FOLIA PARASITOLOGICA (PRAHA) 18: 261—266, 1971.

STRATIFICATION OF ENGORGED IXODES RICINUS LARVAE OVERWINTERING IN SOIL

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Abstract. A field experiment to determine the vertical stratification of overwintering engorged larvae of *Ixodes ricinus* in soil was conducted in the winter period 1969—1970 in the region of southern Moravia. More than 95 % of specimens were found to be overwintering in the upper soil strata 5 cm deep.

The exposure of ticks to the effects of different microclimate becomes apparent in their vitality, speed of development and other physiological functions, as already revealed by our previous studies on the importance of bushes in the survival of engorged females of *I. ricinus* (L.) (Černý 1959). From this aspect an extensive field experiment was set up aiming to study the developmental cycle of this species in closely situated habitats in different types of vegetation with consequent different microclimates. One of the studied problems was the observation of the course of overwintering and of the factors directly or indirectly influencing it. As a part of these studies also stratification of ticks in soil was investigated.

Due to the fact that the studies which were carried out a few years ago in our laboratories and which consisted in the processing of soil samples in Tullgren's apparatus, did not yield satisfactory results, we decided to conduct a field experiment with engorged larvae of *I. ricinus*. The experiment took place in southern Moravia (at the Valtice locality in the district of Břeclav) under strict maintenance of natural conditions.

MATERIAL AND METHODS

Two sites situated in different biotopes were chosen for the experiment. The first one was located in the open terrain overgrown with dense turfy grass (meadow biotope). The second site was located in deciduous forest of thermophilic oak type with a thick layer of leaf litter and humus. Pedologic characteristics are given in Table 1. In both sites a cylinder made of galvanized metal sheet 36 cm in diameter and 60 cm high was inserted in soil 40 cm deep. The dimensions of the cylinder were chosen in such a way that the limited area should cover roughly 0.1 m². When the cylinders were being inserted in the soil attention was paid to the fact that the soil column inside be left intact and its structure unchanged and also the surrounding environment be kept in the original state. The upper inside periphery of each cylinder (5 cm wide) was smeared with lanolin in order to prevent the ticks from escaping. On September 23, 1969 a total of 498 engorged larvae of *I. ricinus* were released in cylinders located in the meadow and 497 larvae in cylinders located in the forest; their development (i.e. from oviposition until engorgement prior to their transfer to cylinders) took place in the same biotope.

On January 29, 1970, when the localization of overwintering larvae became definitive in the course of winter, the cylinders were taken out together with the soil column. First, the litter layer (forest sample) and vegetation detritus (meadow sample) were separated and processed (further ground layers). Afterwards each column was divided into layers 5 cm thick. The stratification of the overwintering engorged larvae was ascertained by flotation method using saturated solution of crystalline calcium chloride p.a. ($CaCl_2 . 6 H_2O$) which had proved to be effective at 50% concentration in previous laboratory experiments. (The solutions $Na_2S_2O_3$ and K_2CO_3 showed similar effects in laboratory; their choice was influenced by practical aspects.)

During the experiment continuous measurements were taken of the temperature near the ground and at the height of 20 cm above the ground by means of thermistor and platinum probes until October 8. From October 9 measurements were carried out at the ground level and 10 cm deep in soil in both biotopes in close vicinity of built-in cylinders. At the same time recordings were made by the standard meteorological box situated in the forest growth and precipitation was observed in the open terrain.

CLIMATE AND MICROCLIMATE DURING THE EXPERIMENT

The recordings made by meteorological box in the vicinity of experimental sites are given in Table 2. The coldest months were December and January. One icy and four frosty days were recorded in November, 25 and 31 respectively in December, 18 and 19 in January until the end of experiment. A certain peculiarity of the winter in the given region was the continuous snow cover which had been lasting since November 26. It had been snowing for 15 days in December and for two days in January. The snow cover greatly influenced the course of temperatures near the ground and 10 cm deep in soil as shown in Table 3. While the 10-day-differences between minimum and maximum temperatures taken 10 cm deep in soil were generally small, near the ground they dropped to 2 °C and lower only since the beginning of December after the snowfall. With the progress of winter these temperature differences got gradually balanced and in the last ten days of January did not reach even 1 °C. Throughout the experiment maximum daily temperatures in individual 10-day-periods, except one, were in both soil zones higher in the meadow, as well as the minimum temperatures taken 10 cm deep in soil. Minimum temperatures near the ground were higher in the meadow at first, then for about two months in the forest and at the end of our studies in the meadow again. Protective influence of snow cover was evident. While the lowest temperature

Table 1. Analysis of soil in experimental sites*

Biotope	Soil layer in cm	Percentage of individual categories** of soil granules				Classification of soil after Kopecký	active pH
		I	II	III	IV		
Meadow	0—5	27.14	23.06	13.16	36.64	sandy loam	7.00
	5—10	27.20	21.06	20.70	31.04	sandy loam	6.75
	10—15	47.76	20.40	5.04	26.80	mud loam	7.20
	15—20	54.26	19.18	22.50	4.06	clay-loam soil	7.50
	20—25	32.66	26.70	19.44	21.20	sandy loam	7.60
Forest	0—5	38.16	15.82	6.44	39.58	sand-clay-loam soil	7.50
	5—10	20.10	50.16	15.80	13.94	loam	7.55
	10—15	24.66	53.08	13.40	8.86	loam	7.55
	15—20	24.16	51.72	13.64	10.48	loam	7.70
	20—25	19.10	47.78	18.06	15.06	sandy loam	7.75

* Carried out by the Research Institute of Forestry and Gamekeeping, Opočno.

** Details see in: Klika, Novák, Gregor (1954).

recorded in the meteorological box was —18.2 °C, the lowest value near the ground was —0.7 °C or —1.2 °C. With the progress of winter also a gradual chilling of soil 10 cm deep could be observed. At the end of experiment the soil in the meadow was chilled 12 cm deep, in the forest only 5 cm deep.

Table 2. Weather characteristics at Valtice during the period of the experiment

Month	IX	X	XI	XII	I
maximum temperatures in °C	15.5–23.0	10.3–21.2	0.6–18.0	–12.8–1.5	–7.0–6.0
minimum temperatures in °C	5.0–10.2	0.3–10.2	5.3–7.7	–18.2–(–1.0)	10.2–(–0.9)
number of icy days with T max ≤0.1 °C	0	0	1	25	18
number of frosty days with T min ≤0.1 °C	0	0	4	31	29

RESULTS

A survey of the tick numbers found is given in Table 4. It shows that more than 95 % of specimens found passed the winter in the upper soil strata 5 cm deep. Only single specimens occurred deeper, the lowest layer containing them having been 15—20 cm. Strata deeper than that were always negative.

But there was a marked difference in the stratification of engorged larvae released in the meadow and in the forest. The former occurred primarily on the ground, the latter in the soil 0—5 cm deep. The larvae were not dispersed in a certain layer regularly, but were concentrated in various sections of the area delimited by the cylinder, apparently due to the structure of the soil favourable for penetration. Of the total number found 71.1 % of larvae were mobile in the meadow, 87.5 % in the forest. Immobile larvae were partly dead specimens, partly specimens in the state of immobile stage of metamorphosis. Remarkable was the finding of 17 nymphs in the meadow area, representing 7 % of the total number of flotated ticks. These nymphs evidently originated from the engorged larvae which had undergone metamorphosis as early as before hibernation. The state of larvae found was determined after the experiment was completed.

DISCUSSION

It is known from literature that the engorged tick individuals, due to negative phototaxis and positive thigmotaxis, try to find shelter in vegetation litter, soil crevices and other possible places (Balashov 1967 and others). It has not been demonstrated, however, whether the sites sought after for metamorphosis immediately after falling off are also used for hibernation, or whether they are exchanged later on with the advent of winter. Our findings show that the large majority of engorged *I. ricinus* larvae remain on the ground or in the uppermost layer of 5 cm at least in sites with similar characteristics as in biotopes studied. There are certain differences in the stratification of larvae in both zones, depending on the general character of the given biotope.

If we assume that the larvae take their position for overwintering shortly after falling off their host, then in our case the temperature should not have had a decisive effect on the selection of suitable zone, because it varied within the range allowing the

Table 3. Survey of minimum and maximum temperatures in °C taken daily at 7,14 and 21 hours in individual 10-day-periods

Month	10-day period	Meadow		Forest	
		ground level	—10 cm	ground level	—10 cm
IX.	3	10.0—20.8	—	9.1—16.3	—
X.	1	8.1—18.0	—	8.1—14.2	—
	2	6.1—15.1	11.0—12.8	9.0—14.0	10.8—12.1
	3	4.8—14.8	10.1—12.8	8.0—12.9	9.2—11.7
XI.	1	—0.4—12.0	6.1—11.4	3.7—11.6	6.8—10.2
	2	2.3—10.2	6.0—11.0	4.0—10.0	5.9—9.4
	3	1.4—9.0	4.2—9.0	2.1—8.9	3.7—8.2
XII.	1	—0.6—1.5	2.8—4.1	—0.1—1.9	1.9—3.1
	2	0.3—2.1	2.8—3.8	0.3—1.5	1.5—2.4
	3	—0.3—0.8	2.1—2.9	—0.7—0.4	0.6—1.5
I.	1	0.0—0.8	2.2—3.0	—0.5—0.7	0.5—1.6
	2	—0.4—0.5	2.0—2.8	—1.0—0.3	—0.1—1.1
	3	—0.7—(—0.1)	1.8—2.1	—1.2—(—0.5)	—

Table 4. Numbers of overwintering ticks at individual depths

Depth \ Location	Meadow						Forest	
	L	%	N	%	L + N	%	L	%
ground level	143	63.5	7	41.1	150	62.0	21	21.9
0—5 cm	76	33.8	6	35.3	82	33.9	73	76.1
5—10 cm	0	0.0	2	11.8	2	0.8	1	1.0
10—15 cm	4	1.8	2	11.8	6	2.5	1	1.0
15—20 cm	2	0.9	0	0.0	2	0.8	0	0.0
20—25 cm	0	0.0	0	0.0	0	0.0	0	0.0
in total	225	100.0	17	100.0	242	100.0	96	100.0

L — engorged larvae, N — unengorged nymphs

movement activity of ticks. Another explanation ought to be looked for in the properties of the soil basis on which the engorged larvae occurred. Although the granularity of the soil particles as well as pH in both sites did not differ on the whole there were considerable differences in the compactness of the basis in both sites. The upper soil layer in the meadow was considerably turfy, compressed and penetrable with difficulty, while in the forest it was light and crumbly, with addition of humus and consequently easily penetrable. These properties probably played a decisive role in the stratification as demonstrated in our experiment.

If we admit that after falling off the engorged larvae still move about for a longer period before they find a definitive site to survive the winter, a certain influence of temperature must be anticipated which conditions the selection of such a place. It may be assumed that the ticks will select a layer with optimum temperature. The problem of temperatures preferred by larvae and nymphs of *I. ricinus* were studied under laboratory conditions by Totze (1933) and MacLeod (1935) who found out that certain temperatures preferred depended on temperatures at which the ticks had lived previously. The ascertained optimum temperatures ranged between 14° and 23 °C. Our previous findings of active unengorged nymphs at the temperature of 1.5 °C (with the night minima of —1.0 °C, (Černý 1957)) indicate that under natural conditions the ticks can move about even at lower temperatures than given by both above-mentioned authors. In this case the period until the end of November (the onset of freezing and snow cover) characterized by higher mean temperatures, should be taken into consideration as the time limit for seeking out a favourable zone for survival in winter. In the mentioned period a considerable fluctuation of daily temperatures near the ground were observed especially in the meadow, while the temperatures ascertained 10 cm deep in soil varied within the range of ground temperatures. Throughout this period higher daily minimum temperatures taken 10 cm deep in soil both in the meadow and forest areas were registered and the same results were obtained while measuring the mean daily temperatures. In view of the minimum temperatures in both cases the lower layer proved to be more favourable. The maximum daily temperatures taken in the forest and meadow areas in the individual 10-day-period were higher at the ground level, but on individual days there were some deviations. The mean daily temperatures and their maxima observed in 10-day-periods were markedly higher in the layer 10 cm deep in the meadow area, while in the forest area the maxima of mean daily temperatures were somewhat higher near the ground at first, but in the layer 10 cm deep in soil in November. In view of the maximum temperatures the conditions in the meadow area were more favourable in the soil layer, while in the forest area they were favourable near the ground. If we compare these conclusions with the actual findings of ticks, it may be said that from the above aspect the temperature was not a decisive factor for their stratification. The explanation could be found either in the properties of the soil, as mentioned above, or in the influence of other factors, such as humidity, concentration of CO_2 etc., which had not been studied. The elucidation of all causes conditioning the selection of certain stratum for survival in winter, will require further investigations. Remarkable was the finding of nymphs in the meadow area, indicating that a certain number of larvae engorged in the second half of September are capable of metamorphosing into nymphs in the same season. The recent studies on the developmental cycle of *I. ricinus* under natural conditions in this country showed that only larvae which have fed in August and earlier are moulting in the same season (Černý 1958, Chmela 1969). Our finding confirms Chmela's observations (1969) to the effect that in some specimens of the tick population the whole development may be completed within two years.

CONCLUSIONS

In the winter season 1969/1970 a field experiment was carried out in order to find out the vertical stratification of overwintering engorged larvae of *I. ricinus* in the soil. In two biotopes (thermophilic oak forest and meadow) a total of 995 specimens were released (23. 9. 1969) in two metal sheet cylinders inserted 40 cm deep in soil and each covering an area of 0.1 m². In mid-winter (29. 1. 1970) soil samples were collected from each area and processed by flotation method in saturated solution of $CaCl_2$. More than 95 % of specimens found by us passed the winter in the upper layers (5 cm deep in soil). Only single ticks were found deeper than that. Layers deeper than 20 cm were quite negative. A marked difference was observed between the stratification in the forest (most larvae passed the winter at the depth of 5 cm) and in the meadow (most larvae passed the winter directly on the ground level).

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Received 6 January 1971.

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