Ecological studies on ixodid ticks

7. The effects of humidity on oviposition, development and survival of *Ixodes ovatus* Neumann (Acarina: Ixodidae)

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Abstract: The oviposition, development and survival of *Ixodes ovatus* were studied at six different humidities (52, 68, 74, 86, 95 and 100%) and at 20 and 25°C. At both 20 and 25°C, egg production occurred at 86 to 100% RH, but the mean number of deposited eggs per mg body weight of engorged female was scarce at 86% RH; accordingly, the duration of oviposition was shorter than that at 95–100% RH. The preoviposition period increased with decreasing relative humidity. Hatching of eggs held at 100% RH and 20–25°C averaged 57.2–63.8%, but reduced to 1.6–17.7% at 95% RH, and none hatched at 86% RH and below. At 95–100% RH and 20–25°C, 45.1–74.3% of engorged larvae molted, but none molted at 86% RH and below. At both 20 and 25°C, molting of engorged nymphs to adults occurred at 86 to 100% RH, but the percentage of molting success was few at 86–95% RH. Weight loss of engorged nymphs increased with decreasing relative humidity. Longevity of unfed larvae, nymphs and adults decreased considerably as the relative humidity was lowered. Unfed larvae and nymphs were less tolerant to desiccating conditions than unfed adults. These results suggest that *I. ovatus* is more susceptible to desiccating conditions compared with other ixodid ticks, and this would play an important role in the distribution and abundance of this species.

INTRODUCTION

In order to clarify the factors that define the distribution and habitats of *Ixodes ovatus*, Fujimoto (1989) has studied the effects of temperature on oviposition, development and survival of this tick under laboratory conditions. The laboratory results showed that each stage of *I. ovatus* was more adversely affected by high (27–30°C) and low (15°C)

temperatures compared with common Japanese *Haemaphysalis* species, *H. longicornis* and *H. flava* (Fujimoto and Yamaguti, 1987). Many researchers have reported that humidity as well as temperature plays an important role in the development and survival of ticks: *Amblyomma americanum* (Lancaster and McMillan, 1955), *Derma-centor variabilis* (Sonenshine and Tigner, 1969), *H. longicornis* (Fujisaki *et al.*, 1974; Heath, 1979, 1981), *H. flava* (Fujimoto, 1988), *Ixodes hexagonus* (Arthur, 1951), *I. ricinus* (Lees, 1946) and *Rhipicephalus sanguineus* (Koch and Tuck, 1986). For *I.*

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ovatus, however, there is no information concerning the effects of humidity. The purpose of the present study was to observe the effects of different relative humidities on development, survival and oviposition of *I. ovatus* under laboratory conditions and compare them with similar studies on other ixodid ticks.

MATERIALS AND METHODS

Ticks used in this study were derived from adults collected by the flagging method in Saitama Prefecture. Larvae and nymphs were fed on albino mice, and adults on ears of domestic rabbits in the laboratory maintained at $25 \pm 3^\circ\text{C}$ and with a photoperiod of L:D 16:8. Each host was used only once.

Laboratory experiments were conducted under six different relative humidities (52, 68, 74, 86, 95 and 100%) and constant temperatures of 20 and 25°C . Each relative humidity was obtained by filling the bottom of a desiccator with a certain saturated salt solution of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, NaNO_2 , NaCl , KCl and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, for 52, 68, 74, 86 and 95%, respectively. The desiccators, in which tick containers (Petri dishes of 3–4.5 cm in diameter) were placed, were kept in incubators maintained at 20 and 25°C with a photoperiod of L:D 16:8. To obtain 100% RH, the bottom of a tick container was covered with a double sheet of filter paper on which drops of water were added at appropriate intervals to maintain the wet condition; the containers were also placed in the incubators as above. Temperature and relative humidity in each desiccator were checked regularly with a thermohyrometer.

Each engorged female was placed in a tick container and kept in different humidities after being weighed on a chemical balance (Shimadzu Co. Ltd., NL-200P), and examined daily to determine their preoviposition and oviposition periods. Some engorged females were allowed to oviposit without disturbance by daily examination. These undisturbed females were compared with females disturbed during the preoviposition and oviposition periods by daily examination. The number of deposited eggs was finally

determined by counting hatched larvae and unhatched eggs. Observations of incubation period and hatching percentage were made on those eggs which were collected daily from ovipositing females kept at 100% RH. After a majority of larvae had hatched from the eggs, the number of larvae and unhatched eggs were counted to determine hatching percentage. Unhatched eggs were carefully examined under a stereomicroscope to determine if they could continue further embryonic development. They were taken to be dead when either depression of the surface was observed or there were no signs of normal embryonic development such as formation of rectal sac, Malpighian tubule, etc. Engorged larvae and nymphs dropped off from the host on the same day were placed together in batches of one to 10 specimens in Petri dishes at different humidities, and developmental period (days from drop-off to following ecdysis) and molting percentage were observed. Some engorged nymphs reared at various humidities were weighed in a batch of six specimens every day or every other day, and their weight loss was calculated. Unfed nymphs or adults molted on the same day from each preceding stage which had been held at 100% RH were placed together in a tick container as a group of 1–10 individuals, and were observed for their longevity (days from ecdysis to death) under the different humidities.

RESULTS

1. Effect of humidity on oviposition

The effect of relative humidity on the oviposition of females is summarized in Tables 1 and 2. At both 20 and 25°C , egg production occurred at 86 to 100% RH, but no eggs were produced at 74% RH and below. The percentage of oviposition of the females ranged from 81–93% at 95–100% RH, but decreased to 50–62% at 86% RH. The duration of the preoviposition period was prolonged in the lower humidities, ranging from a mean of 12.4 to 18.1 days at 86–100% RH and 20°C , and from a mean of 9.6 to 13.2 days at the same RH and 25°C . The mean days of the oviposition at 95–100% RH and 20°C was 24.5–28.4, about twice that at 86% RH. The mean number

Table 1 Percent oviposition of engorged females of *I. ovatus* held at different humidities at 20 and 25°C.

Temp. (°C)	R.H. (%)	No. of females observed	No. of females oviposited	Percent oviposition (%)
20	68	5	0	0
	74	5	0	0
	86	13	8	61.5
	95	15	14	93.3
	100	20	18	90.0
25	68	3	0	0
	74	4	0	0
	86	14	7	50.0
	95	16	14	87.5
	100	36	29	80.6

Table 2 Fertility, preoviposition and oviposition periods of *I. ovatus* females held at different humidities at 20 and 25°C.

Temp. (°C)	R.H. (%)	No. of females observed	Preoviposition period in days±S.D.	Oviposition period in days±S.D.	Mean no. of eggs per mg body weight±S.D.
20	68	5	No oviposition	No oviposition	0
	74	5	No oviposition	No oviposition	0
	86	4	18.1±3.2	13.4±4.0	2.7±2.2
		(4)			(2.3±0.9)
	95	6	12.9±1.9	24.5±2.6	9.4±1.3
		(8)			(11.5±0.8)
100	8	12.4±0.7	28.4±9.6	12.3±1.6	
	(10)				(10.5±4.1)
25	68	3	No oviposition	No oviposition	0
	74	4	No oviposition	No oviposition	0
	86	3	13.2±4.3	12.3±1.5	3.6±1.9
		(4)			(0.6±0.8)
	95	6	10.6±1.3	19.0±10.8	5.9±3.5
		(8)			(11.2±1.2)
100	17	9.6±1.7	14.5±6.8	10.6±4.8	
	(12)				(9.1±3.7)

Figures in parentheses show the data of undisturbed females.

of deposited eggs per mg body weight of engorged female (both disturbed and undisturbed females) was considerably decreased at 86% RH and 20°C. The mean duration of the oviposition at 95–100% RH and 25°C was 14.5–19.0 days, which was longer than that at 86% RH. The mean number of deposited eggs per mg body weight of disturbed female at 95–100% RH and 25°C

was 5.9–10.6 (undisturbed females=9.1–11.2), about 1.5- to 3-times that at 86% RH.

The pattern of oviposition at 25°C did not vary among three humidities (86, 95 and 100% RH) except that the duration of the oviposition was shorter at 86% RH. Egg production reached a peak between the 12th to 14th day after drop-off at all humidities

(86–100% RH), and then gradually declined until oviposition ceased (Fig. 1). The pattern of oviposition at 20°C was similar to that at 25°C except that egg production at 86% RH had two peaks due to disturbance by repeated handlings. The maximum egg production was seen between the 17th and 19th day after drop-off at all humidities (86–100% RH) and was followed by a gradual decrease.

2. Effect of humidity on egg hatching

The hatching percentage and the incubation period of eggs held at different humidities at 20 and 25°C are shown in Table 3. Hatching of disturbed eggs held at 100% RH and 20–25°C averaged 57.2–63.8%, but

reduced to 1.6–17.7% at 95% RH, and all eggs failed to hatch at 86% RH and below, though signs of embryonic development were observed. Undisturbed eggs also hatched in a humidity range of 95 to 100% RH, but the hatchability did not decrease at 95% RH. The mean incubation period of eggs at 20°C was shortened at 95% RH (56.4 days) compared to 100% RH (74.5 days).

3. Effect of humidity on survival of engorged immature ticks and on molting success

The developmental period and molting success of engorged larvae and nymphs incubated at humidities from 74 to 100% RH are shown in Table 4. At 95–100% RH and

Fig. 1 Typical oviposition patterns of *I. ovatus* held at different humidities at 20 and 25°C.

20–25°C, 45.1–74.3% of engorged larvae molted to nymphs, but no molting occurred at 74–86% RH. Development from the engorged nymph to an unfed adult occurred in a humidity range of 86 to 100% RH at both 20 and 25°C. Relatively high molting percentage was observed at 100% RH and 25°C, where 45.6% of engorged nymphs

molted to adults, but only 22.2–22.4% molted at 86–95% RH. At 100% RH and 20°C, 21.9% of engorged nymphs molted to adults, but only a small percentage (1.5–4.2%) molted at 86–95% RH. At 95–100% RH and 20°C, the developmental period of engorged larvae ranged from a mean of 39.6 to 45.8 days and from a mean of 34.4 to

Table 3 Percentage of hatchability and incubation period of eggs of *I. ovatus* held at different humidities at 20 and 25°C.

Temp. (°C)	R.H. (%)	No. of eggs examined	No. of larvae hatched	Hatchability (%)	Incubation period (days) ± S.D.
20	68	1,010	0	0	No hatch
	74	2,844	0	0	No hatch
	86	1,959 (1,134)	0 (0)	0 (0)	No hatch
	95	1,708 (10,299)	302 (6,589)	17.7 (64.0)	74.5 ± 4.1
	100	7,557 (9,861)	4,321 (4,993)	57.2 (50.6)	56.4 ± 5.9
25	68	353	0	0	No hatch
	74	735	0	0	No hatch
	86	1,826 (431)	0 (0)	0 (0)	No hatch
	95	2,746 (11,646)	43 (3,402)	1.6 (29.2)	—
	100	17,647 (14,800)	11,266 (4,169)	63.8 (28.2)	47.2 ± 2.6

Figures in parentheses show the hatchability of eggs deposited by undisturbed females.

Table 4 Developmental period and percentage molting success of engorged larvae and nymphs of *I. ovatus* held at different humidities at 20 and 25°C.

Temp. (°C)	R.H. (%)	Larvae			Nymphs		
		No.	% molting	Developmental period* (days) ± S.D.	No.	% molting	Developmental period* (days) ± S.D.
20	74	46	0	—	34	0	—
	86	50	0	—	65	1.5	—
	95	60	60.0	45.8 ± 4.69	48	4.2	52.5 ± 2.12
	100	304	74.3	39.6 ± 2.95	169	21.9	48.4 ± 6.99
25	74	26	0	—	32	0	—
	86	53	0	—	27	22.2	46.3 ± 5.68
	95	51	45.1	41.4 ± 3.89	107	22.4	37.9 ± 4.32
	100	793	69.6	34.4 ± 2.77	169	45.6	41.6 ± 4.06

* Developmental period was monitored from time dropped off host to molting.

41.4 days at the same RH and 25°C. At both 20 and 25°C, engorged larvae held at 95% RH took longer to molt than those held at 100% RH. The developmental period of engorged nymphs ranged from a mean of 48.4 to 52.5 days at 95–100% RH and 20°C and from a mean of 41.6 to 46.3 days at 86–100% RH and 25°C. At 20°C, the developmental period of engorged nymphs was more prolonged at 95% RH than at 100% RH, but at 25°C, the minimum developmental period was observed at 95% RH.

4. Effect of humidity on weight loss of engorged nymphs

Weight loss of engorged nymphs was observed under different humidities, and the mean percentage of the loss was compared between *I. ovatus* and *H. flava* (Fig. 2). The difference in weight losses of the two species grew greater with decreasing humidity. At 95% RH, the rates of weight loss did not differ distinctly between these two species, but at 86% RH the loss of the original weight up to the 15th day after drop-off was 36% in *I. ovatus*, while 26% in *H. flava*. At 74%

Fig. 2 Weight loss of engorged nymphs of *I. ovatus* compared with *H. flava* at different relative humidities at 25°C.

Weight loss is indicated by data from a batch of six engorged nymphs. *H. flava* nymphs were collected by flagging method and fed on rabbits.

Table 5 Longevity of unfed adults, nymphs and larvae of *I. ovatus* held at different humidities at 20 and 25°C.

Temp. (°C)	R.H. (%)	Larvae		Nymphs		Adults	
		No.	Longevity in days (mean)	No.	Longevity in days (mean)	No.	Longevity in days (mean)
20	74	40	1-3 (1.5)	30	1-3 (1.6)	15	1-10 (3.6)
	86	41	1-3 (1.7)	30	1-6 (3.4)	15	1-55 (14.8)
	95	65	1-12 (3.3, <i>n</i> =46)	31	1-12 (3.1, <i>n</i> =10)	18	60<
			80< (<i>n</i> =19)		42< (<i>n</i> =21)		
25	74	50	1-2 (1.1)	57	1-2 (1.1)	20	2-16 (6.2)
	86	50	1-2 (1.2)	45	1-5 (1.2)	18	1-25 (6.3)
	95	40	1-8 (2.7, <i>n</i> =36)	34	1-2 (1.3, <i>n</i> =6)	15	56<
			80< (<i>n</i> =4)		40< (<i>n</i> =28)		

RH, furthermore, *I. ovatus* lost 31% of the original weight up to the 7th day, and 41% up to the 15th day, while *H. flava* lost only 20 and 30%, respectively. At 52% RH, *I. ovatus* lost 38% up to the second day, and 47% up to the 5th day, whereas *H. flava* lost only 16 and 21%, respectively.

5. Effect of humidity on the longevity of unfed ticks

The mean longevity of unfed larvae, nymphs and adults in relation to the relative humidity is shown in Table 5. At 74–86% RH, unfed larvae survived for only 1–3 days at 20°C and 1–2 days at 25°C. At 95% RH, however, some larvae survived for longer than 80 days at both 20 and 25°C. All unfed nymphs held at 74–86% RH had died after 6 days at both 20 and 25°C. At 95% RH, however, some nymphs survived for 40 days and more. Unfed adults survived 2.2- to 6-times longer than nymphs and larvae at 74–86% RH. At 74–86% RH, the length of survival for adults was 1–55 days at 20°C and 1–25 days at 25°C, and at 95% RH all adults survived for longer than 56 days at both 20 and 25°C.

DISCUSSION

It is known that many unfed ticks can withstand long periods of starvation in the absence of suitable hosts. The longevity of a tick depends primarily on the relative humidity of the air (Knülle, 1966). Many researchers have reported that the survival, development and molting of engorged ticks are also much influenced by the relative humidity to which they are exposed (MacLeod, 1934, 1935; Feldman-Muhsam, 1947; Arthur, 1951; Sonenshine and Tigner, 1969; Fujisaki *et al.*, 1974; Fujimoto, 1988). Thus, humidity, together with temperature, has considerable influence on the distribution and abundance of ticks (Heath, 1979, 1981; Koch and Tuck, 1986).

In the present study, the development and survival of *I. ovatus* were almost impossible under low relative humidities. Engorged *I. ovatus* females oviposited under a narrow range of high relative humidity; also, the eggs and postembryonic immature stages were strongly affected by the relative humidity.

Especially, ixodid ticks of the genus *Ixodes* seem to be susceptible to desiccation (Lees, 1946). However, the lower humidity limit for the survival and development of *I. ovatus* was higher than that for other species of the same genus, such as *I. ricinus* (MacLeod, 1934, 1935), *I. hexagonus* (Arthur, 1951) and *I. aulacodi* (Ntiemoa-Baidu, 1987).

Unfed ticks generally have the ability to absorb water from the atmosphere to maintain water balance in the body (Lees, 1946). However, this active absorption has functional limits (generally between 74 and 95% RH). Thus, if unfed ticks were exposed to an extremely desiccating condition, they would lose water rapidly through the cuticle and spiracles and die of dehydration. It may be due to high rates of water loss that the longevity of unfed larvae, nymphs and adults in the present study was markedly shortened at 74–86% RH (Table 5). The longevity of unfed larvae and nymphs was shorter than that of unfed adults at all humidities as listed in Table 5. This may be correlated with the surface/volume ratio; the ratio is larger in larvae and nymphs than in adults, and, consequently, immature stages are ready to suffer from a high water loss.

It was generally assumed that engorged ixodid ticks and the eggs do not have the ability to absorb water from the atmosphere (Lees, 1946; Sauer and Hair, 1971; Heath, 1979; Knülle and Rudolph, 1982). Kahl and Knülle (1988), however, have shown that engorged larvae and nymphs of some ixodid ticks consistently take up substantial amount of atmospheric water vapour. In the present study, engorged *I. ovatus* larvae and nymphs were hardly able to molt at 74–86% RH (Table 4). In addition, the engorged nymphs of *I. ovatus*, compared with *H. flava*, lost water more rapidly with decreasing humidity (Fig. 2). It is not clear from these experiments whether engorged *I. ovatus* larvae and nymphs can take up water vapour from the atmosphere, but the present results suggest that the minimum humidity limit for survival of engorged *I. ovatus* larvae and nymphs is above 86% RH, which is higher than that for engorged *H. flava*. Lees (1946) described that the differential susceptibility to desiccation seen among tick species can essentially be attributed to differences in water perme-

ability of the tick integument which is primarily due to the molecular nature of waxy lipids of the epicuticle. Further, Knülle and Rudolph (1982) pointed out that the transition temperature of the epicuticular lipids of ticks is associated with the degree of cuticular impermeability to water. In the present study, the low tolerance of engorged *I. ovatus* to desiccation may be related to the nature of waxy lipids of the epicuticle.

These results suggest that each stage of *I. ovatus* can survive only in highly moist condition and the longevity rapidly decreases with decreasing RH's below 86% RH.

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摘 要

マダニ類の生態学的研究

7. ヤマトマダニの産卵, 発育および生存における湿度の影響

ヤマトマダニの産卵, 発育および生存における湿度の影響を, 20°Cと25°Cの温度条件下で調べた。産卵は20°C, 25°Cとも相対湿度(以下, 単に湿度)86%から100%の間でみられた。しかし, 雌体重1mgあたりの産卵数は86%の湿度で極端に減少し, 産卵期間

も短くなった。産卵前期間は湿度が低下するにつれて長くなった。20～25°Cでの卵の孵化率は、湿度100%では57.2～63.8%であったが、95%の湿度では1.6～17.7%に低下し、86%以下の湿度ではまったく孵化しなかった。20～25°Cでの飽血幼虫の脱皮率は湿度95～100%では45.1～74.3%であったが、86%以下の湿度ではまったく脱皮がみられなかった。飽血若虫の脱皮は20°C、25°Cとも湿度86%から100%までみられた

が、86～95%の湿度では脱皮率が極端に低下した。飽血若虫の体重の減少率は湿度が低下するにつれて大きくなった。未吸血の幼虫、若虫、成虫の寿命は湿度が低下するにつれて短くなったが、とくに幼・若虫において顕著であった。

以上の結果はヤマトマダニが乾燥条件下にきわめて弱いことを示しており、湿度条件は本種の分布や生息密度に重大な影響を及ぼすものと思われた。