protected from predators and could not be picked-up by hosts. Therefore, the general decrease in tick number was probably due to tick death by exhaustion of their energy reserves. The decrease in the proportion of questing ticks was influenced by drought. Indeed, the proportion of questing ticks dropped considerably after the first large drought in June. The proportion of questing adults, but not nymphs, was partially restored after each drought. Drought events consisted either in drops in the maximum relative humidity (mostly occurring at night) or in increases of the maximum saturation deficit (mostly occurring during the day). The observed impact of climate on tick questing can be explained by two factors: the reduced maximal relative humidity prevented ticks from rehydrating at their questing site during the night and the increased saturation deficit during the warmer periods of the day increased water loss at questing sites, thus forcing ticks to search for a humid place to rehydrate. Adult activity was prolonged until September while nymphal activity strongly decreased already in June. These differences may be explained by the bigger size of adults which possess more energy reserves than nymphs. In addition, nymphs and adults did not immediately react to climatic conditions[4]. This shows that in nature ticks use their water content as a buffer to survive dessicating conditions during short periods. Adults by their size have a higher water content than nymphs. In addition, the surface to volume ratio is smaller for adults than nymphs, so that adults lose proportionally less water than nymphs. More energy, higher water content and smaller water loss in adult ticks enabled a higher proportion of adults to quest for longer periods than nymphs. Buffered reactions of ticks to climate was also observed by Gigon (Gigon, 1985) who observed that questing was influenced by a combination of relative humidity

[3] the cows access this high location after staying in an other area where they were reported to be free of ticks. It is therefore improbable that those cows brought all ticks reported with them when ascending the mountain

[4] no significant variations in the proportion of questing ticks were observed between the morning and the afternoon

and temperature, in other words: saturation deficit.

The density of questing ticks at the field in 2000, as recorded in two locations close to the field arenas ("Chaumont low" and "Neuchâtel extended surface sampling"), showed that nymphal and adult questing densities declined at the end of May, during the first drought event, but before the strong decrease in the proportion of questing ticks in the arenas. This suggest that either the decrease in the density of questing ticks in the field was due to a reduction in the tick **population size** rather than a decrease in the proportion of questing ticks, or that different cohorts of ticks coexist in the field, following different questing histories, so that the behaviour of the single group of ticks studied in the arenas does not relate the proportion of questing ticks in the mixed population. However, since ticks observed in the arenas were collected very early in the season it is not expected that these ticks would quest longer than ticks present in the field later in spring. Therefore we think that the decline in questing ticks in the field during May 2000 in Neuchâtel was primarily due to tick removal either by hosts or by predation of ticks rather than by limitation of the proportion of questing ticks by climatic conditions.

No increase in the proportion of questing nymphs was observed in the arenas during autumn 2000. On the other hand, an autumn peak of nymphs was observed at the two sampling locations close to the arenas. This suggests that the autumn peak of nymphs observed in nature during 2000 was due to newly emerged spring-fed larvae and not to reactivated spring active nymphs. Randolph et al. (Randolph et al., 2002) hypothesised that the occurrence of an autumn peak in tick activity is dependent upon the development duration of spring fed ticks. Our results in the arenas strengthen this explanation.

In *I. ricinus* the duration of development is inversely proportional to the temperature (MacLeod, 1934), (Campbell, 1948) cited in (Randolph et al., 2002). Under such a scenario we would expect an autumnal peak when temperatures are high in summer and no autumn peak otherwise. Over three years, we observed that indeed the relative importance of the autumnal peaks of tick activity decreased with altitude (3.7.2 on page 91). Since temperature decreases with altitude, this observation strengthens the explanation of Randolph et al. about the occurrence of the autumn tick activity (Randolph et al., 2002). This relation between temperature and the importance of the autumn activity of nymphal ticks was also observed over the whole of Western Switzerland, taking into account areas as diverse as an alpine valley (Viège), the Alp foothills (Interlaken) or the Swiss Plateau (Brochus-Creuson) (3.7.3.2 page 127). In addition, the onset of tick activity in spring was dependent upon temperature in winter (1. January to the 1. February) (see page 125 and (Perret et al., 2000)). Therefore ticks feeding at the warmest locations not only moult faster during summer, but also gain time to do so in spring. In summary, at the highest altitudes few ticks may feed in early spring and the fed ticks moult slowly compared to the lower altitudes, so that few ticks have the chance to develop to the next stage before the arrival of winter. At lower altitudes however, spring fed ticks are able to develop and quest before the arrival of winter, as in Neuchâtel during 1998 (Perret et al., 2000), 2000 and 2001. In contrast during 1996, 1997 (Perret et al., 2000) and 1999 no autumn peak was observed. This means that the number of ticks fed during one year may vary considerably at lower altitude depending on the presence or not of an autumn peak. At higher altitudes on the other hand, since an autumn peak rarely occurs, the number of fed ticks during one year will be smaller, but much more constant between

years. This mechanism could explain why strong variations in tick density, especially in spring, were observed among years at lower altitudes, while peak tick density was almost constant at the highest altitudes.

Qualitatively the impact of climate on tick phenology was similar throughout Western Switzerland (the tick activity onset in spring was related to temperature in January, see 3.7.3.2 on page 125, and the relative importance of the autumn peak was related to temperature from March to June see 3.7.3.2 on page 127). However, much of the variance in the onset of tick activity and in the importance of the autumn peak was not explained by the mesoclimatic variables. Microclimatic variable may have provided much better correlations between climate and tick phenology. The nature of the soil, its color, its permeability, the vegetation structure, the slope, its orientation, are all factors affecting the microclimate experienced locally by ticks and these factors are not assessed by mesoclimatic variables. One particularly interesting example of the importance of microclimate is the effect of hurricane Lothar at Interlaken. During winter 1999-2000 the forest was partly destroyed, leaving big holes in the canopy under which ticks experienced almost bare ground. The phenology of ticks was disrupted in 2000, with very low nymphal activity in spring, but with the occurrence of an autumn peak in both nymph and adult ticks. The amount of sunlight reaching the ground was increased just after Lothar due to tree fall. We therefore expect that ticks experienced higher temperatures and lower humidities during the year following the storm. During spring higher saturation deficit reduced tick questing, but during summer higher temperature led to a faster development of spring fed ticks therefore explaining the reduced questing density in spring and the occurrence of an autumn peak of activity at Interlaken during 2000. In 2001 however, the vegetation coverage on the ground increased thanks to the increased light at ground level and nymphs recovered a more usual phenology with a main spring peak, even if tick density was much lower than before the hurricane. This illustrates the role of the habitat micro-environment and sets the limits to the use of mesoclimatic variables for the correlation of climate with tick phenology. At Neuchâtel, comparison between the microclimate measured in the forest and the mesoclimate measured by the Meteosuisse weather station showed a good correlation for temperature with colder temperatures within the forest, but a lower correlation for relative humidity.

Another interesting and extreme event was observed during 2000 in Vaumarcus where the forest was entirely destroyed by the building of a highway. In order to reduce ground erosion, the chopped vegetation was left on the ground for 10 months. Ticks which survived the forest destruction experienced much drier conditions on the bare landscape (up to 30.6°C and 22 mm Hg saturation deficit on May 10, 2000) than in the forest. This resulted in a dramatic reduction in the density of questing nymphs. In contrast, the peak density of questing adults remained at a similar level to the peak adult density during the year before forest destruction. However, most ticks were found within the few patches of herbaceous vegetation which survived in this arid environment. This illustrates the robustness of adult ticks towards dessication as compared to nymphs and suggest that ticks, especially adults, may be able to move horizontally to find a favourable microclimate to quest and to rehydrate.

Exceptionally dry conditions in spring are not only due to catastrophic events. In 1998 at Neuchâtel for example, saturation deficit reached very high levels during spring (Perret et al.,

2000). The questing nymph density during this period remained extremely low compared to the other years. We believe that the duration of questing events in nymphs was strongly reduced by the highly dessicating conditions, thus reducing the proportion of questing nymphs and therefore also reducing the questing nymph density. Since *I. ricinus* quests to grab hosts, we hypothesise that a shortening of questing events also reduces the number of ticks fed, and therefore has an impact on the evolution of the tick population size.

The stable phenology of *I. ricinus* observed as high as 900m, and the instable phenology observed at the lowest altitudes in Switzerland may have important consequences for the establishment of diseases like tick-borne encephalitis (TBE). Indeed it was shown that the crucial factor for the establishment of TBE foci is the synchronous feeding of nymphs and larvae on rodents under the control of climatic conditions (Randolph et al., 2000). Synchronising factors for the timepoint of tick feeding are on the one hand cold temperatures in winter preventing questing and synchronising the development of ticks, and on the other hand warm temperatures in summer reducing questing but enhancing the development of spring fed ticks. Increased temperatures may have different consequences at different altitudes. While new TBE foci may appear at higher altitudes, the impact of temperature increase at low altitudes may be much more complicated. We observed strong instabilities in tick phenology and density at low altitudes. Using a general circulation model that predicts how climatic variables may change in the future, Randolph and Rogers (Randolph and Rogers, 2000) suggested that TBE foci might even disappear from Switzerland in 2020.

I. ricinus has a very wide geographic distribution, occurring throughout Europe and extending to North Africa (Gern and Humair, 2002). The climatic, photoperiod and vegetational conditions experienced by this tick species, as well as its host range, are extremely heterogeneous with diverse effects on tick populations. Under these conditions, different *I. ricinus* populations may have developed different physiological and behavioural adaptations to optimize energy use in a particular ecozone. The video tracking system developed for this study might help to estimate such local adaptations in populations of *I. ricinus* adapted to different climatic and photoperiod conditions.

Our observations on the behaviour and on the phenology of ticks in Neuchâtel, along an altitude gradient, over Western Swizerland and in laboratory has led us to conclude that the phenology of *I. ricinus* in Switzerland depends on the winter temperature determining the onset of tick activity, on spring saturation-deficit limiting the duration of questing periods, and on summer temperature influencing the development speed of spring-fed ticks and the occurrence of an autumn peak of questing tick density.

We demonstrated that the questing behaviour in *I. ricinus* is a form of appetitive search under the influence of light, climate and *B. burgdorferi* infection. We discovered that while ticks indeed stay immobile in long questing periods limited by dessicating conditions, they also show significant walking periods during darkness. Our results clarified apparently contradictory results concerning the diurnal rythms in *I. ricinus* and the walking activity of this tick. We also provide data on quiescence interruption which is shown to be independant of climatic conditions. *I. ricinus*, which was thougt to be a rather passive ambusher waiting for a passing host may in fact

rather be a clever ambusher hunter tick optimising its position and minimising the energy costs to reach its hosts. These results demonstrate the adaptations enabling the survival of *I. ricinus* over its very broad distribution and under varying climatic conditions despite its high sensitivity to dessication.

Finally, the tracking system developed for this study could easily be used to study the behaviour of other parasitic and non-parasitic animals.

Appendix A

CD-ROM

Here is a list of all files provided on the attached cd-rom (full path to the files on the cd-rom are in bold font).

index.html: web page presenting the content of this cd-rom.

Posters

posters/sc$_2$$001.pdf$:$<<Lestiquessontparminous>>$, $posteronticks(infrench)$**posters/syst**$_2$$000.pdf$**posters**

Comma separated files

rawdata/lieux.csv: *Description of locations*. Fields include: location code (=codelieu), name, swiss grid coordinates and altitude.

rawdata/drapeau.csv: *List of sampling sessions*. Fields include: sampling code (=iddrapeau), location code (=codelieu), date, start time, ground temperature, air temperature at 60cm, air temperature at 10cm, relative humidity at 10cm above ground (%), wind (Beaufort), clouds (0-8/8).

rawdata/drags.csv: *Counts of ticks collected on each single drag* (raw data). Fields include: location code (=codelieu), sampling sode (=iddrapeau), surface of the drag (m^2), number of larvae, nymphs, females, males collected.

Homemade Programs

rawdata/loadclimatthese.for: Fortran program run on the wawona.ethz.ch server to retrieve hourly climatic data for one year.

Appendix B

Computer code

This chapter shows some of the computer code which was used or developed for this thesis. Some of the computer programs are much too long to be printed here, but are listed here for referrence.

B.1 Video tracking

camtud3: video tracking software

vtrackanalysis: cut a track of vertical positions into QUIESCENCE / WALKING / QUESTING events

vtrackanalysisall: apply vtrackanalysis to all tracks in an experiment

B.2 Statistics

B.2.1 Jonckheere test

Luciano Molinari (Luciano.Molinari@kispi.unizh.ch)
programmed in R (Ihaka and Gentleman, 1996).

```
jonckheere<-function(y, groups)
{
 if(!is.ordered(groups)){
     groups <- as.factor(groups)
     cat("groups not an ordered factor.\n")
   }
   cat("Ordering Used \n\n")
   cat(paste(levels(groups),collapse=" < "),"\n\n")
   lev <- levels(groups)
   nlev <- length(lev)
```

```
  if(nlev < 2) {
    cat("No case for Jonckeree \n")
    return(NULL)
  }
  jonck <- 0
  ties.p <- F
  for(i in 1:(nlev - 1))
  for(j in (i + 1):nlev) {
    xx <- y[groups == lev[i]]
    yy <- y[groups == lev[j]]
    xy <- c(xx, yy)
    ties.p <- ties.p | (length(xy) !=length(unique(xy)))
    sss <- length(xx) * length(yy) + (length(xx) *
      (length(xx) + 1))/2 - sum(rank(xy)[1:length(xx)])
    jonck <- jonck + sss
  }
  #for ties see Lehmann p.235ff
  names(jonck) <- NULL
  ns <- tabulate(as.numeric(groups))
  nn <- length(y)
  expj <- (nn * nn - sum(ns * ns))/4
  if(!ties.p) {
    varj <- (nn * nn * (2 * nn + 3)
     - sum(ns * ns * (2 * ns + 3)))/72
  }
  else {
    nun <- as.vector(table(y))
    varj <- (nn * (nn - 1) * (2 * nn + 5) -
    sum(ns * (ns - 1) * (2 * ns + 5)) -
    sum(nun * (nun - 1) * (2 * nun + 5)))/72 +
    (sum(ns * (ns - 1) * (ns - 2)) * sum(nun * (nun - 1) *
    (nun - 2)))/(36 * nn * (nn - 1) * (nn - 2)) +
    (sum(ns * (ns - 1)) * sum(nun * (nun - 1)))/(8 * nn * (nn - 1))
  }
  c(Jonckheere = jonck, ExpJ = expj, VarJ = varj,
    P = 1 - pnorm((jonck- expj)/sqrt(varj))
   )
}
```

Appendix C

Tick phenology: descriptive statistics

The phenology of ticks is represented graphically as timeplots of the questing tick density. From this phenology we extracted several descriptive statistics that each characterize a particular aspect of the phenology. Some descriptive statistics describe the whole year such as the cumulated tick density, others only describe the spring peak (for example the date of onset of tick activity), and others only describe the autumn peak (for example the maximum tick density reached in autumn). A full list of the definitions of these statistics is given below and is illustrated in figure C.1.

Tables C.7 to C.62 show the descriptive statistics calculated for all areas and years sampled during this work.

Descriptive statistics for the whole year:

Timing of peak (TP) : The timing of the questing peak was defined as the sampling date corresponding to the maximal questing tick density over one year, calculated separately for nymphs (TPN) and for adults (TPA).

Peak tick density (PTD) : Maximal tick density recorded over one year, calculated separately for nymph (PTDN) and adult (PTDA) peak density

Duration 25% (D25) : Duration in days for which questing tick density was at least 25% of the peak tick density, calculated separately for nymphs (D25N) and adults (D25A).

Duration 10% (D10) : Duration in days for which questing tick density was at least 10% of the peak tick density, calculated separately for nymphs (D10N) and adults (D10A).

Span 25% (S25) : Time span covered by the period(s) when questing tick density was over 25% of the peak tick density, equals F25-O25, calculated separately for nymphs (S25N) and adults (S25A).

Span 10% (S10) : Time span covered by the period(s) when questing tick density was over 10% of the peak tick density, equals F10-O10, calculated separately for nymphs (S10N) and adults (S10A).

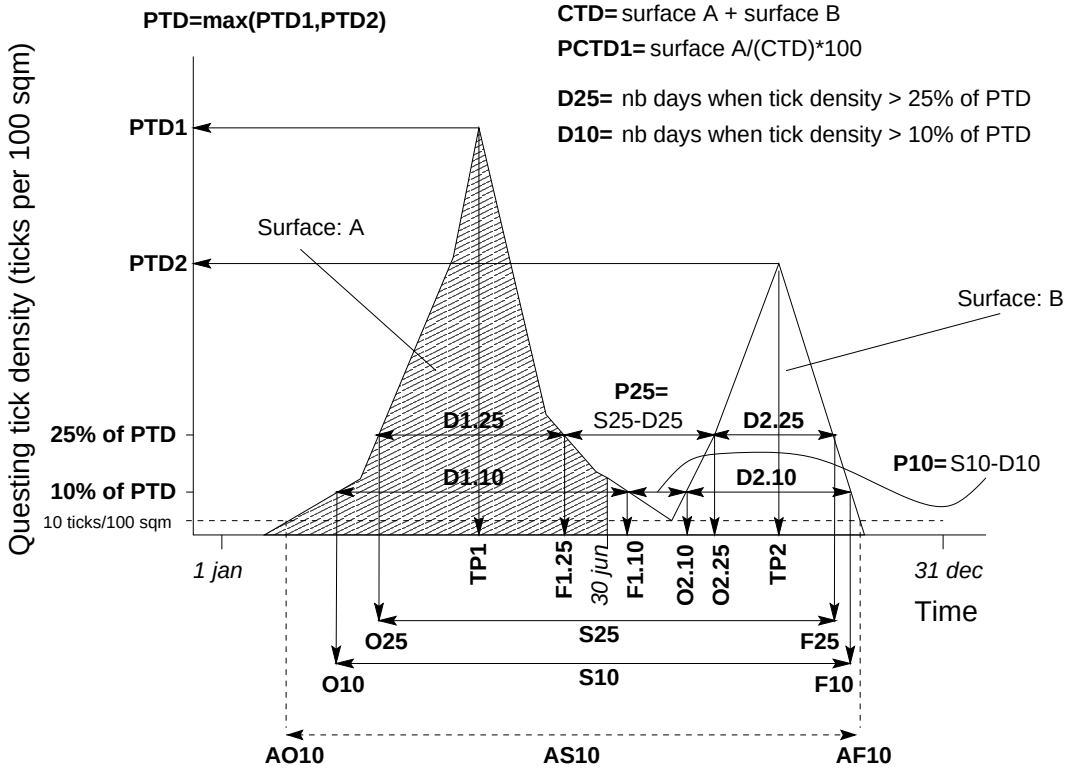


Figure C.1: **Descriptive statistics calculated to describe tick phenology**
(figure: Jean-Luc Perret 2002)

Pauses 25% (P25) : Total number of days, within the period delimited by O25 and F25, when tick density was below 25% of the peak tick density. Equivalent to the duration of the summer pause when tick density showed a secondary peak in autumn, equals S25-D25, calculated separately for nymphs (P25N) and adults (P25A).

Pauses 10% (P10) : Total number of days, within the period delimited by O10 and F10, when tick density was below 10% of the peak tick density. Equals to S10-D10. Equivalent to the duration of the summer pause when tick density showed a secondary peak in autumn, calculated separately for nymphs (P10N) and adults (P10A).

Onset 25% (O25) : First date when tick density increased above 25% of the peak tick density, calculated separately for nymphs (O25N) and adults (O25A).

Onset 10% (O10) : First date when questing tick density increased above 10% of the peak tick density, calculated separately for nymphs (O10N) and adults (O10A).

Fall 25% (F25) : Last date when questing tick density decreased below 25% of the peak tick density, calculated separately for nymphs (F25N) and adults (F25A).

Fall 10% (F10) : Last date when questing tick density decreased below 10% of the peak tick density, calculated separately for nymphs (F10N) and adults (F10A).

Absolute onset 10 sqm (AO10) : First date when questing tick density increased above 10 ticks per 100m^2, calculated separately for nymphs (AO10N) and adults (AO10A).

Absolute fall 10 sqm (AF10) : Last date when questing tick density decreased below 10 ticks per 100m^2, calculated separately for nymphs (AF10N) and adults (AF10A).

Absolute duration 10 sqm (AD10) : Duration in days for which questing tick density was at least 10 ticks per 100m^2, calculated separately for nymphs (AD10N) and adults (AD10A).

Absolute span 10 sqm (AS10) : Time span covered by the period(s) when questing tick density was over 10 ticks per 100m^2, calculated separately for nymphs (AS10N) and adults (AS10A).

Cumulative tick density (CTD) : The cumulative tick density (in number of ticks per 100m^2 and per year) was estimated by integrating the questing tick density curve over one year[1]. This statistic was calculated separately for nymphs (CTDN) and adults (CTDA).

Proportion of cumulative tick density in the first half-year (PCTD1) : The proportion (in %) of cumulative tick density during the first half-year was calculated by the following ratio:

$$\%\ tick\ presence\ in\ first\ 1/2\ year = \frac{\int_{1\ January}^{30\ June} questing\ tick\ density\ curve}{\int_{1\ January}^{31\ December} questing\ tick\ density\ curve} * 100$$

This statistic was calculated separately for nymphs (PCTD1N) and adults (PCTD1A).

Proportion of cumulative tick density in the second half-year (PCTD2) : The proportion (in %) of cumulative tick density during the second half-year was calculated as 100-PCTD1

This statistic was calculated separately for nymphs (PCTD2N) and adults (PCTD2A).

Descriptive statistics for the spring peak:

Timing of peak 1 (TP1) : The sampling date corresponding to the maximal questing tick density before June 30, calculated separately for nymphs (TP1N) and adults (TP1A).

Peak tick density 1 (PTD1) : maximal tick density recorded before June 30, calculated separately for nymphs (PTD1N) and adults (PTD1A).

Duration 1 25% (D1.25) : Duration of the first peak for which questing tick density was at least 25% of the peak tick density, calculated separately for nymphs (D1.25N) and adults (D1.25A).

[1]Leap years induce a slight error in the estimation of the cumulative tick density, but this error was considered as negligible and ignored

Duration 1 10% (D1.10) : Duration of the first peak for which questing tick density was at least 10% of the peak tick density, calculated separately for nymphs (D1.10N) and adults (D1.10A).

Fall 1 25% (F1.25) : First date after TP1 when questing tick density decreased below 25% of the peak tick density, calculated separately for nymphs (F1.25N) and adults (F1.25A).

Fall 1 10% (F1.10) : First date after TP1 when questing tick density decreased below 10% of the peak tick density, calculated separately for nymphs (F1.10N) and adults (F1.10A).

Descriptive statistics for the autumn peak:

Timing of peak 2 (TP2) : The sampling date corresponding to the maximal questing tick density after F1.25N or if there is no spring peak, after June 30, calculated separately for nymphs (TP2N) and adults (TP2A).

Peak tick density 2 (PTD2) : maximal tick density recorded after F1.25N or if there is no spring peak, after June 30, calculated separately for nymphs (PTD2N) and adults (PTD2A).

Duration 2 25% (D2.25) : Duration of the second peak for which questing tick density was at least 25% of the peak tick density, alculated separately for nymphs (D2.25N) and adults (D2.25A).

Duration 2 10% (D2.10) : Duration of the second peak for which questing tick density was at least 10% of the peak tick density, calculated separately for nymphs (D2.10N) and adults (D2.10A).

Onset2 25% (O2.25) : First date after F1.25 when questing tick density increased above 25%, calculated separately for nymphs (O2.25N) and adults (O2.25A).

Onset2 10% (O2.10) : First date after F1.10 when questing tick density increased above 10%, calculated separately for nymphs (O2.10N) and adults (O2.10A).

Statistics	Nymphs	Adults
Location	**Chaumont High**	**Chaumont High**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	04-25-99	06-01-99
PTD (ind/100sqm)	93	7
Duration 25% (days)	120	169
Duration 10% (days)	205	266
Span 25% (days)	120	169
Span 10% (days)	214	245
Pause 25% (days)	0	0
Pause 10% (days)	9	0
Onset 10% (m-d-y)	03-17-99	02-14-99
Onset 25% (m-d-y)	03-23-99	03-07-99
Fall 25% (m-d-y)	07-21-99	08-23-99
Fall 10% (m-d-y)	10-17-99	10-17-99
Absolute Onset 10 (m-d-y)	03-18-99	NA
Absolute Fall 10 (m-d-y)	11-03-99	NA
Absolute Duration 10 (days)	196	0
Absolute Span 10 (days)	230	0
Timing of Peak 1(m-d-y)	04-25-99	06-01-99
PTD1 (ind/100sqm)	93	7
Duration 1 25% (days)	120	169
Duration 1 10% (days)	168	203
Fall1 25% (m-d-y)	07-21-99	08-23-99
Fall1 10% (m-d-y)	09-01-99	09-05-99
Timing of Peak 2(m-d-y)	10-17-99	10-17-99
PTD2 (ind/100sqm)	13	1
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	16	16
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	10-01-99	10-01-99
CTD (ind/(100sqm*year)	10006	704
% in 1st half-year (%)	76	62

Table C.1: Descriptive statistics of the questing tick timeplots at Chaumont High in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont Middle**	**Chaumont Middle**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	04-25-99	06-29-99
PTD (ind/100sqm)	116	16
Duration 25% (days)	146	147
Duration 10% (days)	226	232
Span 25% (days)	213	147
Span 10% (days)	235	239
Pause 25% (days)	67	0
Pause 10% (days)	9	7
Onset 10% (m-d-y)	02-24-99	02-20-99
Onset 25% (m-d-y)	03-18-99	03-18-99
Fall 25% (m-d-y)	10-17-99	08-12-99
Fall 10% (m-d-y)	10-17-99	10-17-99
Absolute Onset 10 (m-d-y)	02-22-99	04-26-99
Absolute Fall 10 (m-d-y)	11-24-99	07-14-99
Absolute Duration 10 (days)	240	34
Absolute Span 10 (days)	275	79
Timing of Peak 1(m-d-y)	04-25-99	06-29-99
PTD1 (ind/100sqm)	116	16
Duration 1 25% (days)	122	148
Duration 1 10% (days)	166	193
Fall1 25% (m-d-y)	07-18-99	08-13-99
Fall1 10% (m-d-y)	08-09-99	09-01-99
Timing of Peak 2(m-d-y)	10-17-99	10-17-99
PTD2 (ind/100sqm)	41	3
Duration 2 25% (days)	9	NA
Duration 2 10% (days)	86	13
Onset2 25% (m-d-y)	10-08-99	NA
Onset2 10% (m-d-y)	07-23-99	10-04-99
CTD (ind/(100sqm*year)	12832	1536
% in 1st half-year (%)	73	64

Table C.2: Descriptive statistics of the questing tick timeplots at Chaumont Middle in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont Low**	**Chaumont Low**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	04-25-99	04-25-99
PTD (ind/100sqm)	295	24
Duration 25% (days)	143	152
Duration 10% (days)	224	235
Span 25% (days)	143	152
Span 10% (days)	249	245
Pause 25% (days)	0	0
Pause 10% (days)	25	10
Onset 10% (m-d-y)	02-10-99	02-14-99
Onset 25% (m-d-y)	02-24-99	03-07-99
Fall 25% (m-d-y)	07-17-99	08-06-99
Fall 10% (m-d-y)	10-17-99	10-17-99
Absolute Onset 10 (m-d-y)	02-05-99	03-22-99
Absolute Fall 10 (m-d-y)	11-29-99	07-15-99
Absolute Duration 10 (days)	287	116
Absolute Span 10 (days)	297	115
Timing of Peak 1(m-d-y)	04-25-99	04-25-99
PTD1 (ind/100sqm)	295	24
Duration 1 25% (days)	143	152
Duration 1 10% (days)	183	200
Fall1 25% (m-d-y)	07-17-99	08-06-99
Fall1 10% (m-d-y)	08-12-99	09-02-99
Timing of Peak 2(m-d-y)	10-17-99	10-17-99
PTD2 (ind/100sqm)	55	4
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	85	16
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	07-24-99	10-01-99
CTD (ind/(100sqm*year)	34144	2633
% in 1st half-year (%)	82	75

Table C.3: Descriptive statistics of the questing tick timeplots at Chaumont Low in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont High**	**Chaumont High**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	05-17-00	05-17-00
PTD (ind/100sqm)	91	16
Duration 25% (days)	156	140
Duration 10% (days)	262	209
Span 25% (days)	217	140
Span 10% (days)	262	257
Pause 25% (days)	61	0
Pause 10% (days)	0	48
Onset 10% (m-d-y)	02-19-00	02-10-00
Onset 25% (m-d-y)	03-15-00	02-24-00
Fall 25% (m-d-y)	10-18-00	07-13-00
Fall 10% (m-d-y)	11-07-00	10-24-00
Absolute Onset 10 (m-d-y)	02-22-00	04-26-00
Absolute Fall 10 (m-d-y)	11-05-00	06-07-00
Absolute Duration 10 (days)	258	43
Absolute Span 10 (days)	257	42
Timing of Peak 1(m-d-y)	05-17-00	05-17-00
PTD1 (ind/100sqm)	91	16
Duration 1 25% (days)	124	140
Duration 1 10% (days)	NA	191
Fall1 25% (m-d-y)	07-17-00	07-13-00
Fall1 10% (m-d-y)	NA	08-19-00
Timing of Peak 2(m-d-y)	09-28-00	NA
PTD2 (ind/100sqm)	28	NA
Duration 2 25% (days)	32	NA
Duration 2 10% (days)	102	NA
Onset2 25% (m-d-y)	09-16-00	NA
Onset2 10% (m-d-y)	07-28-00	NA
CTD (ind/(100sqm*year)	10406	1495
% in 1st half-year (%)	70	79

Table C.4: Descriptive statistics of the questing tick timeplots at Chaumont High in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont Middle**	**Chaumont Middle**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-20-00	04-20-00
PTD (ind/100sqm)	60	18
Duration 25% (days)	217	166
Duration 10% (days)	282	254
Span 25% (days)	260	250
Span 10% (days)	282	276
Pause 25% (days)	43	84
Pause 10% (days)	0	22
Onset 10% (m-d-y)	02-10-00	02-08-00
Onset 25% (m-d-y)	02-24-00	02-19-00
Fall 25% (m-d-y)	11-10-00	10-26-00
Fall 10% (m-d-y)	11-18-00	11-10-00
Absolute Onset 10 (m-d-y)	02-17-00	03-13-00
Absolute Fall 10 (m-d-y)	11-14-00	05-26-00
Absolute Duration 10 (days)	272	75
Absolute Span 10 (days)	271	74
Timing of Peak 1(m-d-y)	04-20-00	04-20-00
PTD1 (ind/100sqm)	60	18
Duration 1 25% (days)	139	114
Duration 1 10% (days)	NA	134
Fall1 25% (m-d-y)	07-12-00	06-12-00
Fall1 10% (m-d-y)	NA	06-21-00
Timing of Peak 2(m-d-y)	10-17-00	08-23-00
PTD2 (ind/100sqm)	41	6
Duration 2 25% (days)	78	76
Duration 2 10% (days)	105	120
Onset2 25% (m-d-y)	08-24-00	08-11-00
Onset2 10% (m-d-y)	08-05-00	07-13-00
CTD (ind/(100sqm*year)	8002	1962
% in 1st half-year (%)	59	73

Table C.5: Descriptive statistics of the questing tick timeplots at Chaumont Middle in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont Low**	**Chaumont Low**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-20-00	04-20-00
PTD (ind/100sqm)	144	38
Duration 25% (days)	189	105
Duration 10% (days)	256	169
Span 25% (days)	273	106
Span 10% (days)	287	169
Pause 25% (days)	84	1
Pause 10% (days)	31	0
Onset 10% (m-d-y)	02-06-00	02-05-00
Onset 25% (m-d-y)	02-15-00	02-11-00
Fall 25% (m-d-y)	11-14-00	05-27-00
Fall 10% (m-d-y)	11-19-00	07-23-00
Absolute Onset 10 (m-d-y)	02-06-00	02-13-00
Absolute Fall 10 (m-d-y)	12-10-00	05-22-00
Absolute Duration 10 (days)	309	100
Absolute Span 10 (days)	308	99
Timing of Peak 1(m-d-y)	04-20-00	04-20-00
PTD1 (ind/100sqm)	144	38
Duration 1 25% (days)	123	106
Duration 1 10% (days)	157	169
Fall1 25% (m-d-y)	06-17-00	05-27-00
Fall1 10% (m-d-y)	07-12-00	07-23-00
Timing of Peak 2(m-d-y)	10-17-00	NA
PTD2 (ind/100sqm)	99	NA
Duration 2 25% (days)	66	NA
Duration 2 10% (days)	146	NA
Onset2 25% (m-d-y)	09-09-00	NA
Onset2 10% (m-d-y)	06-26-00	NA
CTD (ind/(100sqm*year)	18779	3440
% in 1st half-year (%)	67	93

Table C.6: Descriptive statistics of the questing tick timeplots at Chaumont Low in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont High**	**Chaumont High**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-08-01	03-27-01
PTD (ind/100sqm)	97	11
Duration 25% (days)	54	174
Duration 10% (days)	185	204
Span 25% (days)	54	174
Span 10% (days)	167	204
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	03-30-01	02-16-01
Onset 25% (m-d-y)	04-05-01	02-23-01
Fall 25% (m-d-y)	05-29-01	08-16-01
Fall 10% (m-d-y)	09-13-01	09-08-01
Absolute Onset 10 (m-d-y)	03-31-01	03-26-01
Absolute Fall 10 (m-d-y)	09-29-01	05-09-01
Absolute Duration 10 (days)	183	45
Absolute Span 10 (days)	182	44
Timing of Peak 1(m-d-y)	05-08-01	03-27-01
PTD1 (ind/100sqm)	97	11
Duration 1 25% (days)	54	202
Duration 1 10% (days)	NA	209
Fall1 25% (m-d-y)	05-29-01	09-13-01
Fall1 10% (m-d-y)	NA	09-13-01
Timing of Peak 2(m-d-y)	07-03-01	NA
PTD2 (ind/100sqm)	20	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	103	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	06-02-01	NA
CTD (ind/(100sqm*year)	5765	1278
% in 1st half-year (%)	61	67

Table C.7: Descriptive statistics of the questing tick timeplots at Chaumont High in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont Middle**	**Chaumont Middle**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-08-01	05-08-01
PTD (ind/100sqm)	126	44
Duration 25% (days)	67	96
Duration 10% (days)	225	186
Span 25% (days)	67	96
Span 10% (days)	237	223
Pause 25% (days)	0	0
Pause 10% (days)	12	37
Onset 10% (m-d-y)	02-23-01	02-07-01
Onset 25% (m-d-y)	03-21-01	02-23-01
Fall 25% (m-d-y)	05-27-01	05-30-01
Fall 10% (m-d-y)	10-18-01	09-18-01
Absolute Onset 10 (m-d-y)	02-20-01	02-19-01
Absolute Fall 10 (m-d-y)	10-28-01	05-31-01
Absolute Duration 10 (days)	251	102
Absolute Span 10 (days)	250	101
Timing of Peak 1(m-d-y)	05-08-01	05-08-01
PTD1 (ind/100sqm)	126	44
Duration 1 25% (days)	67	96
Duration 1 10% (days)	96	178
Fall1 25% (m-d-y)	05-27-01	05-30-01
Fall1 10% (m-d-y)	05-30-01	08-04-01
Timing of Peak 2(m-d-y)	09-13-01	NA
PTD2 (ind/100sqm)	16	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	124	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	06-16-01	NA
CTD (ind/(100sqm*year)	8089	3332
% in 1st half-year (%)	72	75

Table C.8: Descriptive statistics of the questing tick timeplots at Chaumont Middle in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Chaumont Low**	**Chaumont Low**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-09-01	02-14-01
PTD (ind/100sqm)	208	16
Duration 25% (days)	183	112
Duration 10% (days)	255	209
Span 25% (days)	238	112
Span 10% (days)	251	235
Pause 25% (days)	55	0
Pause 10% (days)	0	26
Onset 10% (m-d-y)	02-09-01	02-02-01
Onset 25% (m-d-y)	02-22-01	02-04-01
Fall 25% (m-d-y)	10-18-01	05-27-01
Fall 10% (m-d-y)	10-18-01	09-25-01
Absolute Onset 10 (m-d-y)	02-06-01	02-10-01
Absolute Fall 10 (m-d-y)	11-14-01	04-29-01
Absolute Duration 10 (days)	279	79
Absolute Span 10 (days)	281	78
Timing of Peak 1(m-d-y)	05-09-01	02-14-01
PTD1 (ind/100sqm)	208	16
Duration 1 25% (days)	129	112
Duration 1 10% (days)	175	184
Fall1 25% (m-d-y)	07-01-01	05-27-01
Fall1 10% (m-d-y)	08-03-01	08-05-01
Timing of Peak 2(m-d-y)	10-18-01	07-03-01
PTD2 (ind/100sqm)	78	4
Duration 2 25% (days)	43	NA
Duration 2 10% (days)	73	110
Onset2 25% (m-d-y)	09-05-01	NA
Onset2 10% (m-d-y)	08-06-01	06-07-01
CTD (ind/(100sqm*year)	22067	1716
% in 1st half-year (%)	62	82

Table C.9: Descriptive statistics of the questing tick timeplots at Chaumont Low in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 1**	**Neuchâtel Zone 1**
Year	**1996**	**1996**
Timing of Peak (m-d-y)	05-29-96	04-21-96
PTD (ind/100sqm)	84	6
Duration 25% (days)	85	126
Duration 10% (days)	124	158
Span 25% (days)	85	127
Span 10% (days)	146	159
Pause 25% (days)	0	1
Pause 10% (days)	22	1
Onset 10% (m-d-y)	03-14-96	02-14-96
Onset 25% (m-d-y)	03-28-96	03-06-96
Fall 25% (m-d-y)	06-21-96	07-11-96
Fall 10% (m-d-y)	08-07-96	07-22-96
Absolute Onset 10 (m-d-y)	03-17-96	NA
Absolute Fall 10 (m-d-y)	08-04-96	NA
Absolute Duration 10 (days)	115	0
Absolute Span 10 (days)	140	0
Timing of Peak 1(m-d-y)	05-29-96	04-21-96
PTD1 (ind/100sqm)	84	6
Duration 1 25% (days)	85	127
Duration 1 10% (days)	103	159
Fall1 25% (m-d-y)	06-21-96	07-11-96
Fall1 10% (m-d-y)	06-25-96	07-22-96
Timing of Peak 2(m-d-y)	07-29-96	NA
PTD2 (ind/100sqm)	13	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	21	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	07-17-96	NA
CTD (ind/(100sqm*year)	5378	493
% in 1st half-year (%)	89	92

Table C.10: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 1 in 1996 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 2**	**Neuchâtel Zone 2**
Year	**1996**	**1996**
Timing of Peak (m-d-y)	05-29-96	06-27-96
PTD (ind/100sqm)	81	10
Duration 25% (days)	134	89
Duration 10% (days)	213	201
Span 25% (days)	134	89
Span 10% (days)	232	249
Pause 25% (days)	0	0
Pause 10% (days)	19	48
Onset 10% (m-d-y)	03-03-96	02-17-96
Onset 25% (m-d-y)	03-17-96	04-23-96
Fall 25% (m-d-y)	07-29-96	07-21-96
Fall 10% (m-d-y)	10-21-96	10-23-96
Absolute Onset 10 (m-d-y)	03-11-96	06-28-96
Absolute Fall 10 (m-d-y)	10-12-96	06-28-96
Absolute Duration 10 (days)	186	1
Absolute Span 10 (days)	215	00:00:00
Timing of Peak 1(m-d-y)	05-29-96	06-27-96
PTD1 (ind/100sqm)	81	10
Duration 1 25% (days)	134	89
Duration 1 10% (days)	165	160
Fall1 25% (m-d-y)	07-29-96	07-21-96
Fall1 10% (m-d-y)	08-15-96	07-26-96
Timing of Peak 2(m-d-y)	09-26-96	09-26-96
PTD2 (ind/100sqm)	14	2
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	48	41
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	09-03-96	09-12-96
CTD (ind/(100sqm*year)	9330	766
% in 1st half-year (%)	76	68

Table C.11: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 2 in 1996 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 3**	**Neuchâtel Zone 3**
Year	**1996**	**1996**
Timing of Peak (m-d-y)	05-29-96	05-29-96
PTD (ind/100sqm)	309	39
Duration 25% (days)	95	164
Duration 10% (days)	154	228
Span 25% (days)	95	252
Span 10% (days)	154	279
Pause 25% (days)	0	88
Pause 10% (days)	0	51
Onset 10% (m-d-y)	03-09-96	02-10-96
Onset 25% (m-d-y)	03-22-96	02-25-96
Fall 25% (m-d-y)	06-25-96	11-03-96
Fall 10% (m-d-y)	08-10-96	11-15-96
Absolute Onset 10 (m-d-y)	02-13-96	02-26-96
Absolute Fall 10 (m-d-y)	11-18-96	11-02-96
Absolute Duration 10 (days)	260	161
Absolute Span 10 (days)	279	250
Timing of Peak 1(m-d-y)	05-29-96	05-29-96
PTD1 (ind/100sqm)	309	39
Duration 1 25% (days)	95	158
Duration 1 10% (days)	154	188
Fall1 25% (m-d-y)	06-25-96	08-01-96
Fall1 10% (m-d-y)	08-10-96	08-16-96
Timing of Peak 2(m-d-y)	07-29-96	10-31-96
PTD2 (ind/100sqm)	64	11
Duration 2 25% (days)	NA	6
Duration 2 10% (days)	41	40
Onset2 25% (m-d-y)	NA	10-28-96
Onset2 10% (m-d-y)	06-30-96	10-06-96
CTD (ind/(100sqm*year)	24748	4139
% in 1st half-year (%)	83	74

Table C.12: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 3 in 1996 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 4**	**Neuchâtel Zone 4**
Year	**1996**	**1996**
Timing of Peak (m-d-y)	05-29-96	03-11-96
PTD (ind/100sqm)	102	30
Duration 25% (days)	90	65
Duration 10% (days)	161	136
Span 25% (days)	90	66
Span 10% (days)	161	137
Pause 25% (days)	0	1
Pause 10% (days)	0	1
Onset 10% (m-d-y)	03-01-96	02-04-96
Onset 25% (m-d-y)	03-27-96	02-10-96
Fall 25% (m-d-y)	06-25-96	04-16-96
Fall 10% (m-d-y)	08-09-96	06-20-96
Absolute Onset 10 (m-d-y)	03-01-96	02-15-96
Absolute Fall 10 (m-d-y)	11-01-96	04-12-96
Absolute Duration 10 (days)	163	58
Absolute Span 10 (days)	245	57
Timing of Peak 1(m-d-y)	05-29-96	03-11-96
PTD1 (ind/100sqm)	102	30
Duration 1 25% (days)	90	66
Duration 1 10% (days)	161	137
Fall1 25% (m-d-y)	06-25-96	04-16-96
Fall1 10% (m-d-y)	08-09-96	06-20-96
Timing of Peak 2(m-d-y)	NA	05-29-96
PTD2 (ind/100sqm)	NA	6
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	58
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	04-23-96
CTD (ind/(100sqm*year)	7454	1747
% in 1st half-year (%)	80	92

Table C.13: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 4 in 1996 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 1**	**Neuchâtel Zone 1**
Year	**1997**	**1997**
Timing of Peak (m-d-y)	05-03-97	05-03-97
PTD (ind/100sqm)	170	10
Duration 25% (days)	86	130
Duration 10% (days)	156	182
Span 25% (days)	86	130
Span 10% (days)	156	182
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-26-97	02-14-97
Onset 25% (m-d-y)	03-28-97	03-12-97
Fall 25% (m-d-y)	06-22-97	07-20-97
Fall 10% (m-d-y)	08-01-97	08-15-97
Absolute Onset 10 (m-d-y)	02-18-97	05-03-97
Absolute Fall 10 (m-d-y)	08-10-97	05-05-97
Absolute Duration 10 (days)	174	3
Absolute Span 10 (days)	173	2
Timing of Peak 1(m-d-y)	05-03-97	05-03-97
PTD1 (ind/100sqm)	170	10
Duration 1 25% (days)	86	130
Duration 1 10% (days)	156	182
Fall1 25% (m-d-y)	06-22-97	07-20-97
Fall1 10% (m-d-y)	08-01-97	08-15-97
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	10453	728
% in 1st half-year (%)	72	68

Table C.14: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 1 in 1997 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 2**	**Neuchâtel Zone 2**
Year	**1997**	**1997**
Timing of Peak (m-d-y)	05-03-97	05-03-97
PTD (ind/100sqm)	215	22
Duration 25% (days)	153	173
Duration 10% (days)	209	242
Span 25% (days)	153	173
Span 10% (days)	209	242
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-13-97	02-07-97
Onset 25% (m-d-y)	03-02-97	02-13-97
Fall 25% (m-d-y)	08-02-97	08-05-97
Fall 10% (m-d-y)	09-10-97	10-07-97
Absolute Onset 10 (m-d-y)	02-09-97	02-22-97
Absolute Fall 10 (m-d-y)	10-08-97	07-19-97
Absolute Duration 10 (days)	242	126
Absolute Span 10 (days)	241	147
Timing of Peak 1(m-d-y)	05-03-97	05-03-97
PTD1 (ind/100sqm)	215	22
Duration 1 25% (days)	153	173
Duration 1 10% (days)	209	242
Fall1 25% (m-d-y)	08-02-97	08-05-97
Fall1 10% (m-d-y)	09-10-97	10-07-97
Timing of Peak 2(m-d-y)	NA	09-17-97
PTD2 (ind/100sqm)	NA	4
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	49
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	08-19-97
CTD (ind/(100sqm*year)	21547	2358
% in 1st half-year (%)	65	60

Table C.15: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 2 in 1997 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 3**	**Neuchâtel Zone 3**
Year	**1997**	**1997**
Timing of Peak (m-d-y)	05-03-97	05-03-97
PTD (ind/100sqm)	303	111
Duration 25% (days)	134	149
Duration 10% (days)	221	216
Span 25% (days)	134	149
Span 10% (days)	221	216
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-14-97	02-11-97
Onset 25% (m-d-y)	03-05-97	02-23-97
Fall 25% (m-d-y)	07-17-97	07-22-97
Fall 10% (m-d-y)	09-23-97	09-15-97
Absolute Onset 10 (m-d-y)	02-07-97	02-11-97
Absolute Fall 10 (m-d-y)	10-20-97	09-20-97
Absolute Duration 10 (days)	256	222
Absolute Span 10 (days)	255	221
Timing of Peak 1(m-d-y)	05-03-97	05-03-97
PTD1 (ind/100sqm)	303	111
Duration 1 25% (days)	134	149
Duration 1 10% (days)	221	216
Fall1 25% (m-d-y)	07-17-97	07-22-97
Fall1 10% (m-d-y)	09-23-97	09-15-97
Timing of Peak 2(m-d-y)	09-17-97	NA
PTD2 (ind/100sqm)	35	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	35	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	08-19-97	NA
CTD (ind/(100sqm*year)	23189	9806
% in 1st half-year (%)	66	67

Table C.16: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 3 in 1997 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 4**	**Neuchâtel Zone 4**
Year	**1997**	**1997**
Timing of Peak (m-d-y)	05-03-97	05-03-97
PTD (ind/100sqm)	112	10
Duration 25% (days)	162	174
Duration 10% (days)	234	233
Span 25% (days)	162	227
Span 10% (days)	234	254
Pause 25% (days)	0	53
Pause 10% (days)	0	21
Onset 10% (m-d-y)	02-11-97	02-09-97
Onset 25% (m-d-y)	02-25-97	02-19-97
Fall 25% (m-d-y)	08-06-97	10-04-97
Fall 10% (m-d-y)	10-03-97	10-21-97
Absolute Onset 10 (m-d-y)	02-11-97	05-04-97
Absolute Fall 10 (m-d-y)	10-07-97	05-04-97
Absolute Duration 10 (days)	239	1
Absolute Span 10 (days)	238	00:00:00
Timing of Peak 1(m-d-y)	05-03-97	05-03-97
PTD1 (ind/100sqm)	112	10
Duration 1 25% (days)	162	146
Duration 1 10% (days)	234	177
Fall1 25% (m-d-y)	08-06-97	07-15-97
Fall1 10% (m-d-y)	10-03-97	08-05-97
Timing of Peak 2(m-d-y)	NA	09-17-97
PTD2 (ind/100sqm)	NA	4
Duration 2 25% (days)	NA	28
Duration 2 10% (days)	NA	56
Onset2 25% (m-d-y)	NA	09-06-97
Onset2 10% (m-d-y)	NA	08-26-97
CTD (ind/(100sqm*year)	13329	1122
% in 1st half-year (%)	58	64

Table C.17: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 4 in 1997 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 1**	**Neuchâtel Zone 1**
Year	**1998**	**1998**
Timing of Peak (m-d-y)	06-18-98	04-22-98
PTD (ind/100sqm)	40	18
Duration 25% (days)	217	152
Duration 10% (days)	294	228
Span 25% (days)	235	152
Span 10% (days)	248	228
Pause 25% (days)	18	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-09-98	02-09-98
Onset 25% (m-d-y)	02-22-98	02-21-98
Fall 25% (m-d-y)	10-15-98	07-23-98
Fall 10% (m-d-y)	10-15-98	09-25-98
Absolute Onset 10 (m-d-y)	02-23-98	03-18-98
Absolute Fall 10 (m-d-y)	11-05-98	05-18-98
Absolute Duration 10 (days)	215	62
Absolute Span 10 (days)	255	61
Timing of Peak 1(m-d-y)	06-18-98	04-22-98
PTD1 (ind/100sqm)	40	18
Duration 1 25% (days)	158	152
Duration 1 10% (days)	191	248
Fall1 25% (m-d-y)	07-30-98	07-23-98
Fall1 10% (m-d-y)	08-19-98	10-15-98
Timing of Peak 2(m-d-y)	09-24-98	NA
PTD2 (ind/100sqm)	15	NA
Duration 2 25% (days)	37	NA
Duration 2 10% (days)	55	NA
Onset2 25% (m-d-y)	09-08-98	NA
Onset2 10% (m-d-y)	08-21-98	NA
CTD (ind/(100sqm*year)	5947	1813
% in 1st half-year (%)	61	74

Table C.18: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 1 in 1998 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 2**	**Neuchâtel Zone 2**
Year	**1998**	**1998**
Timing of Peak (m-d-y)	05-25-98	06-18-98
PTD (ind/100sqm)	51	21
Duration 25% (days)	263	195
Duration 10% (days)	303	279
Span 25% (days)	228	195
Span 10% (days)	245	246
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-12-98	02-11-98
Onset 25% (m-d-y)	03-01-98	02-27-98
Fall 25% (m-d-y)	10-15-98	09-10-98
Fall 10% (m-d-y)	10-15-98	10-15-98
Absolute Onset 10 (m-d-y)	02-23-98	03-23-98
Absolute Fall 10 (m-d-y)	12-05-98	08-15-98
Absolute Duration 10 (days)	282	146
Absolute Span 10 (days)	285	145
Timing of Peak 1(m-d-y)	05-25-98	06-18-98
PTD1 (ind/100sqm)	51	21
Duration 1 25% (days)	165	195
Duration 1 10% (days)	NA	246
Fall1 25% (m-d-y)	08-13-98	09-10-98
Fall1 10% (m-d-y)	NA	10-15-98
Timing of Peak 2(m-d-y)	10-15-98	10-15-98
PTD2 (ind/100sqm)	47	4
Duration 2 25% (days)	51	NA
Duration 2 10% (days)	49	16
Onset2 25% (m-d-y)	08-25-98	NA
Onset2 10% (m-d-y)	08-27-98	09-29-98
CTD (ind/(100sqm*year)	8526	3106
% in 1st half-year (%)	46	55

Table C.19: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 2 in 1998 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 3**	**Neuchâtel Zone 3**
Year	**1998**	**1998**
Timing of Peak (m-d-y)	06-18-98	04-22-98
PTD (ind/100sqm)	85	43
Duration 25% (days)	236	156
Duration 10% (days)	295	229
Span 25% (days)	235	156
Span 10% (days)	248	229
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-09-98	02-09-98
Onset 25% (m-d-y)	02-22-98	02-21-98
Fall 25% (m-d-y)	10-15-98	07-27-98
Fall 10% (m-d-y)	10-15-98	09-26-98
Absolute Onset 10 (m-d-y)	02-12-98	02-20-98
Absolute Fall 10 (m-d-y)	12-05-98	07-28-98
Absolute Duration 10 (days)	288	159
Absolute Span 10 (days)	296	158
Timing of Peak 1(m-d-y)	06-18-98	04-22-98
PTD1 (ind/100sqm)	85	43
Duration 1 25% (days)	160	156
Duration 1 10% (days)	189	248
Fall1 25% (m-d-y)	08-01-98	07-27-98
Fall1 10% (m-d-y)	08-17-98	10-15-98
Timing of Peak 2(m-d-y)	09-24-98	NA
PTD2 (ind/100sqm)	45	NA
Duration 2 25% (days)	42	NA
Duration 2 10% (days)	53	NA
Onset2 25% (m-d-y)	09-03-98	NA
Onset2 10% (m-d-y)	08-23-98	NA
CTD (ind/(100sqm*year)	12791	4642
% in 1st half-year (%)	55	74

Table C.20: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 3 in 1998 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 4**	**Neuchâtel Zone 4**
Year	**1998**	**1998**
Timing of Peak (m-d-y)	06-18-98	06-18-98
PTD (ind/100sqm)	76	60
Duration 25% (days)	208	131
Duration 10% (days)	296	220
Span 25% (days)	231	131
Span 10% (days)	246	220
Pause 25% (days)	23	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-11-98	02-19-98
Onset 25% (m-d-y)	02-26-98	03-19-98
Fall 25% (m-d-y)	10-15-98	07-28-98
Fall 10% (m-d-y)	10-15-98	09-27-98
Absolute Onset 10 (m-d-y)	02-15-98	03-05-98
Absolute Fall 10 (m-d-y)	11-29-98	09-04-98
Absolute Duration 10 (days)	282	184
Absolute Span 10 (days)	287	183
Timing of Peak 1(m-d-y)	06-18-98	06-18-98
PTD1 (ind/100sqm)	76	60
Duration 1 25% (days)	154	131
Duration 1 10% (days)	NA	238
Fall1 25% (m-d-y)	07-30-98	07-28-98
Fall1 10% (m-d-y)	NA	10-15-98
Timing of Peak 2(m-d-y)	10-15-98	NA
PTD2 (ind/100sqm)	33	NA
Duration 2 25% (days)	26	NA
Duration 2 10% (days)	52	NA
Onset2 25% (m-d-y)	09-19-98	NA
Onset2 10% (m-d-y)	08-24-98	NA
CTD (ind/(100sqm*year)	9994	5706
% in 1st half-year (%)	56	59

Table C.21: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 4 in 1998 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 1**	**Neuchâtel Zone 1**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	04-26-99	05-25-99
PTD (ind/100sqm)	66	6
Duration 25% (days)	137	134
Duration 10% (days)	178	246
Span 25% (days)	137	134
Span 10% (days)	178	246
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-10-99	02-15-99
Onset 25% (m-d-y)	02-25-99	03-08-99
Fall 25% (m-d-y)	07-12-99	07-20-99
Fall 10% (m-d-y)	08-07-99	10-19-99
Absolute Onset 10 (m-d-y)	02-16-99	NA
Absolute Fall 10 (m-d-y)	07-26-99	NA
Absolute Duration 10 (days)	161	0
Absolute Span 10 (days)	160	0
Timing of Peak 1(m-d-y)	04-26-99	05-25-99
PTD1 (ind/100sqm)	66	6
Duration 1 25% (days)	137	134
Duration 1 10% (days)	178	246
Fall1 25% (m-d-y)	07-12-99	07-20-99
Fall1 10% (m-d-y)	08-07-99	10-19-99
Timing of Peak 2(m-d-y)	NA	08-27-99
PTD2 (ind/100sqm)	NA	1
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	82
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	07-29-99
CTD (ind/(100sqm*year)	6359	638
% in 1st half-year (%)	88	77

Table C.22: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 1 in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 2**	**Neuchâtel Zone 2**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	04-26-99	04-26-99
PTD (ind/100sqm)	146	8
Duration 25% (days)	129	146
Duration 10% (days)	195	226
Span 25% (days)	129	146
Span 10% (days)	241	272
Pause 25% (days)	0	0
Pause 10% (days)	46	46
Onset 10% (m-d-y)	02-11-99	02-09-99
Onset 25% (m-d-y)	02-26-99	02-22-99
Fall 25% (m-d-y)	07-05-99	07-18-99
Fall 10% (m-d-y)	10-10-99	11-08-99
Absolute Onset 10 (m-d-y)	02-09-99	NA
Absolute Fall 10 (m-d-y)	10-23-99	NA
Absolute Duration 10 (days)	229	0
Absolute Span 10 (days)	256	0
Timing of Peak 1(m-d-y)	04-26-99	04-26-99
PTD1 (ind/100sqm)	146	8
Duration 1 25% (days)	129	146
Duration 1 10% (days)	177	167
Fall1 25% (m-d-y)	07-05-99	07-18-99
Fall1 10% (m-d-y)	08-07-99	07-26-99
Timing of Peak 2(m-d-y)	NA	09-29-99
PTD2 (ind/100sqm)	NA	2
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	59
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	09-10-99
CTD (ind/(100sqm*year)	12471	864
% in 1st half-year (%)	83	80

Table C.23: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 2 in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 3**	**Neuchâtel Zone 3**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	04-26-99	05-25-99
PTD (ind/100sqm)	109	16
Duration 25% (days)	156	145
Duration 10% (days)	178	211
Span 25% (days)	156	145
Span 10% (days)	178	276
Pause 25% (days)	0	0
Pause 10% (days)	0	65
Onset 10% (m-d-y)	02-06-99	02-12-99
Onset 25% (m-d-y)	02-15-99	03-01-99
Fall 25% (m-d-y)	07-21-99	07-24-99
Fall 10% (m-d-y)	08-03-99	11-15-99
Absolute Onset 10 (m-d-y)	02-07-99	04-08-99
Absolute Fall 10 (m-d-y)	08-06-99	07-07-99
Absolute Duration 10 (days)	181	91
Absolute Span 10 (days)	180	90
Timing of Peak 1(m-d-y)	04-26-99	05-25-99
PTD1 (ind/100sqm)	109	16
Duration 1 25% (days)	156	145
Duration 1 10% (days)	178	180
Fall1 25% (m-d-y)	07-21-99	07-24-99
Fall1 10% (m-d-y)	08-03-99	08-11-99
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	14171	1829
% in 1st half-year (%)	86	80

Table C.24: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 3 in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 4**	**Neuchâtel Zone 4**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	03-25-99	03-25-99
PTD (ind/100sqm)	102	10
Duration 25% (days)	146	152
Duration 10% (days)	167	171
Span 25% (days)	146	152
Span 10% (days)	167	171
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-06-99	02-06-99
Onset 25% (m-d-y)	02-14-99	02-14-99
Fall 25% (m-d-y)	07-10-99	07-16-99
Fall 10% (m-d-y)	07-23-99	07-27-99
Absolute Onset 10 (m-d-y)	02-07-99	NA
Absolute Fall 10 (m-d-y)	07-23-99	NA
Absolute Duration 10 (days)	167	0
Absolute Span 10 (days)	166	0
Timing of Peak 1(m-d-y)	03-25-99	03-25-99
PTD1 (ind/100sqm)	102	10
Duration 1 25% (days)	146	152
Duration 1 10% (days)	167	171
Fall1 25% (m-d-y)	07-10-99	07-16-99
Fall1 10% (m-d-y)	07-23-99	07-27-99
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	10572	951
% in 1st half-year (%)	92	91

Table C.25: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 4 in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 1**	**Neuchâtel Zone 1**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-27-00	04-27-00
PTD (ind/100sqm)	79	23
Duration 25% (days)	111	107
Duration 10% (days)	255	160
Span 25% (days)	111	107
Span 10% (days)	255	160
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-28-00	03-01-00
Onset 25% (m-d-y)	03-06-00	03-11-00
Fall 25% (m-d-y)	06-25-00	06-26-00
Fall 10% (m-d-y)	11-09-00	08-08-00
Absolute Onset 10 (m-d-y)	03-01-00	03-24-00
Absolute Fall 10 (m-d-y)	11-03-00	06-01-00
Absolute Duration 10 (days)	200	70
Absolute Span 10 (days)	247	69
Timing of Peak 1(m-d-y)	04-27-00	04-27-00
PTD1 (ind/100sqm)	79	23
Duration 1 25% (days)	111	107
Duration 1 10% (days)	NA	160
Fall1 25% (m-d-y)	06-25-00	06-26-00
Fall1 10% (m-d-y)	NA	08-08-00
Timing of Peak 2(m-d-y)	09-26-00	NA
PTD2 (ind/100sqm)	19	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	128	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	07-04-00	NA
CTD (ind/(100sqm*year)	7793	1784
% in 1st half-year (%)	78	83

Table C.26: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 1 in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 2**	**Neuchâtel Zone 2**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-27-00	06-03-00
PTD (ind/100sqm)	87	31
Duration 25% (days)	211	196
Duration 10% (days)	268	225
Span 25% (days)	244	196
Span 10% (days)	268	225
Pause 25% (days)	33	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-27-00	02-28-00
Onset 25% (m-d-y)	03-03-00	03-06-00
Fall 25% (m-d-y)	11-02-00	09-18-00
Fall 10% (m-d-y)	11-21-00	10-10-00
Absolute Onset 10 (m-d-y)	02-28-00	03-11-00
Absolute Fall 10 (m-d-y)	11-19-00	09-11-00
Absolute Duration 10 (days)	266	185
Absolute Span 10 (days)	265	184
Timing of Peak 1(m-d-y)	04-27-00	06-03-00
PTD1 (ind/100sqm)	87	31
Duration 1 25% (days)	142	196
Duration 1 10% (days)	NA	225
Fall1 25% (m-d-y)	07-23-00	09-18-00
Fall1 10% (m-d-y)	NA	10-10-00
Timing of Peak 2(m-d-y)	09-26-00	NA
PTD2 (ind/100sqm)	54	NA
Duration 2 25% (days)	69	NA
Duration 2 10% (days)	111	NA
Onset2 25% (m-d-y)	08-25-00	NA
Onset2 10% (m-d-y)	08-02-00	NA
CTD (ind/(100sqm*year)	11892	4031
% in 1st half-year (%)	63	68

Table C.27: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 2 in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 3**	**Neuchâtel Zone 3**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-27-00	04-27-00
PTD (ind/100sqm)	145	42
Duration 25% (days)	148	120
Duration 10% (days)	263	209
Span 25% (days)	230	120
Span 10% (days)	263	210
Pause 25% (days)	82	0
Pause 10% (days)	0	1
Onset 10% (m-d-y)	02-27-00	02-29-00
Onset 25% (m-d-y)	03-05-00	03-10-00
Fall 25% (m-d-y)	10-21-00	07-08-00
Fall 10% (m-d-y)	11-16-00	09-26-00
Absolute Onset 10 (m-d-y)	02-26-00	03-11-00
Absolute Fall 10 (m-d-y)	11-21-00	07-13-00
Absolute Duration 10 (days)	270	125
Absolute Span 10 (days)	269	124
Timing of Peak 1(m-d-y)	04-27-00	04-27-00
PTD1 (ind/100sqm)	145	42
Duration 1 25% (days)	111	120
Duration 1 10% (days)	NA	210
Fall1 25% (m-d-y)	06-24-00	07-08-00
Fall1 10% (m-d-y)	NA	09-26-00
Timing of Peak 2(m-d-y)	09-26-00	NA
PTD2 (ind/100sqm)	52	NA
Duration 2 25% (days)	37	NA
Duration 2 10% (days)	136	NA
Onset2 25% (m-d-y)	09-14-00	NA
Onset2 10% (m-d-y)	07-03-00	NA
CTD (ind/(100sqm*year)	15434	3964
% in 1st half-year (%)	72	78

Table C.28: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 3 in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 4**	**Neuchâtel Zone 4**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-27-00	04-27-00
PTD (ind/100sqm)	109	46
Duration 25% (days)	164	138
Duration 10% (days)	266	233
Span 25% (days)	235	138
Span 10% (days)	266	233
Pause 25% (days)	71	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-27-00	02-26-00
Onset 25% (m-d-y)	03-05-00	03-03-00
Fall 25% (m-d-y)	10-26-00	07-19-00
Fall 10% (m-d-y)	11-19-00	10-16-00
Absolute Onset 10 (m-d-y)	02-28-00	03-02-00
Absolute Fall 10 (m-d-y)	11-20-00	09-27-00
Absolute Duration 10 (days)	267	153
Absolute Span 10 (days)	266	209
Timing of Peak 1(m-d-y)	04-27-00	04-27-00
PTD1 (ind/100sqm)	109	46
Duration 1 25% (days)	117	138
Duration 1 10% (days)	NA	233
Fall1 25% (m-d-y)	06-30-00	07-19-00
Fall1 10% (m-d-y)	NA	10-16-00
Timing of Peak 2(m-d-y)	09-26-00	NA
PTD2 (ind/100sqm)	54	NA
Duration 2 25% (days)	47	NA
Duration 2 10% (days)	97	NA
Onset2 25% (m-d-y)	09-09-00	NA
Onset2 10% (m-d-y)	08-14-00	NA
CTD (ind/(100sqm*year)	12757	5445
% in 1st half-year (%)	69	79

Table C.29: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 4 in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 1**	**Neuchâtel Zone 1**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	04-27-01	03-27-01
PTD (ind/100sqm)	114	8
Duration 25% (days)	109	209
Duration 10% (days)	224	271
Span 25% (days)	109	236
Span 10% (days)	248	271
Pause 25% (days)	0	27
Pause 10% (days)	24	0
Onset 10% (m-d-y)	02-28-01	02-22-01
Onset 25% (m-d-y)	03-14-01	02-28-01
Fall 25% (m-d-y)	07-01-01	10-22-01
Fall 10% (m-d-y)	11-03-01	11-20-01
Absolute Onset 10 (m-d-y)	02-28-01	NA
Absolute Fall 10 (m-d-y)	11-08-01	NA
Absolute Duration 10 (days)	235	0
Absolute Span 10 (days)	253	0
Timing of Peak 1(m-d-y)	04-27-01	03-27-01
PTD1 (ind/100sqm)	114	8
Duration 1 25% (days)	109	165
Duration 1 10% (days)	170	292
Fall1 25% (m-d-y)	07-01-01	08-12-01
Fall1 10% (m-d-y)	08-17-01	12-11-01
Timing of Peak 2(m-d-y)	NA	09-27-01
PTD2 (ind/100sqm)	NA	3
Duration 2 25% (days)	NA	44
Duration 2 10% (days)	NA	79
Onset2 25% (m-d-y)	NA	09-08-01
Onset2 10% (m-d-y)	NA	09-02-01
CTD (ind/(100sqm*year)	11037	1105
% in 1st half-year (%)	60	57

Table C.30: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 1 in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 2**	**Neuchâtel Zone 2**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	04-27-01	05-28-01
PTD (ind/100sqm)	141	12
Duration 25% (days)	189	195
Duration 10% (days)	265	274
Span 25% (days)	231	195
Span 10% (days)	274	274
Pause 25% (days)	42	0
Pause 10% (days)	9	0
Onset 10% (m-d-y)	02-24-01	02-14-01
Onset 25% (m-d-y)	03-13-01	02-27-01
Fall 25% (m-d-y)	10-30-01	09-10-01
Fall 10% (m-d-y)	11-25-01	11-15-01
Absolute Onset 10 (m-d-y)	02-20-01	05-09-01
Absolute Fall 10 (m-d-y)	11-30-01	07-14-01
Absolute Duration 10 (days)	282	67
Absolute Span 10 (days)	283	66
Timing of Peak 1(m-d-y)	04-27-01	05-28-01
PTD1 (ind/100sqm)	141	12
Duration 1 25% (days)	143	195
Duration 1 10% (days)	179	300
Fall1 25% (m-d-y)	08-03-01	09-10-01
Fall1 10% (m-d-y)	08-22-01	12-11-01
Timing of Peak 2(m-d-y)	09-27-01	NA
PTD2 (ind/100sqm)	56	NA
Duration 2 25% (days)	46	NA
Duration 2 10% (days)	86	NA
Onset2 25% (m-d-y)	09-14-01	NA
Onset2 10% (m-d-y)	08-31-01	NA
CTD (ind/(100sqm*year)	16020	1820
% in 1st half-year (%)	50	43

Table C.31: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 2 in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 3**	**Neuchâtel Zone 3**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	04-27-01	04-27-01
PTD (ind/100sqm)	187	33
Duration 25% (days)	117	149
Duration 10% (days)	254	234
Span 25% (days)	205	166
Span 10% (days)	269	254
Pause 25% (days)	88	17
Pause 10% (days)	15	20
Onset 10% (m-d-y)	02-22-01	02-09-01
Onset 25% (m-d-y)	03-12-01	02-22-01
Fall 25% (m-d-y)	10-03-01	08-07-01
Fall 10% (m-d-y)	11-18-01	10-21-01
Absolute Onset 10 (m-d-y)	02-12-01	02-28-01
Absolute Fall 10 (m-d-y)	11-29-01	08-02-01
Absolute Duration 10 (days)	288	126
Absolute Span 10 (days)	290	155
Timing of Peak 1(m-d-y)	04-27-01	04-27-01
PTD1 (ind/100sqm)	187	33
Duration 1 25% (days)	109	126
Duration 1 10% (days)	179	192
Fall1 25% (m-d-y)	06-29-01	06-28-01
Fall1 10% (m-d-y)	08-20-01	08-20-01
Timing of Peak 2(m-d-y)	09-27-01	07-30-01
PTD2 (ind/100sqm)	49	11
Duration 2 25% (days)	65	23
Duration 2 10% (days)	133	106
Onset2 25% (m-d-y)	07-30-01	07-15-01
Onset2 10% (m-d-y)	07-08-01	07-07-01
CTD (ind/(100sqm*year)	18758	3597
% in 1st half-year (%)	57	65

Table C.32: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 3 in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Neuchâtel Zone 4**	**Neuchâtel Zone 4**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	04-27-01	04-27-01
PTD (ind/100sqm)	189	22
Duration 25% (days)	125	157
Duration 10% (days)	231	231
Span 25% (days)	136	157
Span 10% (days)	255	246
Pause 25% (days)	11	0
Pause 10% (days)	24	15
Onset 10% (m-d-y)	02-23-01	02-03-01
Onset 25% (m-d-y)	03-18-01	02-09-01
Fall 25% (m-d-y)	08-01-01	07-16-01
Fall 10% (m-d-y)	11-05-01	10-07-01
Absolute Onset 10 (m-d-y)	02-13-01	02-18-01
Absolute Fall 10 (m-d-y)	11-22-01	06-14-01
Absolute Duration 10 (days)	274	117
Absolute Span 10 (days)	282	116
Timing of Peak 1(m-d-y)	04-27-01	04-27-01
PTD1 (ind/100sqm)	189	22
Duration 1 25% (days)	106	157
Duration 1 10% (days)	176	203
Fall1 25% (m-d-y)	07-02-01	07-16-01
Fall1 10% (m-d-y)	08-18-01	08-25-01
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	16932	2497
% in 1st half-year (%)	59	70

Table C.33: Descriptive statistics of the questing tick timeplots at Neuchâtel Zone 4 in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Aigle**	**Aigle**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-02-99	05-02-99
PTD (ind/100sqm)	17	6
Duration 25% (days)	194	134
Duration 10% (days)	238	189
Span 25% (days)	194	134
Span 10% (days)	238	241
Pause 25% (days)	0	0
Pause 10% (days)	0	52
Onset 10% (m-d-y)	02-10-99	02-10-99
Onset 25% (m-d-y)	02-23-99	02-23-99
Fall 25% (m-d-y)	09-05-99	07-07-99
Fall 10% (m-d-y)	10-06-99	10-09-99
Absolute Onset 10 (m-d-y)	03-28-99	NA
Absolute Fall 10 (m-d-y)	07-11-99	NA
Absolute Duration 10 (days)	106	0
Absolute Span 10 (days)	105	0
Timing of Peak 1(m-d-y)	05-02-99	05-02-99
PTD1 (ind/100sqm)	17	6
Duration 1 25% (days)	194	134
Duration 1 10% (days)	NA	158
Fall1 25% (m-d-y)	09-05-99	07-07-99
Fall1 10% (m-d-y)	NA	07-18-99
Timing of Peak 2(m-d-y)	NA	10-09-99
PTD2 (ind/100sqm)	NA	1
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	3
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	10-06-99
CTD (ind/(100sqm*year)	2193	564
% in 1st half-year (%)	35	48

Table C.34: Descriptive statistics of the questing tick timeplots at Aigle in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Aigle**	**Aigle**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	09-27-00	04-18-00
PTD (ind/100sqm)	7	2
Duration 25% (days)	188	155
Duration 10% (days)	261	199
Span 25% (days)	235	257
Span 10% (days)	277	282
Pause 25% (days)	47	102
Pause 10% (days)	16	83
Onset 10% (m-d-y)	02-22-00	02-06-00
Onset 25% (m-d-y)	03-21-00	02-15-00
Fall 25% (m-d-y)	11-11-00	10-29-00
Fall 10% (m-d-y)	11-25-00	11-14-00
Absolute Onset 10 (m-d-y)	NA	NA
Absolute Fall 10 (m-d-y)	NA	NA
Absolute Duration 10 (days)	0	0
Absolute Span 10 (days)	0	0
Timing of Peak 1(m-d-y)	04-18-00	04-18-00
PTD1 (ind/100sqm)	3	2
Duration 1 25% (days)	103	140
Duration 1 10% (days)	147	158
Fall1 25% (m-d-y)	07-02-00	07-04-00
Fall1 10% (m-d-y)	07-18-00	07-13-00
Timing of Peak 2(m-d-y)	09-27-00	10-19-00
PTD2 (ind/100sqm)	7	1
Duration 2 25% (days)	85	15
Duration 2 10% (days)	107	41
Onset2 25% (m-d-y)	08-18-00	10-14-00
Onset2 10% (m-d-y)	08-10-00	10-04-00
CTD (ind/(100sqm*year)	804	204
% in 1st half-year (%)	34	77

Table C.35: Descriptive statistics of the questing tick timeplots at Aigle in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Aigle**	**Aigle**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-11-01	04-03-01
PTD (ind/100sqm)	21	2
Duration 25% (days)	135	191
Duration 10% (days)	193	242
Span 25% (days)	135	269
Span 10% (days)	193	290
Pause 25% (days)	0	78
Pause 10% (days)	0	48
Onset 10% (m-d-y)	02-21-01	01-20-01
Onset 25% (m-d-y)	03-15-01	01-27-01
Fall 25% (m-d-y)	07-28-01	10-23-01
Fall 10% (m-d-y)	09-02-01	11-06-01
Absolute Onset 10 (m-d-y)	04-10-01	NA
Absolute Fall 10 (m-d-y)	07-01-01	NA
Absolute Duration 10 (days)	83	0
Absolute Span 10 (days)	82	0
Timing of Peak 1(m-d-y)	05-11-01	04-03-01
PTD1 (ind/100sqm)	21	2
Duration 1 25% (days)	135	175
Duration 1 10% (days)	193	197
Fall1 25% (m-d-y)	07-28-01	07-21-01
Fall1 10% (m-d-y)	09-02-01	08-05-01
Timing of Peak 2(m-d-y)	NA	10-15-01
PTD2 (ind/100sqm)	NA	1
Duration 2 25% (days)	NA	16
Duration 2 10% (days)	NA	45
Onset2 25% (m-d-y)	NA	10-07-01
Onset2 10% (m-d-y)	NA	09-22-01
CTD (ind/(100sqm*year)	2025	293
% in 1st half-year (%)	69	79

Table C.36: Descriptive statistics of the questing tick timeplots at Aigle in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bavois**	**Bavois**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-01-99	05-01-99
PTD (ind/100sqm)	85	7
Duration 25% (days)	95	183
Duration 10% (days)	183	253
Span 25% (days)	95	218
Span 10% (days)	200	237
Pause 25% (days)	0	35
Pause 10% (days)	17	0
Onset 10% (m-d-y)	02-08-99	02-13-99
Onset 25% (m-d-y)	02-19-99	03-04-99
Fall 25% (m-d-y)	05-25-99	10-08-99
Fall 10% (m-d-y)	08-27-99	10-08-99
Absolute Onset 10 (m-d-y)	02-10-99	NA
Absolute Fall 10 (m-d-y)	08-20-99	NA
Absolute Duration 10 (days)	168	0
Absolute Span 10 (days)	191	0
Timing of Peak 1(m-d-y)	05-01-99	05-01-99
PTD1 (ind/100sqm)	85	7
Duration 1 25% (days)	95	80
Duration 1 10% (days)	133	200
Fall1 25% (m-d-y)	05-25-99	05-23-99
Fall1 10% (m-d-y)	06-21-99	09-01-99
Timing of Peak 2(m-d-y)	NA	07-23-99
PTD2 (ind/100sqm)	NA	5
Duration 2 25% (days)	NA	109
Duration 2 10% (days)	NA	134
Onset2 25% (m-d-y)	NA	06-21-99
Onset2 10% (m-d-y)	NA	05-27-99
CTD (ind/(100sqm*year)	6861	731
% in 1st half-year (%)	80	49

Table C.37: Descriptive statistics of the questing tick timeplots at Bavois in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bavois**	**Bavois**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-17-00	04-17-00
PTD (ind/100sqm)	11	11
Duration 25% (days)	189	158
Duration 10% (days)	260	243
Span 25% (days)	241	239
Span 10% (days)	256	255
Pause 25% (days)	52	81
Pause 10% (days)	0	12
Onset 10% (m-d-y)	02-05-00	02-06-00
Onset 25% (m-d-y)	02-12-00	02-14-00
Fall 25% (m-d-y)	10-10-00	10-10-00
Fall 10% (m-d-y)	10-18-00	10-18-00
Absolute Onset 10 (m-d-y)	03-22-00	04-06-00
Absolute Fall 10 (m-d-y)	04-25-00	04-20-00
Absolute Duration 10 (days)	35	15
Absolute Span 10 (days)	34	14
Timing of Peak 1(m-d-y)	04-17-00	04-17-00
PTD1 (ind/100sqm)	11	11
Duration 1 25% (days)	113	82
Duration 1 10% (days)	142	94
Fall1 25% (m-d-y)	06-04-00	05-06-00
Fall1 10% (m-d-y)	06-26-00	05-10-00
Timing of Peak 2(m-d-y)	08-08-00	06-07-00
PTD2 (ind/100sqm)	8	8
Duration 2 25% (days)	76	146
Duration 2 10% (days)	128	160
Onset2 25% (m-d-y)	07-26-00	05-17-00
Onset2 10% (m-d-y)	06-12-00	05-11-00
CTD (ind/(100sqm*year)	1485	1172
% in 1st half-year (%)	63	67

Table C.38: Descriptive statistics of the questing tick timeplots at Bavois in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bavois**	**Bavois**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-11-01	05-11-01
PTD (ind/100sqm)	9	5
Duration 25% (days)	103	158
Duration 10% (days)	165	187
Span 25% (days)	103	158
Span 10% (days)	247	187
Pause 25% (days)	0	0
Pause 10% (days)	82	0
Onset 10% (m-d-y)	02-14-01	01-25-01
Onset 25% (m-d-y)	04-12-01	02-06-01
Fall 25% (m-d-y)	07-24-01	07-14-01
Fall 10% (m-d-y)	10-19-01	07-31-01
Absolute Onset 10 (m-d-y)	NA	NA
Absolute Fall 10 (m-d-y)	NA	NA
Absolute Duration 10 (days)	0	0
Absolute Span 10 (days)	0	0
Timing of Peak 1(m-d-y)	05-11-01	05-11-01
PTD1 (ind/100sqm)	9	5
Duration 1 25% (days)	103	158
Duration 1 10% (days)	173	187
Fall1 25% (m-d-y)	07-24-01	07-14-01
Fall1 10% (m-d-y)	08-06-01	07-31-01
Timing of Peak 2(m-d-y)	09-12-01	NA
PTD2 (ind/100sqm)	1	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	38	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	09-11-01	NA
CTD (ind/(100sqm*year)	773	522
% in 1st half-year (%)	65	82

Table C.39: Descriptive statistics of the questing tick timeplots at Bavois in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bière**	**Bière**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-26-99	05-01-99
PTD (ind/100sqm)	13	1
Duration 25% (days)	154	108
Duration 10% (days)	233	124
Span 25% (days)	154	199
Span 10% (days)	205	206
Pause 25% (days)	0	91
Pause 10% (days)	0	82
Onset 10% (m-d-y)	03-18-99	03-17-99
Onset 25% (m-d-y)	03-29-99	03-24-99
Fall 25% (m-d-y)	08-30-99	10-09-99
Fall 10% (m-d-y)	10-09-99	10-09-99
Absolute Onset 10 (m-d-y)	05-07-99	NA
Absolute Fall 10 (m-d-y)	06-16-99	NA
Absolute Duration 10 (days)	41	0
Absolute Span 10 (days)	40	0
Timing of Peak 1(m-d-y)	05-26-99	05-01-99
PTD1 (ind/100sqm)	13	1
Duration 1 25% (days)	154	57
Duration 1 10% (days)	NA	68
Fall1 25% (m-d-y)	08-30-99	05-20-99
Fall1 10% (m-d-y)	NA	05-24-99
Timing of Peak 2(m-d-y)	NA	10-09-99
PTD2 (ind/100sqm)	NA	1
Duration 2 25% (days)	NA	24
Duration 2 10% (days)	NA	28
Onset2 25% (m-d-y)	NA	09-15-99
Onset2 10% (m-d-y)	NA	09-11-99
CTD (ind/(100sqm*year)	1378	54
% in 1st half-year (%)	37	47

Table C.40: Descriptive statistics of the questing tick timeplots at Bière in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bière**	**Bière**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	06-08-00	09-26-00
PTD (ind/100sqm)	4	2
Duration 25% (days)	212	184
Duration 10% (days)	264	230
Span 25% (days)	212	231
Span 10% (days)	264	249
Pause 25% (days)	0	47
Pause 10% (days)	0	19
Onset 10% (m-d-y)	02-24-00	03-16-00
Onset 25% (m-d-y)	03-30-00	03-25-00
Fall 25% (m-d-y)	10-28-00	11-11-00
Fall 10% (m-d-y)	11-14-00	11-20-00
Absolute Onset 10 (m-d-y)	NA	NA
Absolute Fall 10 (m-d-y)	NA	NA
Absolute Duration 10 (days)	0	0
Absolute Span 10 (days)	0	0
Timing of Peak 1(m-d-y)	06-08-00	04-18-00
PTD1 (ind/100sqm)	4	1
Duration 1 25% (days)	212	86
Duration 1 10% (days)	NA	112
Fall1 25% (m-d-y)	10-28-00	06-19-00
Fall1 10% (m-d-y)	NA	07-06-00
Timing of Peak 2(m-d-y)	NA	09-26-00
PTD2 (ind/100sqm)	NA	2
Duration 2 25% (days)	NA	99
Duration 2 10% (days)	NA	118
Onset2 25% (m-d-y)	NA	08-04-00
Onset2 10% (m-d-y)	NA	07-25-00
CTD (ind/(100sqm*year)	537	214
% in 1st half-year (%)	36	32

Table C.41: Descriptive statistics of the questing tick timeplots at Bière in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bière**	**Bière**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-10-01	02-15-01
PTD (ind/100sqm)	8	2
Duration 25% (days)	150	250
Duration 10% (days)	255	282
Span 25% (days)	204	286
Span 10% (days)	260	297
Pause 25% (days)	54	36
Pause 10% (days)	5	15
Onset 10% (m-d-y)	02-20-01	01-19-01
Onset 25% (m-d-y)	04-03-01	01-23-01
Fall 25% (m-d-y)	10-24-01	11-05-01
Fall 10% (m-d-y)	11-07-01	11-12-01
Absolute Onset 10 (m-d-y)	NA	NA
Absolute Fall 10 (m-d-y)	NA	NA
Absolute Duration 10 (days)	0	0
Absolute Span 10 (days)	0	0
Timing of Peak 1(m-d-y)	05-10-01	02-15-01
PTD1 (ind/100sqm)	8	2
Duration 1 25% (days)	71	150
Duration 1 10% (days)	144	169
Fall1 25% (m-d-y)	06-13-01	06-22-01
Fall1 10% (m-d-y)	07-14-01	07-07-01
Timing of Peak 2(m-d-y)	09-12-01	08-14-01
PTD2 (ind/100sqm)	5	1
Duration 2 25% (days)	80	100
Duration 2 10% (days)	111	113
Onset2 25% (m-d-y)	08-05-01	07-28-01
Onset2 10% (m-d-y)	07-19-01	07-22-01
CTD (ind/(100sqm*year)	813	358
% in 1st half-year (%)	53	68

Table C.42: Descriptive statistics of the questing tick timeplots at Bière in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bois-de-Portes**	**Bois-de-Portes**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-02-99	10-08-99
PTD (ind/100sqm)	17	1
Duration 25% (days)	147	191
Duration 10% (days)	182	243
Span 25% (days)	147	230
Span 10% (days)	209	242
Pause 25% (days)	0	39
Pause 10% (days)	27	0
Onset 10% (m-d-y)	03-13-99	02-08-99
Onset 25% (m-d-y)	03-21-99	02-20-99
Fall 25% (m-d-y)	08-15-99	10-08-99
Fall 10% (m-d-y)	10-08-99	10-08-99
Absolute Onset 10 (m-d-y)	04-10-99	NA
Absolute Fall 10 (m-d-y)	05-20-99	NA
Absolute Duration 10 (days)	41	0
Absolute Span 10 (days)	40	0
Timing of Peak 1(m-d-y)	05-02-99	03-11-99
PTD1 (ind/100sqm)	17	1
Duration 1 25% (days)	147	113
Duration 1 10% (days)	172	136
Fall1 25% (m-d-y)	08-15-99	06-13-99
Fall1 10% (m-d-y)	09-01-99	06-24-99
Timing of Peak 2(m-d-y)	10-08-99	10-08-99
PTD2 (ind/100sqm)	2	1
Duration 2 25% (days)	NA	88
Duration 2 10% (days)	6	95
Onset2 25% (m-d-y)	NA	07-12-99
Onset2 10% (m-d-y)	10-02-99	07-05-99
CTD (ind/(100sqm*year)	1550	137
% in 1st half-year (%)	52	47

Table C.43: Descriptive statistics of the questing tick timeplots at Bois-de-Portes in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bois-de-Portes**	**Bois-de-Portes**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	07-18-00	03-10-00
PTD (ind/100sqm)	3	1
Duration 25% (days)	216	165
Duration 10% (days)	276	186
Span 25% (days)	272	246
Span 10% (days)	289	255
Pause 25% (days)	56	81
Pause 10% (days)	13	69
Onset 10% (m-d-y)	02-10-00	02-04-00
Onset 25% (m-d-y)	02-25-00	02-10-00
Fall 25% (m-d-y)	11-23-00	10-13-00
Fall 10% (m-d-y)	11-25-00	10-16-00
Absolute Onset 10 (m-d-y)	NA	NA
Absolute Fall 10 (m-d-y)	NA	NA
Absolute Duration 10 (days)	0	0
Absolute Span 10 (days)	0	0
Timing of Peak 1(m-d-y)	NA	03-10-00
PTD1 (ind/100sqm)	NA	1
Duration 1 25% (days)	NA	111
Duration 1 10% (days)	NA	122
Fall1 25% (m-d-y)	NA	05-31-00
Fall1 10% (m-d-y)	NA	06-05-00
Timing of Peak 2(m-d-y)	NA	09-26-00
PTD2 (ind/100sqm)	NA	1
Duration 2 25% (days)	NA	53
Duration 2 10% (days)	NA	64
Onset2 25% (m-d-y)	NA	08-21-00
Onset2 10% (m-d-y)	NA	08-13-00
CTD (ind/(100sqm*year)	455	87
% in 1st half-year (%)	39	72

Table C.44: Descriptive statistics of the questing tick timeplots at Bois-de-Portes in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Bois-de-Portes**	**Bois-de-Portes**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	06-13-01	05-11-01
PTD (ind/100sqm)	4	2
Duration 25% (days)	112	103
Duration 10% (days)	193	122
Span 25% (days)	165	104
Span 10% (days)	246	122
Pause 25% (days)	53	1
Pause 10% (days)	53	0
Onset 10% (m-d-y)	03-15-01	04-06-01
Onset 25% (m-d-y)	04-09-01	04-12-01
Fall 25% (m-d-y)	09-21-01	07-25-01
Fall 10% (m-d-y)	11-16-01	08-06-01
Absolute Onset 10 (m-d-y)	NA	NA
Absolute Fall 10 (m-d-y)	NA	NA
Absolute Duration 10 (days)	0	0
Absolute Span 10 (days)	0	0
Timing of Peak 1(m-d-y)	06-13-01	05-11-01
PTD1 (ind/100sqm)	4	2
Duration 1 25% (days)	96	104
Duration 1 10% (days)	136	122
Fall1 25% (m-d-y)	07-14-01	07-25-01
Fall1 10% (m-d-y)	07-29-01	08-06-01
Timing of Peak 2(m-d-y)	09-12-01	NA
PTD2 (ind/100sqm)	1	NA
Duration 2 25% (days)	16	NA
Duration 2 10% (days)	119	NA
Onset2 25% (m-d-y)	09-05-01	NA
Onset2 10% (m-d-y)	07-20-01	NA
CTD (ind/(100sqm*year)	331	115
% in 1st half-year (%)	57	72

Table C.45: Descriptive statistics of the questing tick timeplots at Bois-de-Portes in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Brochus-Creuson**	**Brochus-Creuson**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-02-99	09-07-99
PTD (ind/100sqm)	20	3
Duration 25% (days)	125	233
Duration 10% (days)	249	270
Span 25% (days)	125	230
Span 10% (days)	223	242
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-27-99	02-08-99
Onset 25% (m-d-y)	03-18-99	02-20-99
Fall 25% (m-d-y)	07-21-99	10-08-99
Fall 10% (m-d-y)	10-08-99	10-08-99
Absolute Onset 10 (m-d-y)	04-02-99	NA
Absolute Fall 10 (m-d-y)	07-12-99	NA
Absolute Duration 10 (days)	102	0
Absolute Span 10 (days)	101	0
Timing of Peak 1(m-d-y)	05-02-99	06-30-99
PTD1 (ind/100sqm)	20	2
Duration 1 25% (days)	125	230
Duration 1 10% (days)	NA	NA
Fall1 25% (m-d-y)	07-21-99	10-08-99
Fall1 10% (m-d-y)	NA	NA
Timing of Peak 2(m-d-y)	09-07-99	NA
PTD2 (ind/100sqm)	4	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	73	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	07-27-99	NA
CTD (ind/(100sqm*year)	2174	418
% in 1st half-year (%)	50	25

Table C.46: Descriptive statistics of the questing tick timeplots at Brochus-Creuson in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Brochus-Creuson**	**Brochus-Creuson**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	05-10-00	04-17-00
PTD (ind/100sqm)	16	5
Duration 25% (days)	215	201
Duration 10% (days)	263	231
Span 25% (days)	215	201
Span 10% (days)	263	231
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-13-00	02-08-00
Onset 25% (m-d-y)	03-02-00	02-20-00
Fall 25% (m-d-y)	10-03-00	09-08-00
Fall 10% (m-d-y)	11-02-00	09-26-00
Absolute Onset 10 (m-d-y)	04-29-00	NA
Absolute Fall 10 (m-d-y)	08-22-00	NA
Absolute Duration 10 (days)	116	0
Absolute Span 10 (days)	115	0
Timing of Peak 1(m-d-y)	05-10-00	04-17-00
PTD1 (ind/100sqm)	16	5
Duration 1 25% (days)	215	201
Duration 1 10% (days)	NA	231
Fall1 25% (m-d-y)	10-03-00	09-08-00
Fall1 10% (m-d-y)	NA	09-26-00
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	2150	642
% in 1st half-year (%)	43	57

Table C.47: Descriptive statistics of the questing tick timeplots at Brochus-Creuson in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Brochus-Creuson**	**Brochus-Creuson**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-11-01	05-11-01
PTD (ind/100sqm)	20	10
Duration 25% (days)	202	182
Duration 10% (days)	247	243
Span 25% (days)	202	182
Span 10% (days)	247	243
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	01-28-01	01-25-01
Onset 25% (m-d-y)	02-13-01	02-06-01
Fall 25% (m-d-y)	09-03-01	08-07-01
Fall 10% (m-d-y)	10-02-01	09-25-01
Absolute Onset 10 (m-d-y)	03-14-01	NA
Absolute Fall 10 (m-d-y)	07-11-01	NA
Absolute Duration 10 (days)	120	0
Absolute Span 10 (days)	119	0
Timing of Peak 1(m-d-y)	05-11-01	05-11-01
PTD1 (ind/100sqm)	20	10
Duration 1 25% (days)	202	182
Duration 1 10% (days)	247	243
Fall1 25% (m-d-y)	09-03-01	08-07-01
Fall1 10% (m-d-y)	10-02-01	09-25-01
Timing of Peak 2(m-d-y)	NA	09-12-01
PTD2 (ind/100sqm)	NA	2
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	41
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	08-15-01
CTD (ind/(100sqm*year)	2722	1141
% in 1st half-year (%)	66	68

Table C.48: Descriptive statistics of the questing tick timeplots at Brochus-Creuson in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Eclépens**	**Eclépens**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-26-99	05-26-99
PTD (ind/100sqm)	160	18
Duration 25% (days)	103	202
Duration 10% (days)	160	263
Span 25% (days)	103	202
Span 10% (days)	169	235
Pause 25% (days)	0	0
Pause 10% (days)	9	0
Onset 10% (m-d-y)	02-17-99	02-15-99
Onset 25% (m-d-y)	03-13-99	03-09-99
Fall 25% (m-d-y)	06-24-99	09-27-99
Fall 10% (m-d-y)	08-05-99	10-08-99
Absolute Onset 10 (m-d-y)	02-12-99	04-22-99
Absolute Fall 10 (m-d-y)	08-28-99	08-02-99
Absolute Duration 10 (days)	198	103
Absolute Span 10 (days)	197	102
Timing of Peak 1(m-d-y)	05-26-99	05-26-99
PTD1 (ind/100sqm)	160	18
Duration 1 25% (days)	103	213
Duration 1 10% (days)	133	NA
Fall1 25% (m-d-y)	06-24-99	10-08-99
Fall1 10% (m-d-y)	06-30-99	NA
Timing of Peak 2(m-d-y)	07-23-99	NA
PTD2 (ind/100sqm)	19	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	27	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	07-09-99	NA
CTD (ind/(100sqm*year)	12113	2279
% in 1st half-year (%)	65	38

Table C.49: Descriptive statistics of the questing tick timeplots at Eclépens in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Eclépens**	**Eclépens**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	04-17-00	04-17-00
PTD (ind/100sqm)	44	23
Duration 25% (days)	168	217
Duration 10% (days)	266	302
Span 25% (days)	168	217
Span 10% (days)	251	253
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-10-00	02-08-00
Onset 25% (m-d-y)	02-25-00	02-20-00
Fall 25% (m-d-y)	08-11-00	09-24-00
Fall 10% (m-d-y)	10-18-00	10-18-00
Absolute Onset 10 (m-d-y)	02-24-00	03-07-00
Absolute Fall 10 (m-d-y)	08-19-00	08-26-00
Absolute Duration 10 (days)	178	137
Absolute Span 10 (days)	177	172
Timing of Peak 1(m-d-y)	04-17-00	04-17-00
PTD1 (ind/100sqm)	44	23
Duration 1 25% (days)	168	217
Duration 1 10% (days)	NA	253
Fall1 25% (m-d-y)	08-11-00	09-24-00
Fall1 10% (m-d-y)	NA	10-18-00
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	4645	3041
% in 1st half-year (%)	62	54

Table C.50: Descriptive statistics of the questing tick timeplots at Eclépens in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Eclépens**	**Eclépens**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	04-02-01	04-02-01
PTD (ind/100sqm)	63	17
Duration 25% (days)	143	200
Duration 10% (days)	213	254
Span 25% (days)	143	223
Span 10% (days)	217	254
Pause 25% (days)	0	23
Pause 10% (days)	4	0
Onset 10% (m-d-y)	02-15-01	01-28-01
Onset 25% (m-d-y)	02-24-01	02-13-01
Fall 25% (m-d-y)	07-17-01	09-24-01
Fall 10% (m-d-y)	09-20-01	10-09-01
Absolute Onset 10 (m-d-y)	02-20-01	03-09-01
Absolute Fall 10 (m-d-y)	08-03-01	07-18-01
Absolute Duration 10 (days)	165	132
Absolute Span 10 (days)	164	131
Timing of Peak 1(m-d-y)	04-02-01	04-02-01
PTD1 (ind/100sqm)	63	17
Duration 1 25% (days)	143	175
Duration 1 10% (days)	180	254
Fall1 25% (m-d-y)	07-17-01	08-07-01
Fall1 10% (m-d-y)	08-14-01	10-09-01
Timing of Peak 2(m-d-y)	09-12-01	09-12-01
PTD2 (ind/100sqm)	8	6
Duration 2 25% (days)	NA	25
Duration 2 10% (days)	33	54
Onset2 25% (m-d-y)	NA	08-30-01
Onset2 10% (m-d-y)	08-18-01	08-16-01
CTD (ind/(100sqm*year)	7168	2380
% in 1st half-year (%)	77	67

Table C.51: Descriptive statistics of the questing tick timeplots at Eclépens in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Interlaken**	**Interlaken**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-02-99	05-02-99
PTD (ind/100sqm)	101	6
Duration 25% (days)	137	230
Duration 10% (days)	203	269
Span 25% (days)	137	228
Span 10% (days)	241	241
Pause 25% (days)	0	0
Pause 10% (days)	38	0
Onset 10% (m-d-y)	02-10-99	02-10-99
Onset 25% (m-d-y)	02-23-99	02-23-99
Fall 25% (m-d-y)	07-10-99	10-09-99
Fall 10% (m-d-y)	10-09-99	10-09-99
Absolute Onset 10 (m-d-y)	02-11-99	NA
Absolute Fall 10 (m-d-y)	11-06-99	NA
Absolute Duration 10 (days)	208	0
Absolute Span 10 (days)	268	0
Timing of Peak 1(m-d-y)	05-02-99	05-02-99
PTD1 (ind/100sqm)	101	6
Duration 1 25% (days)	137	147
Duration 1 10% (days)	175	NA
Fall1 25% (m-d-y)	07-10-99	07-20-99
Fall1 10% (m-d-y)	08-04-99	NA
Timing of Peak 2(m-d-y)	NA	09-08-99
PTD2 (ind/100sqm)	NA	3
Duration 2 25% (days)	NA	62
Duration 2 10% (days)	NA	76
Onset2 25% (m-d-y)	NA	08-08-99
Onset2 10% (m-d-y)	NA	07-25-99
CTD (ind/(100sqm*year)	9432	791
% in 1st half-year (%)	69	48

Table C.52: Descriptive statistics of the questing tick timeplots at Interlaken in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Interlaken**	**Interlaken**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	09-27-00	06-08-00
PTD (ind/100sqm)	21	15
Duration 25% (days)	162	133
Duration 10% (days)	278	251
Span 25% (days)	197	176
Span 10% (days)	294	255
Pause 25% (days)	35	43
Pause 10% (days)	16	4
Onset 10% (m-d-y)	02-22-00	03-15-00
Onset 25% (m-d-y)	04-27-00	05-06-00
Fall 25% (m-d-y)	11-10-00	10-29-00
Fall 10% (m-d-y)	12-12-00	11-25-00
Absolute Onset 10 (m-d-y)	05-07-00	05-27-00
Absolute Fall 10 (m-d-y)	10-15-00	06-27-00
Absolute Duration 10 (days)	104	32
Absolute Span 10 (days)	161	31
Timing of Peak 1(m-d-y)	06-08-00	06-08-00
PTD1 (ind/100sqm)	20	15
Duration 1 25% (days)	77	73
Duration 1 10% (days)	NA	145
Fall1 25% (m-d-y)	07-13-00	07-18-00
Fall1 10% (m-d-y)	NA	08-07-00
Timing of Peak 2(m-d-y)	09-27-00	09-27-00
PTD2 (ind/100sqm)	21	7
Duration 2 25% (days)	85	60
Duration 2 10% (days)	144	122
Onset2 25% (m-d-y)	08-17-00	08-30-00
Onset2 10% (m-d-y)	07-21-00	07-26-00
CTD (ind/(100sqm*year)	2479	1372
% in 1st half-year (%)	31	34

Table C.53: Descriptive statistics of the questing tick timeplots at Interlaken in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Interlaken**	**Interlaken**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-10-01	05-10-01
PTD (ind/100sqm)	37	16
Duration 25% (days)	136	137
Duration 10% (days)	176	178
Span 25% (days)	136	137
Span 10% (days)	202	236
Pause 25% (days)	0	0
Pause 10% (days)	26	58
Onset 10% (m-d-y)	03-01-01	02-21-01
Onset 25% (m-d-y)	03-22-01	03-11-01
Fall 25% (m-d-y)	08-05-01	07-26-01
Fall 10% (m-d-y)	09-19-01	10-15-01
Absolute Onset 10 (m-d-y)	03-25-01	04-16-01
Absolute Fall 10 (m-d-y)	08-04-01	06-23-01
Absolute Duration 10 (days)	133	69
Absolute Span 10 (days)	132	68
Timing of Peak 1(m-d-y)	05-10-01	05-10-01
PTD1 (ind/100sqm)	37	16
Duration 1 25% (days)	136	137
Duration 1 10% (days)	163	167
Fall1 25% (m-d-y)	08-05-01	07-26-01
Fall1 10% (m-d-y)	08-11-01	08-07-01
Timing of Peak 2(m-d-y)	09-11-01	NA
PTD2 (ind/100sqm)	5	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	13	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	09-06-01	NA
CTD (ind/(100sqm*year)	3764	1539
% in 1st half-year (%)	62	73

Table C.54: Descriptive statistics of the questing tick timeplots at Interlaken in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**St-Livres**	**St-Livres**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-26-99	07-01-99
PTD (ind/100sqm)	127	13
Duration 25% (days)	131	128
Duration 10% (days)	206	212
Span 25% (days)	131	128
Span 10% (days)	206	203
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	03-17-99	03-20-99
Onset 25% (m-d-y)	03-26-99	04-02-99
Fall 25% (m-d-y)	08-04-99	08-08-99
Fall 10% (m-d-y)	10-09-99	10-09-99
Absolute Onset 10 (m-d-y)	03-16-99	05-27-99
Absolute Fall 10 (m-d-y)	11-06-99	07-10-99
Absolute Duration 10 (days)	236	45
Absolute Span 10 (days)	235	44
Timing of Peak 1(m-d-y)	05-26-99	07-01-99
PTD1 (ind/100sqm)	127	13
Duration 1 25% (days)	131	128
Duration 1 10% (days)	NA	164
Fall1 25% (m-d-y)	08-04-99	08-08-99
Fall1 10% (m-d-y)	NA	08-31-99
Timing of Peak 2(m-d-y)	NA	10-09-99
PTD2 (ind/100sqm)	NA	3
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	21
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	09-18-99
CTD (ind/(100sqm*year)	12152	1277
% in 1st half-year (%)	47	34

Table C.55: Descriptive statistics of the questing tick timeplots at St-Livres in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**St-Livres**	**St-Livres**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	06-07-00	06-07-00
PTD (ind/100sqm)	95	23
Duration 25% (days)	160	128
Duration 10% (days)	236	204
Span 25% (days)	160	128
Span 10% (days)	236	233
Pause 25% (days)	0	0
Pause 10% (days)	0	29
Onset 10% (m-d-y)	03-06-00	03-05-00
Onset 25% (m-d-y)	04-21-00	03-24-00
Fall 25% (m-d-y)	09-28-00	07-30-00
Fall 10% (m-d-y)	10-28-00	10-24-00
Absolute Onset 10 (m-d-y)	03-09-00	04-14-00
Absolute Fall 10 (m-d-y)	10-27-00	07-10-00
Absolute Duration 10 (days)	233	88
Absolute Span 10 (days)	232	87
Timing of Peak 1(m-d-y)	06-07-00	06-07-00
PTD1 (ind/100sqm)	95	23
Duration 1 25% (days)	160	128
Duration 1 10% (days)	NA	192
Fall1 25% (m-d-y)	09-28-00	07-30-00
Fall1 10% (m-d-y)	NA	09-13-00
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	10270	2082
% in 1st half-year (%)	39	54

Table C.56: Descriptive statistics of the questing tick timeplots at St-Livres in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**St-Livres**	**St-Livres**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	05-10-01	05-10-01
PTD (ind/100sqm)	105	24
Duration 25% (days)	108	108
Duration 10% (days)	227	148
Span 25% (days)	108	108
Span 10% (days)	227	148
Pause 25% (days)	0	0
Pause 10% (days)	0	0
Onset 10% (m-d-y)	02-25-01	03-01-01
Onset 25% (m-d-y)	03-14-01	03-22-01
Fall 25% (m-d-y)	06-30-01	07-08-01
Fall 10% (m-d-y)	10-10-01	07-27-01
Absolute Onset 10 (m-d-y)	02-26-01	04-08-01
Absolute Fall 10 (m-d-y)	10-13-01	06-23-01
Absolute Duration 10 (days)	230	77
Absolute Span 10 (days)	229	76
Timing of Peak 1(m-d-y)	05-10-01	05-10-01
PTD1 (ind/100sqm)	105	24
Duration 1 25% (days)	108	108
Duration 1 10% (days)	227	148
Fall1 25% (m-d-y)	06-30-01	07-08-01
Fall1 10% (m-d-y)	10-10-01	07-27-01
Timing of Peak 2(m-d-y)	08-14-01	NA
PTD2 (ind/100sqm)	16	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	84	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	07-18-01	NA
CTD (ind/(100sqm*year)	8667	1859
% in 1st half-year (%)	74	76

Table C.57: Descriptive statistics of the questing tick timeplots at St-Livres in 2001 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Vaumarcus**	**Vaumarcus**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	06-30-99	05-01-99
PTD (ind/100sqm)	166	19
Duration 25% (days)	157	167
Duration 10% (days)	200	234
Span 25% (days)	157	168
Span 10% (days)	200	206
Pause 25% (days)	0	1
Pause 10% (days)	0	0
Onset 10% (m-d-y)	03-10-99	03-16-99
Onset 25% (m-d-y)	03-22-99	03-23-99
Fall 25% (m-d-y)	08-26-99	09-07-99
Fall 10% (m-d-y)	09-26-99	10-08-99
Absolute Onset 10 (m-d-y)	02-24-99	04-08-99
Absolute Fall 10 (m-d-y)	10-06-99	07-11-99
Absolute Duration 10 (days)	225	95
Absolute Span 10 (days)	224	94
Timing of Peak 1(m-d-y)	06-30-99	05-01-99
PTD1 (ind/100sqm)	166	19
Duration 1 25% (days)	157	199
Duration 1 10% (days)	NA	NA
Fall1 25% (m-d-y)	08-26-99	10-08-99
Fall1 10% (m-d-y)	NA	NA
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	17880	1943
% in 1st half-year (%)	40	43

Table C.58: Descriptive statistics of the questing tick timeplots at Vaumarcus in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Vaumarcus**	**Vaumarcus**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	03-10-00	04-17-00
PTD (ind/100sqm)	6	18
Duration 25% (days)	166	97
Duration 10% (days)	213	134
Span 25% (days)	229	97
Span 10% (days)	235	134
Pause 25% (days)	63	0
Pause 10% (days)	22	0
Onset 10% (m-d-y)	02-04-00	02-07-00
Onset 25% (m-d-y)	02-10-00	02-18-00
Fall 25% (m-d-y)	09-26-00	05-25-00
Fall 10% (m-d-y)	09-26-00	06-20-00
Absolute Onset 10 (m-d-y)	NA	03-10-00
Absolute Fall 10 (m-d-y)	NA	05-04-00
Absolute Duration 10 (days)	0	56
Absolute Span 10 (days)	0	55
Timing of Peak 1(m-d-y)	03-10-00	04-17-00
PTD1 (ind/100sqm)	6	18
Duration 1 25% (days)	90	97
Duration 1 10% (days)	154	134
Fall1 25% (m-d-y)	05-10-00	05-25-00
Fall1 10% (m-d-y)	07-07-00	06-20-00
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	531	1224
% in 1st half-year (%)	75	96

Table C.59: Descriptive statistics of the questing tick timeplots at Vaumarcus in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Viège**	**Viège**
Year	**1999**	**1999**
Timing of Peak (m-d-y)	05-27-99	07-01-99
PTD (ind/100sqm)	43	8
Duration 25% (days)	143	145
Duration 10% (days)	188	235
Span 25% (days)	143	146
Span 10% (days)	188	240
Pause 25% (days)	0	1
Pause 10% (days)	0	5
Onset 10% (m-d-y)	02-10-99	02-11-99
Onset 25% (m-d-y)	02-24-99	02-26-99
Fall 25% (m-d-y)	07-17-99	07-22-99
Fall 10% (m-d-y)	08-17-99	10-09-99
Absolute Onset 10 (m-d-y)	02-23-99	NA
Absolute Fall 10 (m-d-y)	07-18-99	NA
Absolute Duration 10 (days)	146	0
Absolute Span 10 (days)	145	0
Timing of Peak 1(m-d-y)	05-27-99	07-01-99
PTD1 (ind/100sqm)	43	8
Duration 1 25% (days)	143	146
Duration 1 10% (days)	188	200
Fall1 25% (m-d-y)	07-17-99	07-22-99
Fall1 10% (m-d-y)	08-17-99	08-30-99
Timing of Peak 2(m-d-y)	NA	NA
PTD2 (ind/100sqm)	NA	NA
Duration 2 25% (days)	NA	NA
Duration 2 10% (days)	NA	NA
Onset2 25% (m-d-y)	NA	NA
Onset2 10% (m-d-y)	NA	NA
CTD (ind/(100sqm*year)	4680	928
% in 1st half-year (%)	63	52

Table C.60: Descriptive statistics of the questing tick timeplots at Viège in 1999 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Viège**	**Viège**
Year	**2000**	**2000**
Timing of Peak (m-d-y)	10-19-00	06-08-00
PTD (ind/100sqm)	18	7
Duration 25% (days)	101	114
Duration 10% (days)	186	128
Span 25% (days)	195	114
Span 10% (days)	223	128
Pause 25% (days)	94	0
Pause 10% (days)	37	0
Onset 10% (m-d-y)	04-13-00	03-13-00
Onset 25% (m-d-y)	05-05-00	03-20-00
Fall 25% (m-d-y)	11-16-00	07-12-00
Fall 10% (m-d-y)	11-22-00	07-19-00
Absolute Onset 10 (m-d-y)	10-07-00	NA
Absolute Fall 10 (m-d-y)	11-05-00	NA
Absolute Duration 10 (days)	30	0
Absolute Span 10 (days)	29	0
Timing of Peak 1(m-d-y)	05-11-00	06-08-00
PTD1 (ind/100sqm)	6	7
Duration 1 25% (days)	43	114
Duration 1 10% (days)	93	128
Fall1 25% (m-d-y)	06-17-00	07-12-00
Fall1 10% (m-d-y)	07-15-00	07-19-00
Timing of Peak 2(m-d-y)	10-19-00	NA
PTD2 (ind/100sqm)	18	NA
Duration 2 25% (days)	58	NA
Duration 2 10% (days)	93	NA
Onset2 25% (m-d-y)	09-19-00	NA
Onset2 10% (m-d-y)	08-21-00	NA
CTD (ind/(100sqm*year)	1190	567
% in 1st half-year (%)	25	66

Table C.61: Descriptive statistics of the questing tick timeplots at Viège in 2000 (dates are in month-day-year format).

Statistics	Nymphs	Adults
Location	**Viège**	**Viège**
Year	**2001**	**2001**
Timing of Peak (m-d-y)	04-03-01	06-14-01
PTD (ind/100sqm)	17	3
Duration 25% (days)	206	53
Duration 10% (days)	257	155
Span 25% (days)	233	54
Span 10% (days)	261	235
Pause 25% (days)	27	1
Pause 10% (days)	4	80
Onset 10% (m-d-y)	02-19-01	03-09-01
Onset 25% (m-d-y)	02-26-01	05-19-01
Fall 25% (m-d-y)	10-17-01	07-12-01
Fall 10% (m-d-y)	11-07-01	10-30-01
Absolute Onset 10 (m-d-y)	03-15-01	NA
Absolute Fall 10 (m-d-y)	07-13-01	NA
Absolute Duration 10 (days)	97	0
Absolute Span 10 (days)	120	0
Timing of Peak 1(m-d-y)	04-03-01	06-14-01
PTD1 (ind/100sqm)	17	3
Duration 1 25% (days)	159	54
Duration 1 10% (days)	176	144
Fall1 25% (m-d-y)	08-04-01	07-12-01
Fall1 10% (m-d-y)	08-14-01	07-31-01
Timing of Peak 2(m-d-y)	09-11-01	10-15-01
PTD2 (ind/100sqm)	7	1
Duration 2 25% (days)	47	NA
Duration 2 10% (days)	81	31
Onset2 25% (m-d-y)	08-31-01	NA
Onset2 10% (m-d-y)	08-18-01	09-29-01
CTD (ind/(100sqm*year)	2135	162
% in 1st half-year (%)	59	50

Table C.62: Descriptive statistics of the questing tick timeplots at Viège in 2001 (dates are in month-day-year format).

Appendix D

Squirrel settings

code	during tick sampling	at field arenas
5.1: Range	150°C	150°C
5.2: Range	150°C	150°C
5.3: Range	150°C	150°C
5.9: Range	L 100.0%	L 100.0%
7.1: Method	ave	ave
8.1: Logging	log	log
8.2: Hours	00	01
8.3: Minutes	05	00
8.4: Seconds	00	00

Table D.1: Settings of the Squirrel 1200 logger (Grant Instruments) for microclimate recordings during tick sampling and at the field arena locations.

Appendix E

Meteosuisse data retrieval: detailed procedure

```
How to get usefull data from SMA:

telnet wawona.ethz.ch
username: JLP
password: xxxxxxxxx

setup_sma_db

run loadclimatthese

choose station number   (Neuchâtel = 23)
choose year
choose filename         (ex: Neuchâtel)

get file by ftp

/home/sdperret/data/bin/addtime.perl filename > filename.clh

/home/sdperret/data/bin/clh2cld.perl filename.clh
(produit le fichier filename.cld)

mv filename.cl* to /home/sdperret/data/meteo/data/
```

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Appendix F

Glossary

This glossary is not meant to give general definitions of words, but aims at defining the vocabulary used in the specific context of this manuscript.

activation and orientation to the host: activation starts when a blood-feeding arthropod comes into contact with a potential host cue, switching from a non-directional behaviour to a host oriented response. Host cues may be olfactory, visual, thermic and mechanical. It follows appetitive search and usually ends with attration to the host (Hamilton and Hurd, 2002).

appetitive search: appetitive search is the search strategy used by blood-feeding arthropods in response to an endogenous stimulus (hunger) to enhance the probability of encountering host cues. It occurs in the absence of hosts. This behaviour is usually followed by activation and orientation to the host (Hamilton and Hurd, 2002).

attraction to the host: attraction is the behaviour leading the arthropod from a distance to the host. It may imply responses to odours, visual cues, heat and water vapor. It follows appetitive search and activation - orientation to the host and leads to landing and probing behaviour (Hamilton and Hurd, 2002).

autocorrelation function (ACF): see explanation on page 90

cumulated tick density (CTD): the integral of the questing tick density curve over one year (see 2.2.2.3 on page 27)

critical equilibrium humidity (CEH): relative humidity at which water gains compensate water loss in arthropods (Needham and Teel, 1991).

critical equilibrium activity (CEA): equivalent to critical equilibrium humidity since relative humidity equals the water activity times 100 (Needham and Teel, 1991).

current image: last digital image acquired by the computer

difference image: reference image substracted from current image (see 2.3.2.2 on page 50)

drag: smallest surface sampled for ticks before collecting ticks from the flag, usually $25m^2$.

landing and probing behaviour: landing and probing occurs in blood-sucking arthropod vectors within a more or less short distance from the host and leads to the recognition of the suitability of the host for feeding (Hamilton and Hurd, 2002). It follows appetitive search, activation, orientation and attraction.

motion: movement made by a tick during time ΔT (see section 2.3.2 for details on motion detection).

movement: see motion.

phenology of ticks: evolution of the questing tick density curve over one year.

pump threshold: relative humidity at or above which active water sorption is possible (Machin, 1979) (O'Donnell and Machin, 1988).

questing: event during which the tick stays immobile (for at least 2 hours) in possibly dessicating conditions, usually at the top of something vertical.

quiescence: event during which the tick stays immobile (for at least 2 hours), usually in the litter zone.

reference image: image acquired ΔT seconds before the current image.

sampling session: single day and location for which ticks were collected in the field (collection of *drags*).

walking: event during which a tick is moving; the event stops at the time of the last tick movement after which the tick was immobile for at least two hours.

Appendix G

Abbreviations

ACF: autocorrelation function (calculated using the acf function in the ts package in R(Ihaka and Gentleman, 1996)).

adu: adult

CH1903: Swiss grid projection system, see 2.2.2.5 on page 29.

CEH: Critical Equilibrium Humidity.

CTD: Cumulated Tick Density (see 2.2.2.3 on page 27)

CTDA: Cumulated Tick Density for adults (see 2.2.2.3 on page 27)

CTDN: Cumulated Tick Density for nymphs (see 2.2.2.3 on page 27)

d-m-y: Date Format as Day-Month-Year

ETHZ: Eidgenossische Technische Hochschule Zürich.

exp: Experiment.

GPS: Global Positioning System: USA satellite based navigation system.

H0: Null hypothesis.

ind: Individual(s).

IR: Infra-red.

ISM: Institut Suisse de Météorologie (= SMA).

L:D: description of the light cycle: L = hours of light, D = hours of darkness. Transition time, if any, is included in the light period. For example a 14:10 LD light cycle with 2h transitions means: 2h gradual light increase -> 10h light -> 2h gradual light decrease -> 10h darkness.

m-d-y: Date format as Month-Day-Year

nb: Number.

nym: nymph

PCTD1: Proportion of the CTD in the first half-year (see 2.2.2.3 on page 27)

PCTD1A: Proportion of the CTD for adults in the first half-year (see 2.2.2.3 on page 27)

PCTD1N: Proportion of the CTD for nymphs in the first half-year (see 2.2.2.3 on page 27)

R0: Basic reproductive number (Randolph and Craine, 1995).

RH: Relative Humidity (units = %).

SMA: Schweizerische Meteorologische Anstalt (= ISM).

sqm: Square meters.

temp: Temperature.

Appendix H

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