Temporal Variations in the Usefulness of Normalized Difference Vegetation Index as a Predictor for *Ixodes ricinus* (Acari: Ixodidae) in a *Borrelia lusitaniae* Focus in Tuscany, Central Italy

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ABSTRACT Host-seeking ticks were collected during monthly dragging sessions from November 2004 through October 2006 in Tuscany, central Italy. Normalized difference vegetation index (NDVI), which was calculated from Landsat ETM+ 7 remote sensing data recorded in August 2001, was significantly correlated with numbers of host-seeking immature *Ixodes ricinus* L. (Acari: Ixodidae) during periods of relatively low rainfall such as summer 2005 (Spearman's $\rho = 0.78$, $P < 0.001$ for nymphs in July) and to a lower extent in spring–summer 2006. In spring 2005, when rainfall was relatively high, the correlation was weak and not statistically significant. Generalized estimating equations (GEEs), taking into account repeated sampling of the same dragging sites, were used to model the effects of NDVI and season on counts of host-seeking *I. ricinus* nymphs. Seasonal variations of the effect of NDVI yielded a significant NDVI-by-season interaction in the first year of the study (November 2004–October 2005), but not in the second year (November 2005–October 2006) when there was a 2.5-fold increase of the number of nymphs per 100-m dragging for every 0.1 unit increase in NDVI (95% confidence interval = 1.6, 3.0). Risk maps that were obtained based on GEE results confirmed that the predicted number of *I. ricinus* nymphs per 100 m was relatively homogeneous through the study area during the 2005 spring peak of activity. Conversely, in 2006, the predicted abundance of nymphs was greater in moist bottomland habitat (characterized by high NDVI) than in dry, typically Mediterranean, upland habitat.

KEY WORDS *Ixodes ricinus*, normalized difference vegetation index, ticks, Italy

The hard tick *Ixodes ricinus* L. (Acari: Ixodidae) transmits microbic agents of zoonotic diseases in a wide geographic area ranging from northern Europe to northern Africa. Environmental factors such as climate, land morphology, and vegetation cover affect temperature and relative humidity at the microclimatic level, and, together with the availability of vertebrate hosts, they determine ticks' distribution, abundance, and seasonal pattern of activity. These factors, in turn, affect the transmission of pathogens among ticks and competent reservoir hosts (Daniel and Dusbábek 1994, Gray et al. 1998, Randolph and Storey 1999, Randolph 2002, Barandika et al. 2006). Dry habitat conditions may limit the distribution of *I. ricinus* in the southern part of its geographic range (Estrada-Peña et al. 2004). Accordingly, on the Tyrrhenian coast of Tuscany, central Italy, relatively moist, deciduous woods were more favorable for immature *I. ricinus* than dry pine woods (*Pinus pinaster* Aiton, *P. pinea* L.) (Mannelli et al. 1999).

In another area of Tuscany (Le Cerbaie Hills, in the province of Pisa), *Borrelia lusitaniae* was the dominant genospecies of *B. burgdorferi* s.l. (the spirochetes including the etiological agents of Lyme borreliosis) in host-seeking *I. ricinus,* and it was associated with lizards (*Podarcis muralis* Laurenti) as the most likely reservoir hosts (Bertolotti et al. 2006, Amore et al. 2007). *B. lusitaniae* is mostly found in southern Europe and the Mediterranean area, and it was recently identified in the skin of a patient with Lyme borreliosis (Sarih et al. 2003, Collares-Pereira et al. 2004, Younsi et al. 2005). Environmental conditions favorable to lizards (that are a major component of wildlife in Mediterranean habitats) but with enough moisture to allow development and activity of *I. ricinus* probably underlie the geographic focality of *B. lusitaniae.*

In the identification of disease foci, direct habitat characterization (i.e., land morphology and vegetation cover) can be replaced by the use of remote sensing data. Normalized difference vegetation index

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Table 1. Structure of the vegetation cover visually evaluated, in August 2005, in 10- by 10-m quadrats in host-seeking tick collection sites, in bottomland and upland habitat types, in Le Cerbaie Hills, Tuscany, Italy

Ht level (m)	% cover (min., max)	Bottomland habitat % species composition (median, min., max)	% cover (min., max)	Upland habitat % species composition (median, min., max)
>25	0 (0, 25)	*Quercus* sp. (0, 0, 100)	0 (0, 10)	*P. pinaster* (0, 0, 100)
>12–25	70 (20, 95)	*Quercus* sp. (80, 40, 100), *C. betulus* (50 10, 70)	50 (10, 80)	*P. pinaster* (65, 50, 80), *Q. cerris* (35, 0, 100)
>5–12	75 (30, 90)	*Quercus* sp. (50, 40, 100), *C. betulus* (32.5, 10, 75)	75 (30, 90)	*Q. cerris* (50, 10, 100), *Q. ilex* (42.5, 25, 60)
>2–5	60 (20, 70)	*C. betulus* (30, 20, 100), *Quercus* sp. (30, 10, 70)	60 (20, 70)	*Q. ilex* (50, 40, 60), *Q. cerris* (40, 20, 80)
>0.5–2	40 (10, 70)	*P. aquilinum* (40, 40, 60), *C. betulus* (30, 20, 100)	40 (10, 70)	*Q. cerris* (40, 10, 80), *P. aquilinum* (25, 10, 80)
0–0.5	40 (20, 80)	*P. aquilinum* (40, 10, 100), herbs (20, 10, 100)	40 (30, 90)	Herbs (50, 20, 90), *P. aquilinum* (50, 10, 100)
Leaves	100 (70, 100)		90 (60, 100)	-

(NDVI), as an example, has been used as a predictor of moisture availability and therefore of favorable habitat for *I. ricinus* (Kitron 1998, Eisen et al. 2006, Estrada-Peña et al. 2006). In this study, we evaluated the association between ground-level habitat characteristics and NDVI in Le Cerbaie. Moreover, during a 2-yr period, we analyzed seasonal variations in the usefulness of NDVI as a predictor for host-seeking *I. ricinus*, taking into account meteorological conditions.

Materials and Methods

Study Area. The study was carried out in a 500-ha enclosed natural reserve on Le Cerbaie Hills in the province of Pisa, Tuscany (43° 45′ N, 10° 42′ E). Land morphology is characterized by bottomlands (minimal elevation, 35 m above sea level) and uplands (maximal elevation, 110 m above sea level). Bottomland habitat is relatively humid, and cover is dominated by deciduous tree species. Upland habitat is relatively dry and vegetation is typically Mediterranean. Rare (<10 heads) roe deer (*Capreolus capreolus* L.) and wild boar (*Sus scrofa* L.) are the only ungulate species in the enclosure. A case of Lyme borreliosis was recorded in a forestry worker in the reserve in 2001.

Habitat Characterization. Percentage of vegetation cover and the relative contribution of each plant species were visually evaluated, at seven height levels, from leaf litter to >25 m, on 10- by 10-m quadrats at the sites of host-seeking tick collection (Table 1) (Mannelli et al. 1999).

Remote Sensing Data. Landsat ETM+ 7 images from bands 3 and 4 (path 193, row 29) were downloaded from the Global Land Cover Facility (GLCF), Institute for Advanced Computer Studies, University of Maryland, College Park, MD (http://glcfapp.umiacs.umd.edu:8080/esdi/ftp?id=36128). The length of pixels' side is 30 m. Satellite images were imported in the geographical information system (GIS) GRASS 6.2 (http://grass.itc.it/), in the Kubuntu Linux operating system, by using the Universal Transverse Mercator (UTM/ED50) projection, zone 32. Landsat images were previously geocoded by GLCF. Further geometric correction was, however, achieved by using corners of buildings in the study area as ground control points of known coordinates, and the *i.rectify* module in GRASS. The GRASS module *r.mapcalc* was used to calculate NDVI (Neteler and Mitasova 2002). For our analyses, we used images that were taken on 1 August 2001 that were freely downloadable and that corresponded to the peak of vegetation cover in summer, when differences in moisture between habitats within the estate were most likely to be detected by NDVI.

Microclimate Data Collection. Two digital microclimate recording stations (HOBO H8 Pro Series) were placed on the ground at one bottomland and one upland tick collection site. Temperature and relative humidity were recorded every 30 min from January 2006 through February 2007. Saturation deficit (*SD*) was calculated using the following equation:

$$SD = \left(1 - \frac{RH}{100}\right) 4.9463e^{0.0621T}$$

where *RH* is relative humidity, $e = 2.718282$, T is temperature (°C) (Randolph and Storey 1999). Ground-level temperature and relative humidity were recorded, simultaneously with tick collection at each sampling site, by using a HI 8564 thermo hygrometer (Hanna Instrument Italia, Milano), and *SD* was calculated.

Meteorological Data. Data on rainfall and temperature, from fall 2004 through fall 2006, collected at the Orentano station (≈6 km from the study area) were provided by La.M.M.A.-Regione Toscana (http://www.lamma.rete.toscana.it/).

Tick Collection. Tick collection sites were selected by subdividing the study area into seven sections of approximately the same size. Sites were selected within each section, including, when possible, both bottomland and upland habitats. An area where the vegetation was destroyed by fire in 2001 was excluded from sampling. At each site, host-seeking ticks were collected by two operators by dragging 1-m^2 cotton cloths on the ground vegetation on 50-m transects. One 100-m transect was the sampling unit. Twenty-four sites that were visited in November 2004 plus a new site ($n = 25$) were sampled monthly from December 2004 through October 2005. During the second year of the study, we reduced the sampling effort in winter. Accordingly, in November 2005, January, and March 2006, 12, 8, and 13 of the original sites were sampled, respectively, including all of the study area's sections. The original 25 sites were sampled from April through August 2006; 20 and 24 sites were visited in September and October 2006, respectively. No dragging was carried out in February and December 2005, and in February 2006 owing to adverse weather conditions. Ticks were preserved in 70% ethanol for later identification using keys by Manilla (1998).

Statistical Analysis. Percentage of vegetation cover at seven height levels and relative (percentage) composition of vegetation cover by plant species were obtained. For each dragging site, we calculated the mean NDVI value for nine contiguous pixels (resulting in 90- by 90-m quadrats), and this value was used for subsequent statistical analysis. Median NDVI for different categories of land morphology types (bottomland versus upland) were compared by Wilcoxon two-sample test (PROC NPAR1WAY, SAS Institute 1999). Wilcoxon signed rank test for paired observations was used to compare *SD* in the same months of 2005 and 2006 (PROC UNIVARIATE).

The proportions of tick-positive transects, where at least one host-seeking tick was collected, and 95% exact binomial confidence intervals (CI) were calculated for dragging session and tick stage (BINOMIAL option, PROC FREQ, SAS Institute 1999). Estimates of monthly mean numbers of host-seeking ticks per transect, and 95% CI, were obtained by intercept-only, generalized log-linear models by using the GENMOD procedure in the SAS system (Exp option in the ESTIMATE statement). Negative binomial error was used in the statistical models to take into account the potential overdispersion of the distributions of host seeking ticks among dragging sites (Littell et al. 2002).

The association between NDVI and numbers of ticks per transect was tested by Spearman's nonparametric correlation coefficient, ρ, for each month (PROC CORR). We subsequently used statistical modeling to evaluate the use of NDVI as a predictor for host-seeking *I. ricinus* nymphs—the most likely stage for the transmission of *B. burgdorferi* s.l. to people and therefore the most appropriate to be included in a risk assessment model. The adjusted effects of NDVI and season on nymph counts were therefore estimated by generalized estimating equations (GEEs) with log-link and negative binomial error. Using GEEs (exchangeable correlation structure), we accounted for correlation arising from repeatedly collecting ticks at the same sites across the study period (Diggle et al. 2002). The seasonal pattern of nymphs was modeled through a sinusoidal fluctuation, with an amplitude of 1 and a period of 1 yr. The peak of the seasonal fluctuation was chosen based upon exploratory analysis and the model's residuals (Mannelli et al. 2003). Linearity of the effect of NDVI on tick counts was checked by component-plus-residual plots by using the *cr.plots* function in the R software (Fox 2002). Model checking was accomplished by goodness-of-fit statistics (deviance and Pearson's χ^2) and by plotting Pearson's residuals against sampling session (Littell et al. 2002). Model predictions of the number of host-seeking nymphs per 100 m were used to draw raster, risk maps for *I. ricinus*, by using *r. mapcalc.*

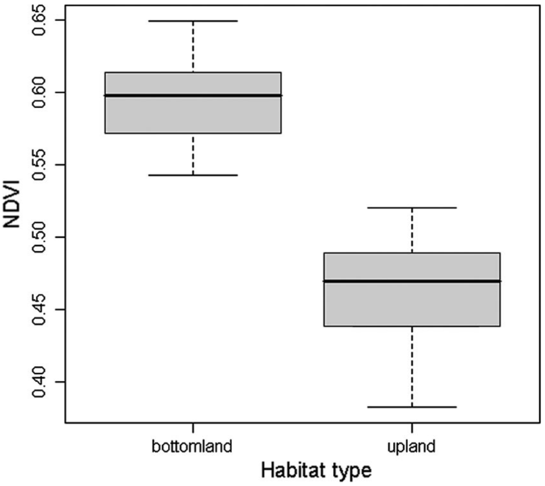

Fig. 1. Boxplots of NDVI at host-seeking tick collection sites that were classified, in the field, as bottomland ($n = 16$) and upland ($n = 9$), in Le Cerbaie Hills, Tuscany, Italy. In the boxplot, the bottom of the box represents the first quartile of the distribution and the top the third quartile. The bar inside the box represents the median.

Results

Median NDVI was significantly greater in tick collection sites that were classified as bottomlands than in upland sites (Wilcoxon two-sample test; $P < 0.001$) (Fig. 1). Structure of the vegetation cover in bottomland and upland sites is reported in Table 1. In bottomland sites, *Quercus* sp. (*Quercus robur* L. and *Quercus petraea* Mattus) dominated the highest levels, and the European hornbeam (*Carpinus betulus* L.) dominated between 2- and 5-m height level. Other deciduous species typically associated with humid habitats were identified in bottomland, including the chestnut (*Castanea sativa* L.) and the common alder (*Alnus glutinosa* L.). In upland habitat, typically Mediterranean, xerophilic tree species were common, including the maritime pine (*Pinus pinaster* Aiton) and the evergreen oak (*Quercus ilex* L.); the Turkey oak (*Q. cerris* L.) also was present.

Based upon data from digital microclimate recording stations, *SD* was greater in the upland than in the bottomland site during most of 2006 and particularly in summer. In fact, in bottomland habitat, maximal 7-d moving average of *SD* was 6.0 on 19 July 2006, whereas in upland habitat, 7-d moving average of SD peaked at 8.9 on 18 July. Rainfall, as measured at the Orentano station, was greater in spring 2005 than in spring 2006, whereas summer 2005 was drier than summer 2006 (Fig. 2). Cumulative rainfall, from 7 d before the start of each dragging session and through the entire session, summed to 80.2 mm for sessions from April through June 2005 and 37.4 mm for the same period of 2006. Cumulative rainfall was 41.7 mm for sessions from July through October 2005 and 121.1 mm during the same period of 2006. Saturation deficit, as calculated from temperature and relative humidity recorded at dragging sites, was significantly lower in May

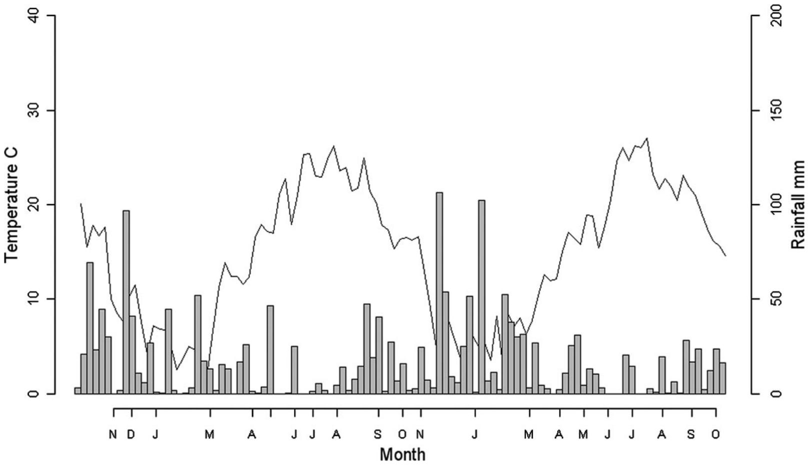

Fig. 2. Weekly rainfall (bars) and 7-d moving average of the mean temperature, from fall 2004 through fall 2006, at the meteorological station of Orentano, ≈6 km from an area of host-seeking tick collection in Le Cerbaie Hills, Tuscany, Italy. Months on the x-axis correspond to the dates of tick collection.

2005 than in May 2006 (Wilcoxon signed rank test; $P < 0.05$). No significant between-year differences were found for other months (Fig. 3).

We collected 9,471 host-seeking *I. ricinus* larvae, 2,335 nymphs, and 196 adults. One *Rhipicephalus* sp. adult was collected in 2006. Larvae were found in 297 of 476 (62.4%) dragging observations, nymphs were collected in 305 observations (64.1%), and adults in 118 observations (24.8%).

I. ricinus larvae were mostly active from April through October but were also found during winter (Fig. 4a). The percent of larvae–positive sites was relatively high in spring 2005 and peaked in April and May of the same year (92.0; 95% CI: 74.0, 99.0). The mean number of larvae per transect was characterized by minor spring peaks of activity in May 2005 and 2006 (95% CI was wider in 2006 than in 2005), followed by major peaks in the months of July 2005 (mean = 44.1; 95% CI: 21.9, 88.6) and 2006 (45.8; 23.6, 88.7) (Fig. 5a). Maximum number of larvae per 100 m was 289 in July 2006.

During spring 2005, nonparametric correlation between NDVI and counts of host-seeking larvae was weak and not statistically significant (Table 2). No correlation was observed in June 2005, whereas relatively high values of Sperman's ρ were found in the following summer. In 2006, there was significant correlation between NDVI and larvae from May through September, but ρ was generally lower than in summer 2005.

The seasonal pattern of host-seeking nymphs was characterized by spring peaks in 2005 and 2006. The percentage of nymph-positive sites was 100% (95% CI: 86.3, 100) in April 2005 and in March 2006 (75.2, 100) (Fig. 4b). Mean numbers of host-seeking nymphs per transect were the same in April and in May 2005

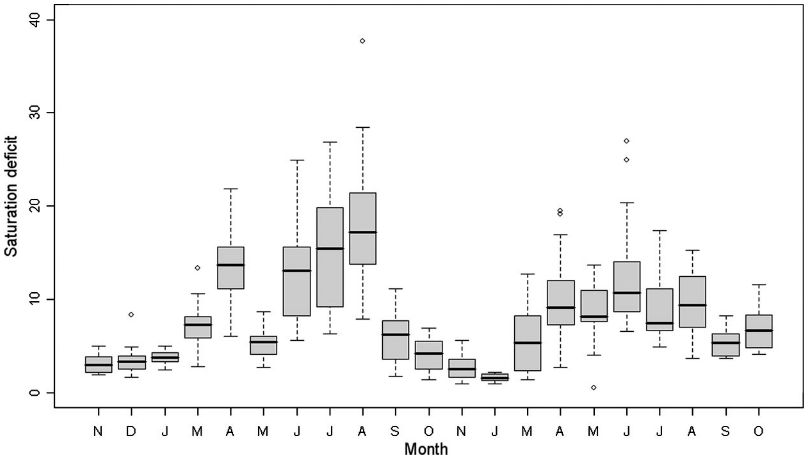

Fig. 3. Boxplots of saturation deficit that was obtained from temperature and relative humidity measured at host-seeking tick collection sites, from November 2004 through October 2006, in Le Cerbaie Hills, Tuscany, Italy. Symbols as in Fig. 1. Small circular symbols indicate values differing from the box >1.5 times the interquartile range.

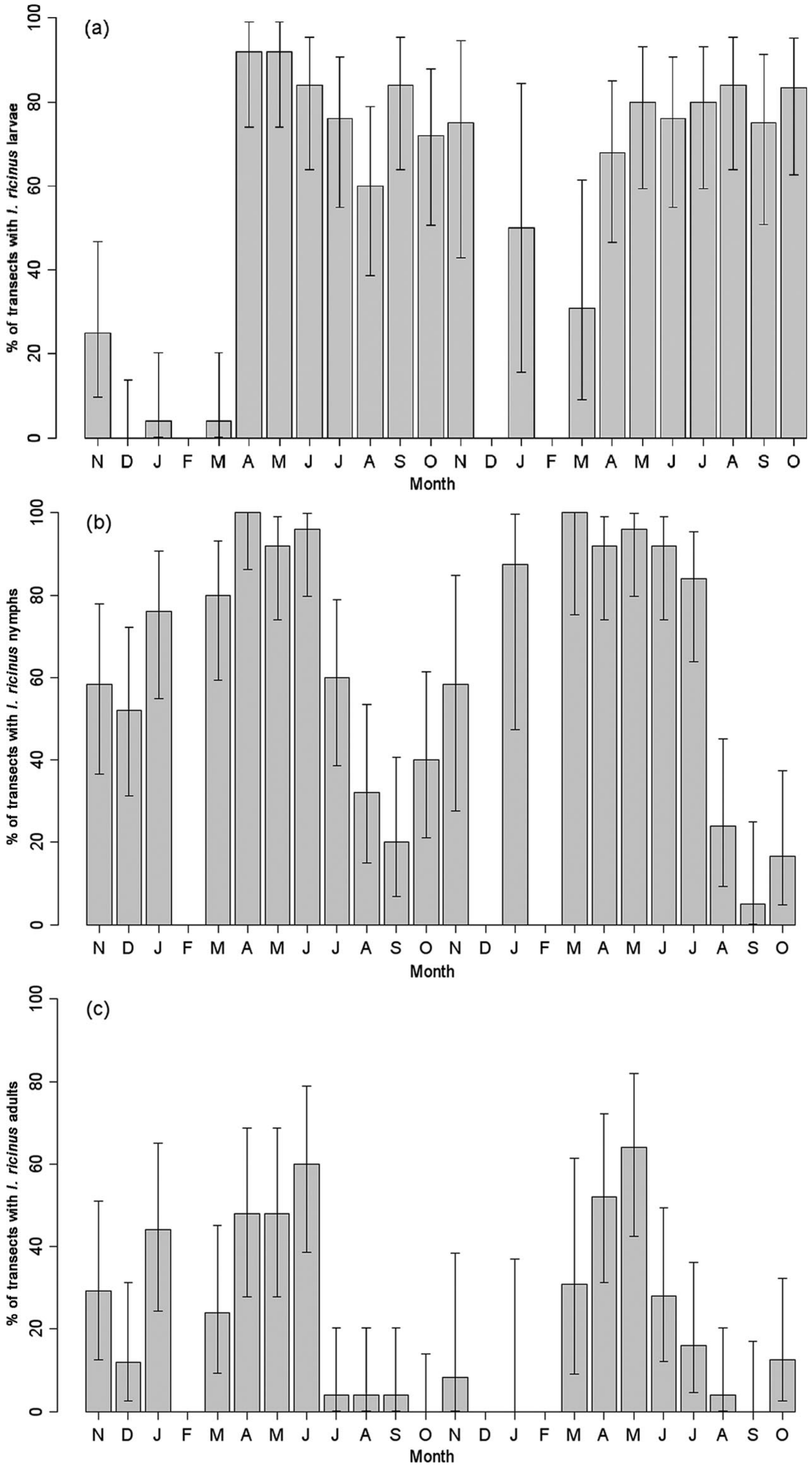

Fig. 4. Percentages and 95% confidence intervals of 100-m transects where at least one host-seeking *I. ricinus* was collected by dragging, from November 2004 through October 2006, in Le Cerbaie Hills, Tuscany, Italy: larvae (a), nymphs (b), and adults (c).

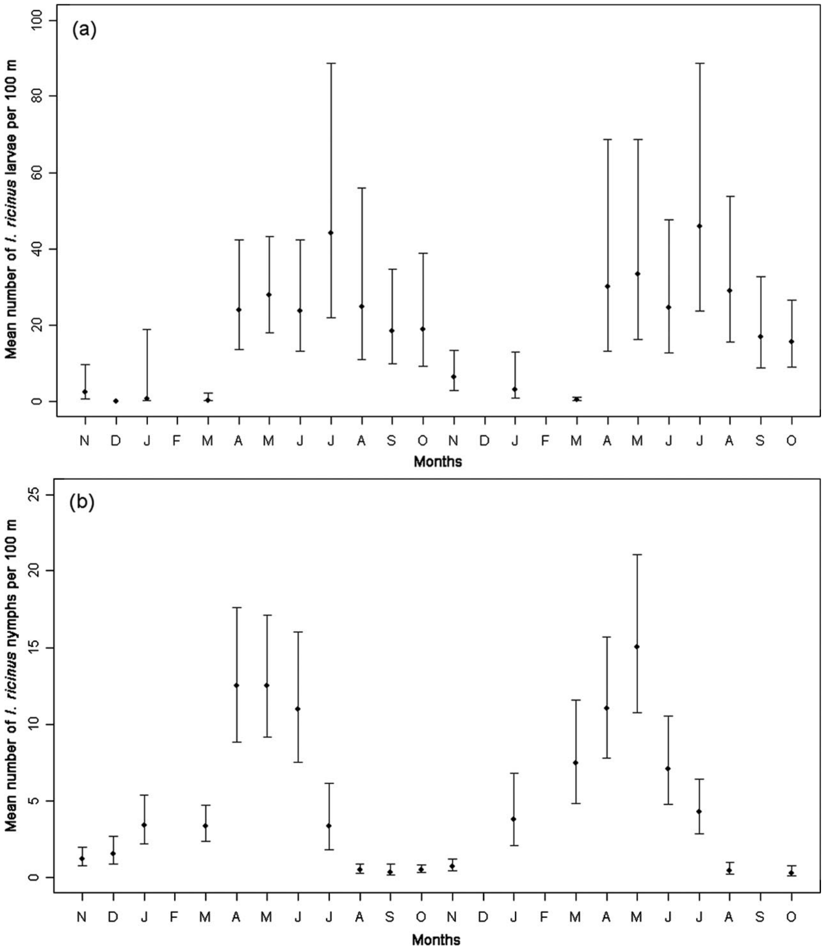

Fig. 5. Mean numbers and 95% confidence intervals of host-seeking *I. ricinus* that were collected by dragging in 100-m transects, from November 2004 through October 2006, in Le Cerbaie Hills, Tuscany, Italy: larvae (a) and nymphs (b).

(12.5), and they remained relatively high in June (Fig. 5b). In 2006, there was a major peak of nymphs in May (15.0, 95% CI: 10.7, 21.1) followed by a drop in June. Maximum number of nymphs per 100 m was 44 in April 2005.

The nonparametric correlation between NDVI and host-seeking nymphs was weak and not statistically significant in spring 2005, whereas it was relatively strong the following summer and fall, and through spring and early summer 2006 (Table 3). We separately analyzed, by GEEs, data from the collection of nymphs during the first 1-yr period of sampling, from November 2004 through October 2005 (period I), and during the second year of sampling, from November 2005 through October 2006 (period II). The varying correlation between nymphs and NDVI in 2005 yielded a significant, negative interaction between seasonal SINE (with a peak in April) and NDVI in the GEE for period I. Such an interaction was not significant in period II (Table 4), when the antilogarithm of the regression parameter for NDVI (0.79; 0.47, 1.1)

Table 2. Correlation between counts of host-seeking *I. ricinus* larvae per 100-m dragging and normalized difference vegetation index during months of major tick activity in Le Cerbaie Hills, Tuscany, Italy

Yr	Value	Mo						
		April	May	June	July	Aug.	Sept.	Oct.
2005	ρ	0.36	0.20	0.09	0.70	0.74	0.73	0.65
	P	0.08	0.33	0.67	<0.001	<0.001	<0.001	<0.001
2006	ρ	0.24	0.50	0.56	0.44	0.67	0.54	0.39
	P	0.25	<0.05	<0.01	<0.05	<0.001	<0.05	0.06

Table 3. Correlation between counts of host-seeking *I. ricinus* nymphs per 100-m dragging and normalized difference vegetation index during months of major tick activity in Le Cerbaie Hills, Tuscany, Italy

Yr	Value	Mo						
		April	May	June	July	Aug.	Sept.	Oct.
2005	ρ	0.26	0.36	0.35	0.78	0.50	0.36	0.47
	P	0.21	0.07	0.08	<0.001	<0.05	0.08	<0.05
2006	ρ	0.53	0.60	0.68	0.71	0.39	0.26	0.06
	P	<0.01	<0.01	<0.001	<0.001	0.06	0.27	0.79

Table 4. **Results of generalized estimating equations, with negative-binomial error and exchangeable correlation among repeated observations, of counts of host-seeking *I. ricinus* nymphs per 100-m dragging in Le Cerbaie Hills, Tuscany, Italy, from November 2004 to October 2006**

Period[a]	Parameter estimates (95% CI)[b]				
	Intercept	Seasonal SINE	NDVI	Inter	Deviance (df)
I	−4.2 (−5.7, −2.7)	3.7 (0.85, 2.1)	0.91 (0.65, 1.2)	−0.40 (−0.08, −0.66)	278.7 (270)
II	−3.6 (−5.4, −1.8)	1.9 (1.7, 2.1)	0.79 (0.47, 1.1)		200.9 (199)

[a] Period I, November 2004–October 2005; period II, November 2005–October 2006.

[b] Seasonal SINE: sinusoidal fluctuation with 1-yr period and peak in the month of April; NDVI, normalized difference vegetation index; INTER, SINE × NDVI interaction.

corresponded to a 2.2 times increase of the number of nymphs per 100-m dragging, every 0.1 U increase in NDVI (95% confidence interval: 1.6, 3.0).

Based upon goodness-of-fit statistics, the GEEs with negative binomial error were considered as appropriate for the analysis of counts of host-seeking nymphs. Residuals plots demonstrated the presence of outliers: in period I, a Pearson residual value = 6.0 was obtained for a bottomland site where 42 nymphs were collected in June 2005 against a model prediction of 6.0 nymphs. In period II, Pearson residual = 4.8 was obtained for another bottomland site with 16 nymphs in July 2006, against a prediction of 2.8 nymphs. These outliers did not affect the overall fit of the models. Component-plus-residual plots showed an overall good linear association between host-seeking nymphs and NDVI (data not shown). Predictive risk maps indicated that the number of *I. ricinus* nymphs per 100 m was generally lower and more homogeneous through the study area during the 2005 peak season of activity, whereas in 2006, nymphs were more strongly associated with bottomland habitat, characterized by high NDVI values (Fig. 6, a and b).

Adult *I. ricinus* were mostly active in spring, but they were also found in fall and winter (Fig. 4c). The maximum number of adults per transect was seven in May 2005. In January 2005, median NDVI was significantly greater in adult-positive sites than in sites where no adult was found (Wilcoxon 2-sample test; $P < 0.01$), whereas no correlation between adults and NDVI was found in other months.

Discussion

At our study area, NDVI was profitably used to distinguish relatively moist bottomland habitat covered by deciduous trees (including *C. betulus*), from dry upland habitat covered by Mediterranean vegetation. NDVI was calculated from remote sensing data recorded in summer 2001, whereas habitat was directly characterized during summer 2005. However, in the natural reserve, vegetation was integrally protected since the late 1970s, and no notable environmental change occurred between 2001 and 2005.

In general, there was a positive correlation between NDVI and host-seeking *I. ricinus*. Therefore, bottomland can be considered the habitat at the greatest risk for this tick species in Le Cerbaie and the European hornbeam as a plant indicator of risk. However, the above-mentioned correlation varied during the same, and different years, and the usefulness of NDVI in a risk assessment model for *I. ricinus* and zoonotic agents should be considered carefully. In fact, during the peak of activity, in spring 2005, nymphs were only weakly, or not correlated with NDVI and they were collected in most of the study area. Rainfall was relatively high during the period of tick collection in spring 2005, probably causing enough moisture to allow tick activity in dry upland habitat. However, *SD*, as evaluated from temperature and relative humidity measured at tick collection sites, was significantly lower in 2005 than in 2006 in May but not in other spring months. In spring 2006, host-seeking nymphs

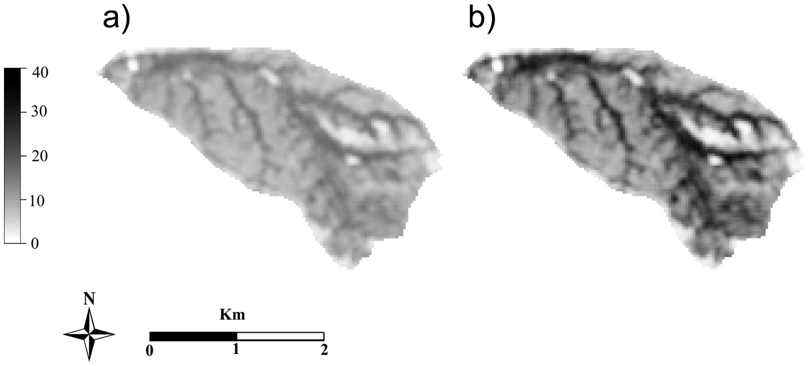

Fig. 6. Predictive maps of the numbers of host-seeking *I. ricinus* nymphs per 100-m dragging in Le Cerbaie Hills, Tuscany, Italy, based upon results of generalized estimating equations including normalized difference vegetation index and seasonal sine as predictors: 2005 peak activity season (a) and 2006 peak activity season (b).

were most abundant in bottomland habitat and positively correlated with NDVI. In summer, the correlation between ticks and NDVI was greatest in 2005, when rainfall was low and *SD* was relatively high. Our results suggest that rainfall preceding tick collection by dragging may modify the association of tick activity with habitat type. Meteorological conditions should therefore be included in predictive models for *I. ricinus* (Barandika et al. 2006).

Our study was carried out within a small, enclosed geographic area, and sampling of tick collection sites was specifically aimed at the comparison of the two main habitat types in the reserve. Therefore, although we provide useful information, our results cannot be generalized to large territories, and further research is necessary to test our hypotheses and to validate our statistical models at a larger geographic scale. Tuscany is characterized by a hilly landscape that might lead to variations in habitat suitability for *I. ricinus* as we found in Le Cerbaie. Moreover, due to intense outdoor working and tourist activities, risk assessment for ticks and tick-borne zoonoses is of major public health interest in this region. Among factors affecting the distribution of foci of zoonoses transmitted by *I. ricinus*, Estrada-Peña (2003) included habitat fragmentation and host movements, which should be also considered for *B. lusitaniae* in central Italy.

Numbers of questing nymphs per 100-m dragging in Le Cerbaie during the spring peak season (Fig. 5b) were greater than those found by Mannelli et al. (2003) in a Lyme borreliosis focus in the northwestern Italian region of Liguria (1.9 nymphs per 100 m dragging in April), but they were lower than those reported from the province of Trento (northeastern Italy), where Rizzoli et al. (2002) found up to 37.5 nymphs per 100 m. Such differences were probably associated with variable population densities of favorite hosts for adult ticks, such as the roe deer (*Capreolus capreolus*). These hosts can be estimated at <2/100 ha in Le Cerbaie, were absent from the study area in Liguria, and they reached 32 head per 100 ha in Trento. The prevalence of *B. burgdorferi* s.l. infection in host-seeking nymphs in Le Cerbaie (16.7%, Bertolotti et al. 2006) was also intermediate between Liguria (8.7%) and Trento (up to 37.5%), in agreement with the hypothesis that prevalence of infection in questing nymphs is positively associate with increased tick abundance. It is only above a certain threshold that increased density of deer, which are not competent reservoirs for *B. burgdorferi* s.l., can reduce prevalence of infection in spite of abundant *I. ricinus* populations (Tälleklint and Jaenson 1996).

Experimental studies on the phenology of *I. ricinus* in Britain and in northern Spain led to contrasting conclusions on the time of emergence of active tick stages. In fact, Randolph (2002) concluded that all stages emerge in autumn and a single cohort is sufficient to describe the life cycle. Conversely, in northern Spain, Estrada-Peña et al. (2004) observed a more complex cycle due to environmental conditions that are more similar to those of central Italy. We, however, did not find the bimodal temporal pattern of nymphs (including autumn peaks) that was observed in Spain, and experimental studies would help to clarify the phenology of *I. ricinus* in Tuscany.

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References Cited

Amore, G., L. Tomassone, E. Grego, C. Ragagli, L. Bertolotti, P. Nebbia, S. Rosati, and A. Mannelli. 2007. *Borrelia lusitaniae* in immature *Ixodes ricinus* (Acari: Ixodidae) feeding on common wall lizards in Tuscany, central Italy. J. Med. Entomol. 44: 303–307.

Barandika, J. F., E. Berriatua, M. Barral, R. A. Juste, P. Anda, and A. L. García-Pérez. 2006. Risk factors associated with ixodid tick species distributions in the Basque region in Spain. Med. Vet. Entomol. 20: 177–188.

Bertolotti, L., L. Tomassone, C. Tramuta, E. Grego, G. Amore, C. Ambrogi, P. Nebbia, and A. Mannelli. 2006. *Borrelia lusitaniae* and spotted fever group rickettsiae in *Ixodes ricinus* (Acari: Ixodidae) in Tuscany, central Italy. J. Med. Entomol. 43: 159–165.

Collares-Pereira, M., S. Couceiro, I. Franca, K. Kurtenbach, S. M. Schäfer, L. Vitorino, L. Goncalves, S. Baptista, M. L. Vieira, and C. Cunha. 2004. First isolation of *Borrelia lusitaniae* from a human patient. J. Clin. Microbiol. 42: 1316–1318.

Daniel, M., and F. Dusbábek. 1994. Micrometeorological and microhabitat factors affecting maintenance and dissemination of tick-borne disease in the environment, pp. 91–138. *In* D. E. Sonenshine and T. N. Mather [eds.], Ecological dynamics of tick-borne zoonoses. Oxford University Press, New York.

Diggle, P., P. Heagerty, K.-Y. Liang, and S. Zeger. 2002. Analysis of longitudinal data. Oxford University Press, New York.

Eisen, R. J., L. Eisen, and R. S. Lane. 2006. Predicting density of *Ixodes pacificus* nymphs in dense woodlands in Mendocino County, California, based on geographic information systems and remote sensing versus field-derived data. Am. J. Trop. Med. Hyg. 74: 632–640.

Estrada-Peña, A. 2003. The relationship between habitat topology, critical scales of connectivity and tick abundance *Ixodes ricinus* in a heterogeneous landscape in northern Spain. Ecography 26: 661–671.

Estrada-Peña, A., J. M. Martinez, C. S. Acedo, J. Quilez, and E. D. Cacho. 2004. Phenology of the tick, *Ixodes ricinus*, in its southern distribution range (central Spain). Med. Vet. Entomol. 18: 387–397.

Estrada-Peña, A., J. M. Venzal, and C. S. Acedo. 2006. The tick *Ixodes ricinus:* distribution and climate preferences in the western Palaearctic. Med. Vet. Entomol. 20: 189–197.

Fox, J. 2002. An R and S-Plus companion to applied regression. Sage Publications, Thousand Oaks, CA.

Gray, J. S., O. Kahl, J. N. Robertson, M. Daniel, A. Estrada-Peña, G. Gettinby, T. G. Jaenson, P. Jensen, F. Jongejan, E. Korenberg, K. Kurtenbach, and P. Zeman. 1998. Lyme borreliosis habitat assessment. Zentr. Bakt. 287: 211–228.

Kitron, U. 1998. Landscape ecology and epidemiology of vector-borne diseases: tools for spatial analysis. J. Med. Entomol. 35: 435–445.

Littell, R. C., W. W. Stroup, and R. Freund. 2002. SAS for linear models. SAS Institute, Cary, NC.

Manilla, G. 1998. Acari, Ixodida (Fauna d'Italia 36). Edizioni Calderini, Bologna, Italy.

Mannelli, A., G. Boggiatto, E. Grego, M. Cinco, R. Murgia, S. Stefanelli, D. De Meneghi, and S. Rosati. 2003. Acarological risk of exposure to agents of tick-borne zoonoses in the first recognized Italian focus of Lyme borreliosis. Epidemiol. Infect. 131: 1139–1147.

Mannelli, A., D. Cerri, L. Buffrini, S. Rossi, S. Rosati, T. Arata, M. Innocenti, M. C. Grignolo, G. Bianchi, A. Iori, and F. Tolari. 1999. Low risk of Lyme borreliosis in a protected area on the Tyrrhenian coast, in central Italy. Eur. J. Epidemiol. 15: 371–377.

Neteler, M., and H. Mitasova. 2002. Open Source GIS: a GRASS GIS approach. Kluwer Academic, Dordrecht, The Netherlands and Springer, Boston, MA.

Randolph, S. 2002. Quantitative ecology of ticks as a basis for transmission models of tick-borne pathogens. Vector Borne Zoonotic Dis. 2: 209–215.

Randolph, S. E., and K. Storey. 1999. Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. J. Med. Entomol. 36: 741–748.

Rizzoli, A., S. Merler, C. Furlanello, and C. Genchi. 2002. Geographical information systems and bootstrap aggregation (bagging) of tree-based classifiers for Lyme disease risk prediction in Trentino, Italian Alps. J. Med. Entomol. 39: 485–492.

Sarih, M., F. Jouda, L. Gern, and D. Postic. 2003. First isolation of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks in Morocco. Vector Borne Zoonotic Dis. 3: 133–139.

SAS Institute. 1999. SAS/STAT® user's guide, version 8. SAS Institute, Cary, NC.

Tälleklint, L., and T. G. Jaenson. 1996. Seasonal variations in density of questing *Ixodes ricinus* (Acari: Ixodidae) nymphs and prevalence of infection with *B. burgdorferi* s.l. in south central Sweden. J. Med. Entomol. 33: 592–597.

Younsi, H., M. Sarih, F. Jouda, E. Godfroid, L. Gern, A. Bouattour, G. Baranton, and D. Postic. 2005. Characterization of *Borrelia lusitaniae* isolates collected in Tunisia and Morocco. J. Clin. Microbiol. 43: 1587–1593.

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