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OVERWINTERING OF THE TICK IXODES RICINUS (L.) UNDER CONDITIONS OF A FIELD EXPERIMENT

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Abstract. In the south-Moravian thermophilic oak forest a field experiment was carried out in which the overwintering conditions of the tick *Ixodes ricinus* (L.) were studied. A special attention was paid to: 1. the way in which various developmental stages of *I. ricinus* (unfed and engorged) survive in different soil depths; 2. the microclimatic conditions they meet with in different soil layers and 3. the way in which the tick microenvironment is affected under experimental conditions, if the ticks are placed in terrain in cages made of different material.

In one of our previous papers (Daniel and Černý 1967) we pointed out the necessity of exact studies of microclimate in the research of tick ecology, because the data supplied by standard meteorological stations are very inaccurate for this purpose. At the same time we recommended for such measurements the method worked up by Daniel (1965) which he used for measuring the nest microclimate of small mammals and birds.

On the basis of this method we decided to analyse the effects of microclimate on the developmental cycle of *Ixodes ricinus*. In the first stage of our studies we directed our attention to the analysis of overwintering of ticks in order to use the results obtained in further studies of the whole cycle. Accordingly, we formulated the main questions: 1. In what way do various developmental stages of *I. ricinus* (unfed and engorged) survive in different soil depth; 2. what microclimatic conditions do they meet with in different soil layers; 3. in what way is the tick microenvironment under experimental conditions affected, if the ticks are placed in terrain in cages and what is the difference, if the cages are made of different material.

The field experiment was carried out with technical assistance of ecology group workers of the Institute of Parasitology, Czechoslovak Academy of Sciences. The mathematical calculations were done by the team of workers of the Mathematical Centre of Biological Institutes, headed by Ing. M. Chytil. To all of them we express our sincere thanks.

MATERIAL AND METHODS

PROCEDURE OF EXPERIMENT

The field experiment was carried out in the winter of 1966—1967 (between November 20, 1966 and March 29, 1967) under conditions of south-Moravian thermophilic oak forest (Valtice locality near Břeclav). A total of 484 adults, nymphs and engorged larvae of *I. ricinus* [60♂ (unfed), 60♀

(unfed), 50 nymphs (engorged), 165 nymphs (unfed), 149 larvae (engorged)] and an unspecified number of unfed larvae were used in the experiment. All ticks were reared in laboratory where they were kept under standard conditions (+22 °C, 90 % RH). The ticks were placed in cylinder-shaped cages 50×25 mm large, each made of a wire frame covered with silon fabric. Each cage contained either 10 adults or engorged nymphs, or 15 unfed nymphs, 25 engorged larvae, or unspecified number of unfed larvae. The cages were deposited 0, 10, 20, 30, 40 and 50 cm deep in undisturbed soil layers. (This was achieved by digging a pit in the experimental site and by drilling tunnels of the same diameter as that of the cages at particular depth. The cages were inserted in the tunnels which were stopped with the dug-out soil.) Pedologically the soil was classified at the depth of 40 cm as loamy sand (pH 7.50—7.75), at 50 cm as loam with pH 7.90 [classification after Kopecký's scale (cit. Klika et al.1954)]. The analysis of soil samples was done by the Research Institute of Forestry and Gamekeeping in Opočno near the Orlické Mountains. In order to trace the influence of cages made of different material, additional cages of similar size and shape made of plastic (novodur), metal (dural), glass and wood were used, containing 385 specimens of *I. ricinus* at the depth of 10 and 20 cm [40♂ (unfed), 60♀ (unfed), 35 nymphs (engorged), 150 nymphs (unfed), 100 larvae (engorged)]. After closing the experiment all cages were taken out and the number of ticks counted. The engorged specimens which survived were observed in laboratory as regards their ability for subsequent metamorphosis. The unfed specimens were allowed to feed within the week since the end of overwitering and their ability for further development was determinated. Those specimens which did not moult and the nymphs which failed to engorge and perished, were regarded as specimens with disturbed development.

MEASUREMENTS AND RECORDING OF MICROCLIMATE

At particular depths temperature was taken and continuously recorded inside the cages as well as in the soil. The measurements were taken with thermistor sensing probes made from a pearl thermistor (type 11 NR 17-282, manufactured by Tesla, Šumperk) and with platinum resistance thermometers (Pt K 100, manufactured by Závody průmyslové automatizace, Ústí n/Lab.), whose resistance at 0°C was 100 ohms. The recordings were carried out by means of multirecorders which marked the data on 6 dotted lines and which were provided with a measuring system consisting of a simple revolving coil (system Depréz) for thermistor thermometers or two lattice-wound coils connected with Wheatstone's bridge used for measuring by platinum resistance thermometers (see Daniel 1965 and 1970). The soil humidity in particular layers was measured once daily by method of plaster blocks (Bouyoucos's method, see Slavík 1965).

MEASUREMENTS AND RECORDINGS OF MESO- AND MACROCLIMATE

The mesoclimate (understood as climate of the oak forest in which the experimental area was situated) was observed by a standard meteorological box. The data on the macroclimate of the surrounding area were obtained from the State meteorological station at Lednice (11 km away from the experimental site).

ASSESSMENT OF DATA OBTAINED BY MICROCLIMATIC MEASUREMENTS

Continuous recording curves were digitalised by Oscar apparatus. Due to their smooth course only 3 values per day (7, 14 and 21 hours) were read, which together with other data, were punched on computer tape. For humidity and temperature of each soil layer the following characteristics were calculated: arithmetic mean, average deviation, variance, value of range observed (not identical with daily maximum and minimum) and as regards temperature — the mean absolute difference for considering the speed at which the mean daily temperature is changing.

While studying the influence of cage on its outer environment we compared the mean daily temperature inside the cage with that in the soil layer at the given depth. As a basis were taken differences of mean daily temperatures inside the cage deducted from mean daily temperatures of soil. These differences, however, are mutually dependent significantly and this fact would make the exact statistical testing very complicated; we therefore gave them up.

EVALUATION OF SURVIVAL AND FURTHER DEVELOPMENT OF TICKS

While studying the survival of ticks it was necessary to determine its dependence on two factors, i.e. the depth of soil and the developmental stage (including different physiological state) of the tick. The most convenient method chosen was the analysis of variance with two-way classification.

Some groups had to be eliminated: unfed larvae (where the number of specimens was not countable) and engorged nymphs (where the cage situated on the soil surface got destroyed.) The problem demanded a simultaneous study of the effects of depth and developmental stages and therefore it was impossible to use the procedure of "adding the missing observations". The method of the analysis of variance did not allow to avoid the missing data in other way than by elimination of the whole group of engorged nymphs. The initial material was the ratio of survived ticks to the number of ticks used in the experiment. As the initial numbers were not always equal and in this way the prerequisite for equal variances of particular variables (i.e. ratios) was disturbed, it was necessary first to carry out the transformation according to the formula:

$$z = \arcsin \frac{y}{n}$$

(where y = number of survived ticks; n = number of ticks used in experiment)

which stabilizes the variances (Scheffe 1963). This transformation was done according to the tables of the function arcsin.

Further development of ticks was evaluated by the same method (the analysis of variance with two-way classification). In both cases the analysis of variance showed significant differences for one factor and insignificant ones for the other. As a next step the analysis of one-way classification (according to significant factor) was therefore carried out and the classification according to insignificant factor was left out of consideration. The importance of classes of significant factor was tested on computer by Duncan test.

While studying the influence of cages on the survival of ticks three factors were actually traced by method of the analysis of variance with three-way classification which was most convenient for this purpose. The influence of the following factors was observed: a) groups of developmental stages; b) material of which the cages were made; c) survival at different depth. Furthermore, the action specific for each of three pairs of mentioned factors, i.e. the interaction was studied. The influence of cage on its internal environment was traced in case of standard cages made of silon fabric.

RESULTS

THE COURSE OF CHANGES IN CLIMATIC CONDITIONS

The organisation of experiment (studies in one biotope with possible control of ticks before and after experiment only) did not offer any possibility to ascertain direct relations between the measured temperatures of macro- and mesoclimate and the tick survival. We were therefore compelled to outline only conditions under which the experiment took place. The relation of meso- and macroclimate is discussed in a separate paper. The course of changes is shown in Figs. 1 and 2.

As far as the soil microclimate in particular layers is concerned, the overwintering ticks showed a low sensitivity to differences registered in the experiment and consequently the relation between the survival and soil microclimate, which is characterized in Tables 1 and 2 and in Figs. 3—7 could not be mathematically expressed.

Table 1. Mean monthly temperatures in particular soil layers (in °C)

month \ layer	surface	10 cm	20 cm	30 cm	40 cm	50 cm
XII	1.57	1.65	2.11	2.67	3.31	3.59
I	—0.40	—0.29	0.14	0.71	1.42	1.50
II	—0.18	—0.42	—0.15	0.30	0.91	0.92
III	3.28	2.48	2.62	2.32	2.57	2.55

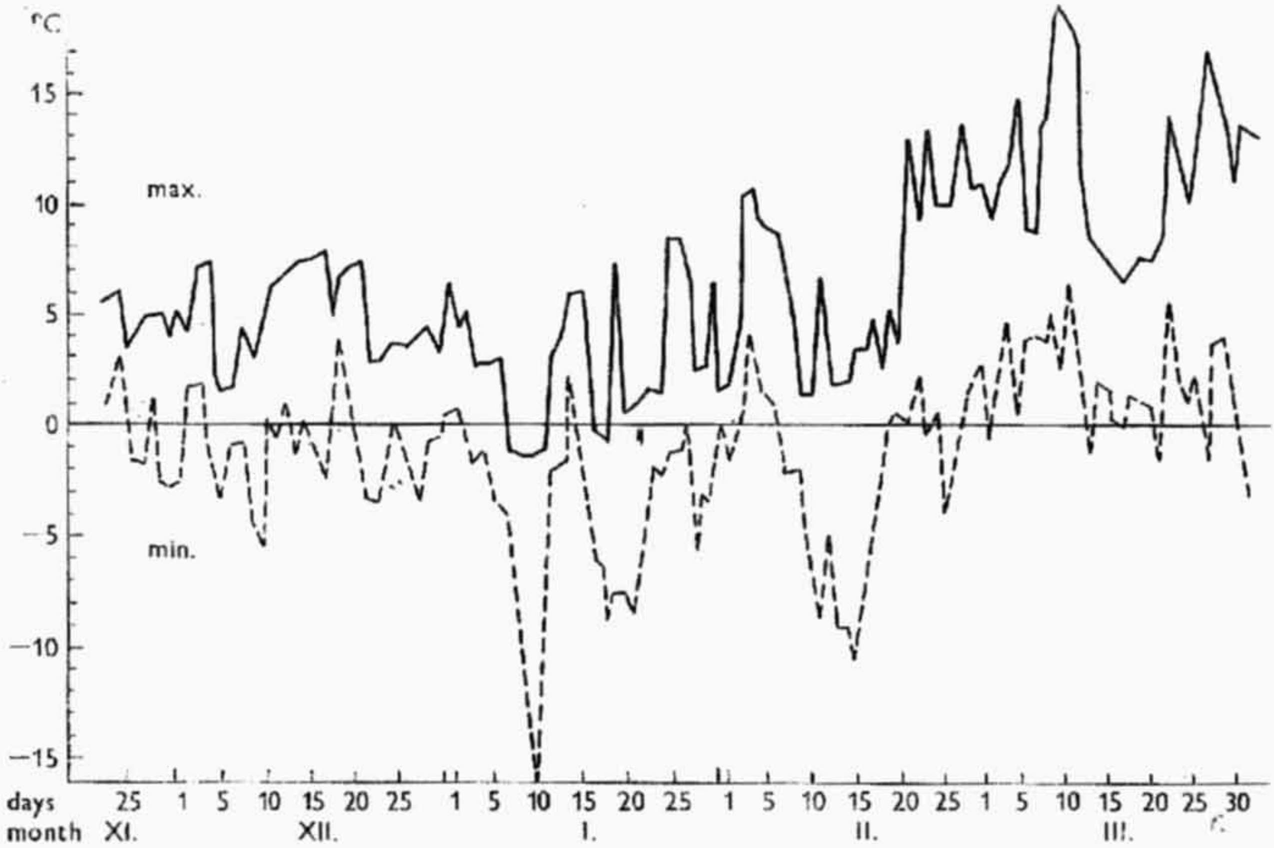

Fig. 1. Macroclimate of experimental area according to data of the State hydrometeorological service in Lednice (— maximum daily temperatures; ---- minimum daily temperatures)

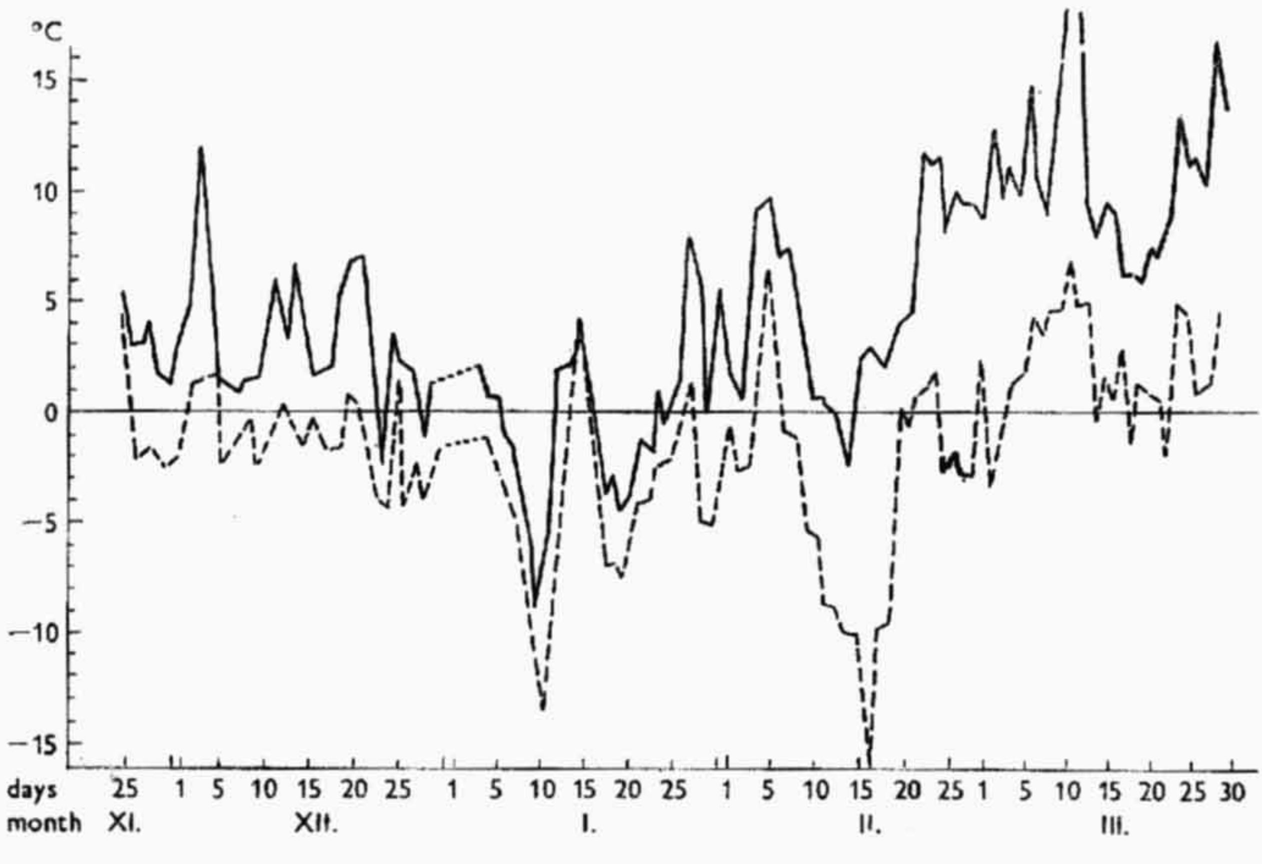

Fig. 2. Mesoclimate of experimental site (— maximum daily temperatures; ---- minimum daily temperatures)

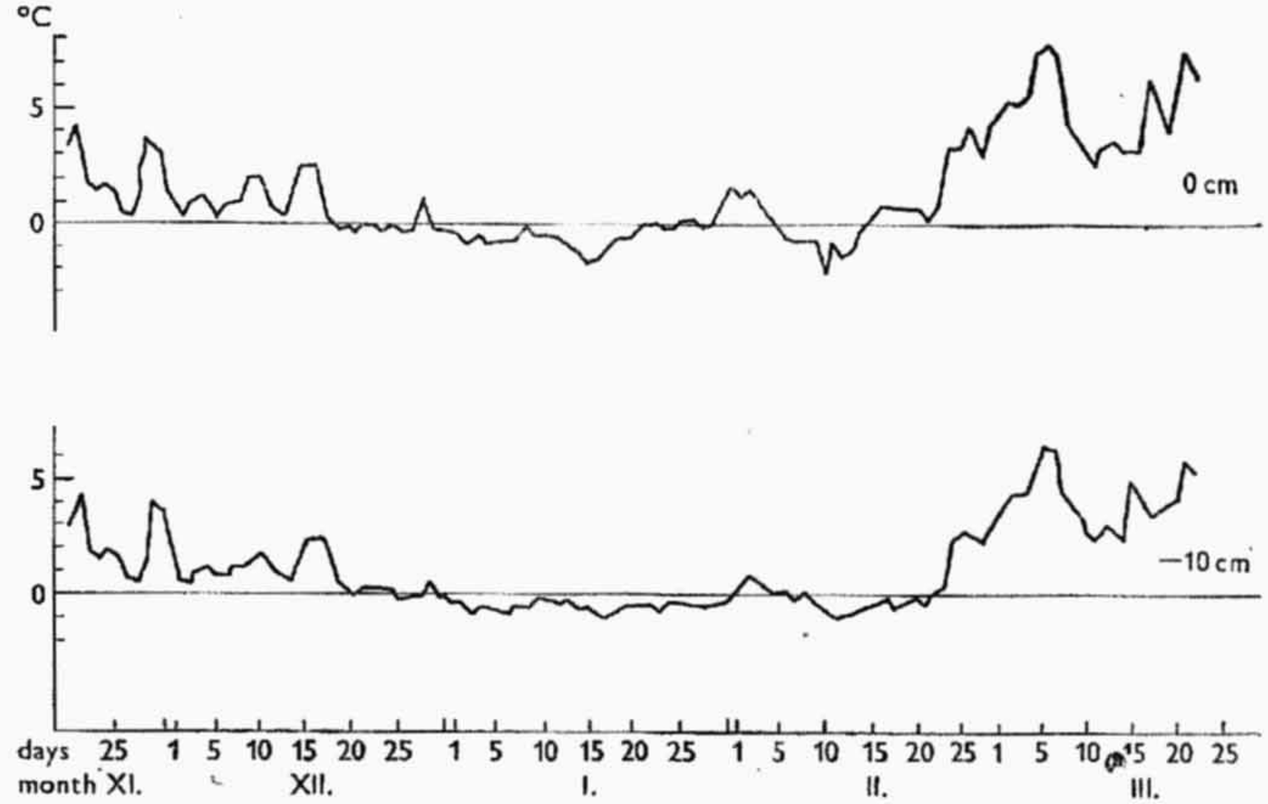

Fig. 3. Soil microclimate: course of changes in daily mean temperatures on surface and at depth of 10 cm

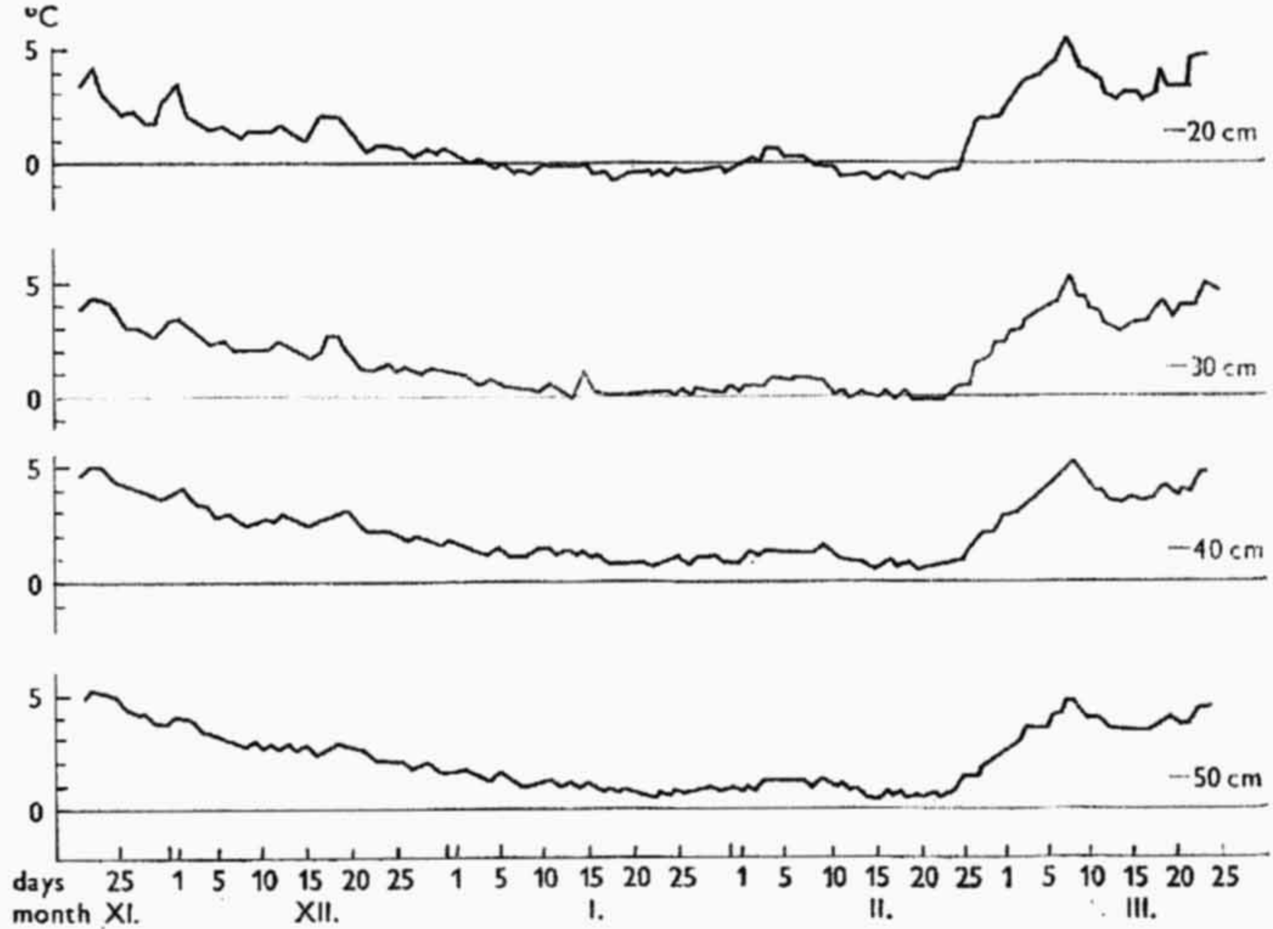

Fig. 4. Soil microclimate: course of changes in daily mean temperatures at depth of 20, 30, 40 and 50 cm

Table 2. Mean humidity in particular soil layers (in %)

soil layer	mean humidity in %
surface	49.9
10 cm	24.8
20 cm	56.4
30 cm	30.4
40 cm	34.6
50 cm	35.8

SURVIVAL OF TICKS DURING OVERWINTERING

The ratios of survived ticks to ticks studied in particular soil layers are given in Table 3. The values obtained were processed by analysis of two-way classification and yielded results compiled in Table 4. One asterisk marks the value significant at 95% level; two asterisks — at 99% level; NS-nonsignificant value (regarding 95% level).

Table 4 shows that the probable survival of ticks *I .ricinus* depends significantly on groups formed according to developmental stages and state of engorgement (engorged larvae, unfed nymphs, unfed females, unfed males). The dependence on the depth has not been demonstrated. In the group of unfed males, nevertless there was a slight dependence. During the general analysis of variance this difference might have been erased by other groups. For the group of unfed males the method χ^2 was therefore used in calculating D = 16.60 with 5 degrees of freedom, which exceeds the 1% critical value, so that the dependence on depth is highly significant. At the depth of 20 cm and deeper more males of *I. ricinus* survived than at the depth of less than 20 cm.

To the result of the analysis of variance was added the arrangement of arithmetical means for groups and with the testing of differences among them. Duncan test, however, yielded no significant differences (all results were close to 5% level). Unfed larvae and engorged nymphs could not be included in statistical classification (see methods).

In case of unfed larvae it could by only estimated that the number of both, the surviving and dead specimens, was roughly the same up to the depth of 20 cm. At deeper layers most specimens survived. 90—100% engorged nymphs survived.

More distinct results were obtained by investigation of further development of larvae and nymphs using the same methods. Table 5 shows ratios of the tick number with disturbed development to the tick number used in the experiment. The value for the depth 0 cm had to be left out because in the group "engorged nymphs" at this depth there was none. The analysis of variance with two-way classification yielded results given in Table 6. The results show that further development towards successful metamorphosis or successful engorgement depends significantly on the physiological state of the overwintering tick stage. The dependence on depth was not demonstrated. In this case we can arrange a decreasing series according to the ratio of specimens with development disturbances:

unfed nymphs	—	engorged larvae	—	engorged nymphs
71%		40.8%		38%

At 5% level there is a significant difference between unfed nymphs on one hand and engorged nymphs and larvae on the other. The unfed overwintering ticks had more disturbances in development.

Table 3. Survival of ticks *Ixodes ricinus* in particular soil layers.

Numerator indicates values of surviving specimens, denominator—values of specimens studied

Tick groups	0	10	20	30	40	50
engorged larvae	12/24	16/25	9/25	19/25	14/25	23/25
unfed nymphs	23/30	15/15	27/30	28/30	20/30	26/30
unfed females	5/10	5/10	6/10	8/10	8/10	7/10
unfed males	1/10	3/10	8/10	5/10	8/10	7/10

Table 4. The analysis of variance, with two-way classification obtained from values given in Table 3

Source of variability	Sum of squares	Degrees of freedom	Quotient	F
Rows (groups)	0.559	3	0.186	3.875*)
Columns (depth)	0.332	5	0.066	1.375 NS
Residual variance	0.720	15	0.048	—
Total variance	1.611	23	—	—

*) The value significant at the level of 95 %.
NS The value statistically insignificant (at the level of 95 %).

Table 5. Influence of overwintering on the development of ticks *Ixodes ricinus* in particular soil layers

Numerator indicates number of ticks with disturbed development, denominator—number of specimens studied

Tick groups	10	20	30	40	50
engorged larvae	10/25	9/25	16/25	6/25	10/25
unfed nymphs	13/15	27/30	24/30	16/30	16/30
engorged nymphs	6/10	4/10	3/10	2/10	4/10

Table 6. The analysis of variance, with two-way classification obtained from values given in Table 5

Source of variability	Sum of squares	Degrees of freedom	Quotient	F
Rows (groups)	1.746	2	0.873	11.19**)
Columns (depth)	0.834	4	0.208	2.67 NS
Residual variance	0.625	8	0.078	—
Total variance	3.205	14	—	—

**) The value significant at the level of 99%.
NS The value statistically insignificant (at the level of 95 %).

For statistical calculations we used variants differing in the cage material, depth of insertion and developmental stages of ticks in combination with various physiological state. In the final assessment we used engorged larvae, unfed nymphs, unfed females and unfed males. Values of the ratio of survived ticks to ticks used in the experiment

Table 7. Survival of ticks *Ixodes ricinus* depending on cage material. Numerator indicates values of surviving specimens, denominator — values of specimens studied

Tick groups	cage material	10 cm	20 cm
Engorged larvae	nylon fabric	0/10	10/10
	glass	4/10	10/10
	metal	8/10	0/10
	plastic	3/10	5/10
	wood	10/10	8/10
Unfed nymphs	nylon fabric	15/15	8/15
	glass	9/15	14/15
	metal	14/15	9/15
	plastic	2/15	10/15
	wood	9/15	9/15
Unfed females	nylon fabric	6/6	1/6
	glass	5/6	4/6
	metal	5/6	6/6
	plastic	5/6	5/6
	wood	5/6	3/6
Unfed males	nylon fabric	1/4	1/4
	glass	0/4	2/4
	metal	1/4	2/4
	plastic	4/4	4/4
	wood	2/4	3/4

Table 8. The analysis of variance, with three-way classification obtained from values given in Table 7

Source of changeability	Sum of squares	Degrees of freedom	Quotient	F
A	0.467	3	0.156	0.657
B	0.148	4	0.037	0.156
C	0.023	1	0.023	0.097
Interaction AB	3.041	12	0.253	1.070
BC	0.650	4	0.162	0.686
AC	0.433	3	0.144	0.609
Residual variance	3.079	13	0.237	—
Total variance	7.841	40	—	—

A — groups (developmental stages)
B — cage material
C — soil depth

are presented in Table 7. Table 8 was calculated from it by the analysis of variance with three-way classification and the tick groups (according to developmental stage and physiological state) were marked A, the cage material B and depth insertion C. None of values F came out as significant at 95% level and was not even close to it. It was impossible statistically to prove the effects of any factors A, B, C, neither the effects of interactions of pairs AB, BC, AC. For the purpose of control also other variant of the model of the analysis of variance was calculated; it involved only possible effects of factors A, B, C and a priori eliminated the possibility of interaction. The results were statistically insignificant again at 95% level and numerically the values F almost coincided with relevant values F in the first three rows in Table 8.

INFLUENCE OF CAGE ON ITS INSIDE ENVIRONMENT

While studying this relationship we compared mean daily temperatures inside the cage and in the soil at certain depth. Exact performance of statistical tests of this kind was prevented by the fact that the series of differences is not formed by independent variables. This knowledge was obtained by a test based on circular correlation coefficient. The cage (i.e. its protective influence) caused an average 1.9 °C increase of mean daily temperature inside in contrast to that of the soil. It would be possible to demonstrate the dependence of temperature inside the cage on surrounding temperature only after a complicated test of time series. We assume that this conclusion would be undoubtely confirmed by such a test.

CONCLUSION AND DISCUSSION

The results presented show that the successful overwintering of *I. ricinus* in our field experiment did not depend on the depth at which the ticks had been placed; only in unfed males there was an exception. Hence it follows that neither temperature nor humidity in the studied range were affecting factors. Thus it can be concluded that *I. ricinus* has apparently a wide ecological amplitude which has evidently not been exceeded in the experiment performed.

The temperatures inside the cage are higher than those in free soil. Their relationship, however, cannot be tested by current methods, because the differences between external and internal temperatures are significantly dependent on one another. This dependence can be understood as a result of the protective influence of the cage (temperature changes inside the cage in contrast to the surrounding soil are not accidental).

As for the effects of different cage materials on the survival of ticks in winter, they proved to be equal. The combination of these effects with other factors studied (depth 10—20 cm, different developmental stages) was not proved either. This conclusion however, might probably have a limited validity seasonally.

Finally, the main result of our experiment, i.e. the fact that a successful overwintering (in the sense of survival and further development as well) does not depend on the depth of insertion in soil, must be compared with conditions in free nature. As demonstrated by the experiment in which ticks *I. ricinus* were placed in the same experimental site in autumn and soil samples were processed by flotation in winter, most ticks overwinter in the surface layers (0—5 cm), while in lower strata they occur to a lesser extent or sporadically (Dusbábek et al., 1971; Daniel et al. 1972).

ЗИМОВАНИЕ ИКСОДОВОГО КЛЕЩА *IXODES RICINUS* (L.) В УСЛОВИЯХ ПОЛЕВОГО ЭКСПЕРИМЕНТА

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Резюме. В южно-моравском теплолюбивом дубняке поставлен опыт, в котором изучались условия зимования иксодового клеща *Ixodes ricinus* (L.). Особое внимание уделялось следующим вопросам: 1. каким способом разные стадии развития клеща *I. ricinus* (голодные и сытые) проводят зиму в почве на разной глубине; 2. с какими микроклиматическими условиями они встречаются в отдельных почвенных слоях; 3. каково воздействие на микросреду клеща в условиях опыта, если клещи помещены в природе в клетках, изготовленных из разного материала.

REFERENCES

DANIEL M., The method of exact continuous measurements of the microclimate in nests of reservoir animals for research of natural disease foci. Theoret. Questions of Nat. Foci of Diseases, Proceedings of a Symposium, pp. 497—506, Czechoslovak Acad. Sci. Publ. House, Praha, 1965.

—, Effects of the microclimate on nest parasites of the bank vole, *Clethrionomys glareolus*, in summer season. Acta Sc. Nat. Brno, 4 (2): 1—48, 1970.

—, ČERNÝ V., To the method of studying the environmental temperature of the tick *Ixodes ricinus* L. Folia parasitol. (Praha) 14: 177 to 183, 1967.

—, —, DUSBÁBEK, F., Stratification of overwintering engorged larvae and nymphs of *Ixodes ricinus* (L.) in soil. Wiadom. paraz., 1972 in press.

DUSBÁBEK F., DANIEL M., ČERNÝ V., Stratification of engorged *Ixodes ricinus* larvae overwintering in soil. Folia parasitol. (Praha) 18: 261—266, 1971.

KLIKA J. et al., Praktikum fytocenologie, ekologie, klimatologie a půdoznalství. 1—773. Czech. Acad. Sci. Publ. House, Praha, 1954.

SLAVÍK B. et al., Metody studia vodního provozu rostlin. 1—302, Czech. Acad. Sci. Publ. House, Praha, 1965.

SCHEFFE O., The analysis of variance. 1—625. Gos. Izd. Fiz.-mat. lit., Moskva, 1963. (In Russian.)

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