

Overwintering Survival of Nymphal *Ixodes scapularis* (Acari: Ixodidae) Under Natural Conditions

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ABSTRACT Blacklegged ticks (*Ixodes scapularis* Say) are exquisitely sensitive to very cold and dry conditions. For this reason it has long been assumed that climatic differences among locations and within microhabitats have a strong influence on variation in their survival over winter. This assumption, however, rests largely on laboratory exposures and on broad-scale associations between climatic variables and the observed distributions of ticks. We present the results of a study of the overwintering survival of *I. scapularis* nymphs in their natural environment from October through May in two locations in New York State using a repeated sampling strategy to determine when mortality occurred, and whether those events coincide with extreme conditions. We then fit these data to a simple, flexible statistical model in which the hazard of mortality varies with measurable conditions, here minimum daily temperature and mean daily relative humidity. Regardless of winter conditions, >80% of ticks survived at both sites. A model with constant hazard (i.e., independent of temperature and humidity) was best supported by the data. Although models with hazard increasing at temperatures below 0 deg C and at >90% relative humidity provided slightly better fits to the data, these models were less parsimonious. These results weaken the expectation that cold-related overwintering mortality necessarily plays a major role in restricting populations of these ticks and thus, risk of tick-borne zoonoses.

KEY WORDS *Ixodes scapularis*, overwintering, survival, likelihood

Most ixodid ticks, including *Ixodes scapularis*, spend that vast majority of their life off of hosts living free in the environment subject to local climatic conditions and weather (Sonenshine 1993). Therefore, these conditions are thought to have a strong influence on the life history, phenology, and survival of these important vectors of zoonotic and animal pathogens.

In North America, larval *I. scapularis* eclose in the late summer and fall (i.e., August to September), quest for a bloodmeal, and molt into nymphs after feeding (Fish 1993). These newly molted nymphs must overwinter before emerging in the late spring or early summer (i.e., June to July) the following year to quest for their next (nymphal) bloodmeal host. Nymphal *I. scapularis* are the most epidemiologically important life stage for Lyme disease, human granulocytic anaplasmosis, and babesiosis because they are numerous, often infected, small, and thus, difficult to detect, and active in the late spring through summer when people spend the most time outside (Barbour and Fish 1993, Centers for Disease Control and Prevention [CDC] 2001). The transition from larva to potentially infectious nymph is thus a key aspect of disease risk. Nymphal overwintering surviving is an important part

of this transition and one that may be very sensitive to climatic changes.

Even short-term exposure to extreme cold in the laboratory can be lethal to *I. scapularis* (−16 to −11°C in cold-acclimated nymphs; Burks et al. 1996, VanDyk et al. 1996), as can several hours at low relative humidity (RH) (≤85% for larvae and nymphs; Stafford 1994, but see Rodgers et al. 2007). Cold, dry winters, among other measures of habitat suitability, have thus been expected to reduce overwintering survival and restrict the distribution and abundance of these important vectors (Estrada-Peña 2002; Brownstein et al. 2003, 2005).

In Europe, for instance, the range of *I. ricinus* has often been thought to be limited by cold temperatures at high latitudes, and the pole-ward and altitudinal spread of Lyme disease associated with climate warming (Lindgren et al. 2000, Daniel et al. 2003). In Switzerland tick densities decrease with increasing elevation, suggesting an altitudinal, presumably temperature-related, limit to tick survival (Jouda et al. 2004). *I. ricinus* was not found at elevations >700 m above sea level (asl) in the Czech Republic during the early 1980s, while more recent surveys have found populations at elevations as high as 1,100 m asl, presumably because of global warming (Daniel et al. 2003). Similarly, in Sweden ticks have spread northward as temperatures have increased over time (Lindgren et al. 2000). See Killilea et al. (2008) for a review of the relationship between the distributions of *I.*

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scapularis and *I. pacificus* and climate in North America.

Extrapolating from winter weather conditions to overwintering mortality, however, is not straightforward. First, overwintering ticks probably never experience the coldest winter temperatures owing to their ability to occupy protected microsites (i.e., leaf litter and soil, under snow) (Burks et al. 1996). Second, studies of broad-scale association between climatic variables and the observed distributions of ticks do not demonstrate whether temperature per se or some other factor correlated with climate is responsible for these changes in abundance and range. Third, climatic conditions in seasons other than winter might be more important in producing the observed patterns.

We present the results of a study of the overwintering survival of *I. scapularis* nymphs in the natural environment in two locations in New York State: at the Cary Institute of Ecosystem Studies in the southern Hudson Valley and at a SUNY College of Environmental Science and Forestry field station near Syracuse, NY. The former site has had abundant tick populations and high incidence of tick-borne disease for at least two decades (Ostfeld et al. 2006), whereas the latter has sparse tick populations (J.L. Brunner, personal observation) and incidence of tick-borne disease two orders of magnitude lower (CDC 2012). Our hypothesis was that if *I. scapularis* nymphs are vulnerable to cold, dry conditions, mortality rates would increase during particularly cold or dry periods.

Materials and Methods

This study was conducted at the Cary Institute of Ecosystem Studies (CIES), in Dutchess County (41° 47' 59.856" N, 73° 43' 44.004" W)—a tick and Lyme disease hotspot (Chen et al. 2005, Waller et al. 2007)—and at SUNY College of Environmental Science and Forestry's (ESF) Lafayette Road Field Station in Onondaga County (42° 59' 27.120" N, 76° 7' 58.020" E) where ticks are much more rare. While there no published data on nymphal densities at ESF, a study by Diuk-Wasser et al. (2006) estimated peak nymphal densities at Verona Beach State Park on Oneida Lake, ≈35 km from our site, at 20–25 nymphs/1,000 m²; nymphal densities at CIES are at least an order of magnitude higher (Ostfeld et al. 2006). These two sites are ≈238 km and ≈1.19° of latitude apart and vary by roughly 3°C in mean winter temperatures (December–February mean monthly temperatures from 2000 to 2007; data from CIES 2012 and National Climate Data Center [NCDC] 2012).

On 25 September (ESF) and 2 October 2009 (CIES) we deployed 23 (ESF) and 21 (CIES) groups of 25 nymphal ticks in soil core enclosures (described below). The nymphs were generated by feeding *I. scapularis* larvae collected from the CIES grounds on *Peromyscus leucopus* purchased from the *Peromyscus* Genetic Stock Center (University of South Carolina, Columbia, SC) then letting them molt in glass scintillation vials with moistened plaster of paris in the bottom. These ticks were in vials less than a month

before being deployed. Nymphs from different mice were mixed before being placed in the enclosures to avoid host-specific effects.

The tick enclosures were designed to maximize the ability of the ticks to respond to changing conditions and select appropriate microhabitat in the soil. Previous studies have found that survival is decreased when ticks do not have access to the soil (Bertrand and Wilson 1996) and cannot descend below the surface (Padgett and Lane 2001). We removed the surface leaf litter and then extracted 10 cm diameter × 5 cm deep soil cores using a lever-action golf hole cutter, which was then pushed intact into a 10 cm diameter × 5 cm tall PVC sheath with four 15 mm holes along its circumference. The cores and sheaths were then enclosed in organically cloth mesh bags and reinserted into the ground. The leaf litter was placed back on top of the soil and 25 nymphs were placed on the litter in the bags. The tops of the bags were sealed with plastic zip ties and then the enclosures were covered with protective cages (cubes ≈45 cm on a side) made from wire fencing arranged in 7.6 × 10.2 cm rectangles. The enclosures were arranged haphazardly within a 10 m-diameter circle in each site located in oak-maple forest stands.

We measured the temperature and RH near eight randomly selected enclosures with Hobo H8 Professional Series data loggers set to record every 30 min. To protect these loggers from precipitation and melting snow, they were glued to the underside of plastic Ziploc boxes (Racine, WI) with large holes cut from their sides such that they were suspended upside down ≈4 cm above the ground. The measurements were averaged among the loggers at a site and used to calculate daily minimum temperatures (T_{min}) and mean RH.

The day after the enclosures were deployed and then every 2 wk thereafter we dug up one core (and in the spring two or three), placed it in a Ziploc bag, and returned it to the laboratory to be searched for live nymphs. The last enclosures were removed 14 May (CIES) or 21 May 2010 (ESF). After being allowed to warm to room temperature for at least several hours each enclosure was searched for nymphs by hand in a white pan for 90 min (by which time we found 90–95% of the ticks, on average; data not shown) and later placed in a Berlese funnel for an hour. Enclosures with few recovered ticks were rechecked over several days at CIES. Enclosures at ESF were replaced in their bags and searched again in May. Two additional enclosures without any nymphs added to them were deployed at each site to determine whether our soil cores had free-living ticks in them already. These were dug up at the last collection date.

We constructed a small set of simple models describing the probability of nymphal survival through time where the hazard of mortality was either constant or changed with T_{min} or RH. Because the relationship between daily hazard of mortality and climatic variables is not necessarily linear (e.g., there may be threshold T_{min} or RH, as well as maximal values of the hazard rate) we made the hazard a three-parameter

Table 1. Parameter estimates and (SEs) for the three models fit to the data from the ESF and CIES sites

	Max hazard (deaths/wk)	Slope (hazard/°C or %RH)	Half-max (°C or %RH)	P_{detect}
ESF				
$\lambda(\cdot)$	0.0073 (0.0014)	—	—	0.965 (0.023)
$\lambda(T_{min})$	0.0024 (0.0005)	-14.614 (0.0001)	-0.305 (0.125)	0.949 (0.019)
$\lambda(RH)$	0.0029 (0.0006)	2.798 (0.003)	91.847 (2.473)	0.951 (0.020)
CIES				
$\lambda(\cdot)$	0.0013 (0.0020)	—	—	0.860 (0.041)
$\lambda(T_{min})$	0.0061 (0.0094)	-4.524 (0.957)	-12.867 (11.450)	0.854 (0.033)
$\lambda(RH)$	0.0138 (0.010)	18.048 (0.000)	94.539 (0.156)	0.978 (0.110)

logistic function of the T_{min} and RH. For instance, the hazard at time t for T_{min} at that time was written as:

$$\lambda(t) = m/[1 + \exp(-4s(T_{min}(t) - h))],$$

where m is the maximum daily hazard, s is the slope of the curve at the half-maximum value, and h is the T_{min} where the half-maximum occurs.

The cumulative hazard experience by each enclosure when it was dug up and returned to the laboratory at time t , $\Lambda(t)$, was calculated by summing up the daily hazards. Cumulative hazard integrates the hazard over the entire period during which cold temperatures are likely to be an important cause of mortality. The proportion of ticks expected to survive to this time was then calculated as $S(t) = \exp[-\Lambda(t)]$. Given the challenge of finding small ticks in soil, roots, and leaf litter we did not expect to be able to find all of the live ticks in enclosures. We thus modified $S(t)$ by multiplying it by a detection probability, $P_{detect} \leq 1$. The expected number of ticks found in each core was fit to the actual number of ticks that survived and were found by minimizing the negative log-likelihood, assuming binomially distributed errors (Bolker 2008).

The models were fit to the data from each site separately using the `mle2()` function in the `bbmle` package (Bolker and R Development Core Team 2011) in R (R Development Core Team 2011). Models were compared using Akaike's information criteria corrected for sample size (AICc; Burnham and Anderson 2002), which measures a model's fit to the data relative to its complexity (number of parameters). Lower AICc values denote stronger support. AICc weights (w_i) indicate the proportion of the evidentiary support for each model relative to the others.

Results

More than 80% of nymphs deployed in the fall of 2009 survived into the spring of 2010, operationally defined as 1 April 2010, regardless of location ($t = 0.834$; $P = 0.415$). At CIES $84.9 \pm 3.4\%$ (mean \pm SE) of the nymphs survived to spring while the percentage at ESF was $80.4 \pm 4.1\%$. Because no ticks were found in any of the control enclosures, which did not have ticks added to them, this high survival cannot be accounted for by contamination from naturally occurring nymphs. In addition, this may represent a slight underestimate of overwintering survival—there was strong support for our models that allowed for imperfect detection of nymphs in the enclosures (not

shown), with detection probabilities estimated at $\approx 95\%$ in the ESF enclosures and 85–98% in the CIES enclosure (Table 1).

The best-fitting model for both datasets, according to the AICc criterion, was the null model with a constant hazard of mortality ($\lambda(\cdot)$; Table 1, Fig. 1). The weekly hazard was significantly different from zero at ESF (95% CI: 0.0041–0.0099), but not at CIES (95% CI: <0 –0.0051), probably because of the larger uncertainty in detecting ticks (Table 1).

At ESF the models where the hazard was a function of minimum daily temperature, $\lambda(T_{min})$, or relative humidity, $\lambda(RH)$, were slightly better fits (lower values of the negative log-likelihood), but not enough to offset the extra parameters in the model. At CIES there was essentially no support for the more complex models as all of the models fit the data equally well (Fig. 1). It is important to note that because the more complex models (e.g., $\lambda(RH)$) provided an adequate fit to the data (goodness-of-fit tests: $\chi^2 = 0.686$, $df = 2$, $P = 0.710$ at ESF, $\chi^2 = 2.191$, $df = 2$, $P = 0.334$ at CIES), we were choosing among reasonable models (Burnham and Anderson 2002).

It is illuminating to look at the parameter estimates from the climate-related hazard models. Again, the models were flexible such that the hazard could increase or decrease, smoothly or suddenly, at any point along the range of conditions. Despite parsimony favoring models with constant hazard of mortality, we did observe some consistent patterns suggesting that certain conditions may increase the hazard of mortality. At ESF there was a sharp increase in estimated hazard when temperature dropped below 0°C, while at CIES this threshold was estimated at about -13°C, but with greater uncertainty (Table 2; Fig. 2). In contrast, hazard at both sites increased sharply when RH was high ($>90\%$; Fig. 2).

Contrary to our expectations, the loggers recorded temperatures that were on average warmer at ESF than CES (Figs. 1 and 2; mean T_{min} at ESF = -2.7°C, mean T_{min} at Cary = -4.9°C over the months of December, January, and February). RH was higher at ESF as well (Figs. 1 and 2; mean RH at ESF = 90.9%, mean RH at Cary = 65.9% during the winter months). The minimum daily air temperatures recorded by the weather station at CIES were $\approx 2.7^\circ\text{C}$ lower than those recorded by our logger whereas at ESF minimum daily air temperatures were 4.3°C cooler than those recorded by our temperature loggers. These data and our unpublished data are consistent with the hypoth-

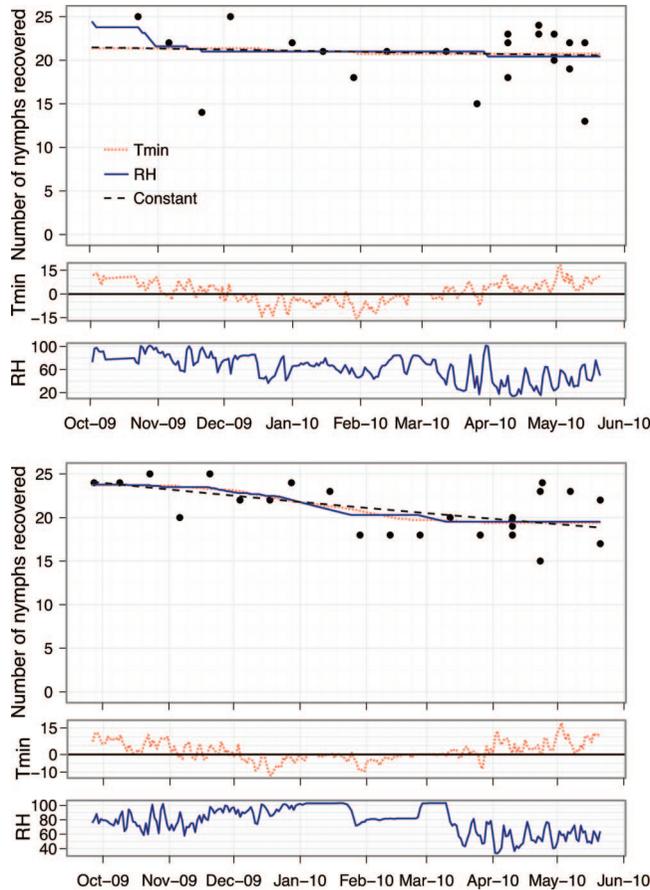


Fig. 1. The number of nymphal ticks recovered from soil core enclosure stocked with 25 newly molted nymphal *Ixodes scapularis* deployed 3 October 2009 at the Cary Institute of Ecosystem Studies (top) and 25 September 2009 at SUNY-ESFs Lafayette Road Experimental Station (bottom). The lines are the predictions from the model where the hazard of mortality is constant humidity (RH). The mean daily values of Tmin (°C) and RH (%) are shown below the figure for each site. (Online figure in color.)

esis that our loggers were insulated by snow more often at ESF than CIES. It is not clear whether the air temperature was different between our sites during our experiment—daily minimum air temperature measurements at weather stations on the Syracuse Airport (≈15 km north of our site) and CIES campuses were not significantly different over the months of December, January, and February (mean of −6.4°C at

ESF and −7.4°C at CIES; $t = 1.207, P = 0.229$)—but it is clear that both sites did experience winter conditions that would be expected to reduce tick survival.

Discussion

Our estimate of nymphal overwintering survival is higher than most found in the literature. This is probably due in part to differences in methodologies and designs of the enclosures. Ticks that can access and move into and out of the soil have much higher survival than those that are housed in bags or enclosures that prevent them from doing so (Bertrand and Wilson 1996, Padgett and Lane 2001), probably because ticks occupying protected microsites (i.e., leaf litter and soil) are protected from the coldest, driest winter conditions (Burks et al. 1996, Lindgren and Gustafson 2001); when restricted to the surface layer they are more likely to die. Ginsberg et al. (2004), for instance, placed fed larvae in the toe end of nylon stockings and then placed the stocking under leaf litter for the winter in deciduous and coniferous forests on Fire Island,

Table 2. Evidentiary support for models of nymphal overwintering survival where the hazard is constant, $\lambda(\cdot)$, is a function of the daily min. temp, $\lambda(\text{Tmin})$, or the daily mean relative humidity, $\lambda(\text{RH})$

Model	df	ESF				CIES			
		$-\ln(\mathcal{L})$	AICc	ΔAICc	w_i	$-\ln(\mathcal{L})$	AICc	ΔAICc	w_i
$\lambda(\cdot)$	2	53.23	111.07	0	0.828	66.33	137.32	0	0.988
$\lambda(\text{Tmin})$	4	51.85	113.93	2.86	0.047	66.33	143.16	5.84	0.003
$\lambda(\text{RH})$	4	51.37	112.96	1.89	0.125	65.77	142.04	4.72	0.009

$-\ln(\mathcal{L})$ is the negative log-likelihood, AICc is the Akaike information criterion, ΔAICc is the difference in AICc from the best model, and w_i is the Akaike wt for each model (Burnham and Anderson 2002).

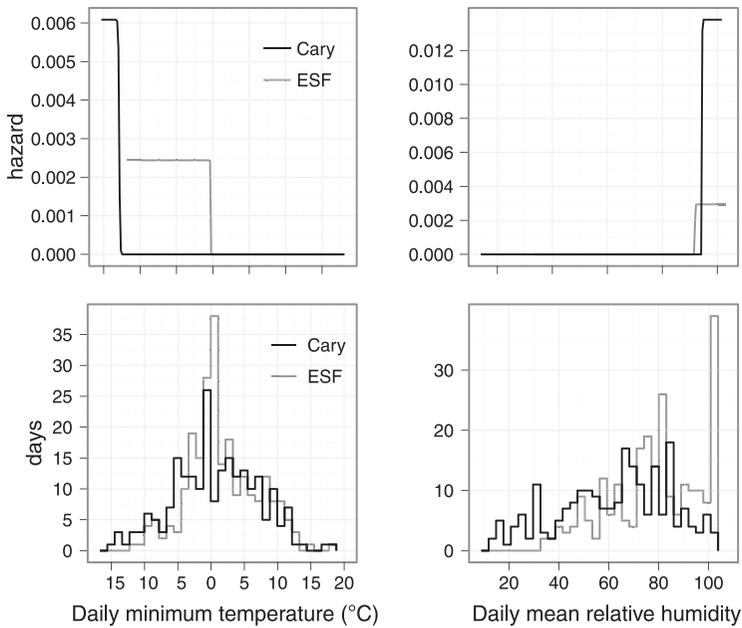


Fig. 2. The estimated relationships between hazard of mortality and daily minimum temperature (left) and mean relative humidity (right) and histograms showing the number of days that experienced those conditions at each site.

NY. They estimated overwintering survival at 26.7–31.3%, although this also included survival through molting. Lindsay et al. (1995, 1998), however, placed newly molted nymphs (and other stages) in polystyrene vials filled with sod plugs and with mesh-covered ends to allow moisture and air to penetrate. These vials were placed in larger outer housings in the ground at several locations in Long Point, Ontario, Canada. Overwintering survival in these nested enclosures ranged from 36.7 to 74.3%, depending on the site, although the mean was closer to 70%.

Lindsay et al. (1995, 1998) attributed difference among sites to habitat type (e.g., sandy dunes vs. maples forests), but more proximately to the availability of refuges from cold conditions. Again, the assumption is that duration and severity of winter conditions can strongly influence overwintering survival, especially in the absence of suitable refuges (Lindsay et al. 1995, 1998; Bertrand and Wilson 1996, 1997). Indeed, relatively warm, wet winters are commonly thought to be important aspects of habitat suitability and survival of *I. scapularis* and *I. ricinus* (Estrada-Peña 2002, Lindgren et al. 2000, Brownstein et al. 2003, Huss and Braun-Fahrlander 2007, Lindgren and Gustafson 2001, but see Ogden et al. 2005).

Based on these prior studies we expected hazard to increase as temperatures decreased. We did, in fact, find apparent low temperature thresholds below which hazard was estimated to increase rapidly (Fig. 2). These thresholds are broadly consistent with previous studies of cold sensitivity of *Ixodes* ticks (Burks et al. 1996, Vandyk et al. 1996), although the precise temperature at which ticks become vulnerable varies with the experimental methods and responses measured (e.g., proportion surviving cold, dry periods

versus temperature of freezing). Burks et al. (1996), for instance, found that the lower lethal temperature for short-term (2 h) exposure to cold was -11°C for nymphal *I. scapularis*, but suggested, based on their experiments with *Amblyomma americanum* (L.) and *Dermacentor variabilis* Say, that inoculative freezing, when ticks were exposed directly to ice crystals, might occur at just a few degrees below zero. This risk would be greatest when RH was high; saturated soils make it difficult for ticks to avoid contact with external ice crystals. Our results support this hypothesis. In both locations the $\lambda(\text{RH})$ model was the best-fitting model, although because of an additional two parameters it was not best supported. These models suggest that the greatest hazard occurred when RH was high (Fig. 2), indicative of saturated soils. Indeed our observations of saturated soils in the enclosures, often with ice crystals throughout, broadly correlate with these periods of high RH, suggesting that inoculative freezing is a real risk. The risk of desiccation, however, seems to be unimportant during the winter; transpirational water loss is reduced at colder temperatures (Burks et al. 1996) and RH presumably remains high in the soil under leaf litter and snow.

In the end, however, these more complex weather-related models received little support from our datasets (Table 2). Rather the hazard of mortality was effectively constant (and low) from late fall through spring (Fig. 1). The nymphal ticks placed in these enclosures presumably were able to find microsites within the soil or leaf litter that protected them from cold, dry, or wet conditions. Our CIES site was well within the range of high blacklegged tick density, but the ESF site does not currently support dense tick populations or frequent tick-borne disease (CDC

2012). Our results suggest that this difference is not related to the severity of winter conditions—indeed CIES was colder than ESF during this study. Of course, our results do not reject the possibility that winter conditions in climates colder than either of our sites affect tick mortality. ESF, however, appears to provide suitable winter conditions for the establishment of blacklegged ticks. More generally, the high overwinter survival of blacklegged ticks in an area (ESF) that currently supports only sparse tick populations and low incidence of tick-borne disease suggests caution in building habitat-suitability models based on climatic conditions within the current range of abundant populations. Scarcity of this species at ESF and its absence outside its current range could be because of dispersal limitation rather than unfavorable conditions.

In summary, we used a novel experimental design and analysis to evaluate the role of cold temperatures and RH on the hazard of mortality in overwintering nymphal *I. scapularis* in seminatural field conditions. Our likelihood based statistical models support a constant hazard of mortality, regardless of measured environmental conditions. Moreover, the strong majority of newly molted nymphs survived to the following spring. Our results suggest that this life-history transition is not limiting to populations of blacklegged ticks, at least at two very different locations in New York State.

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