DEVELOPMENT OF *ONCHOCERCA VOLVULUS* LARVAE IN *SIMULIUM OCHRACEUM* AT VARIOUS ALTITUDES IN GUATEMALA WITH SPECIAL REFERENCE TO THE AMBIENT TEMPERATURE

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Abstract: Females of *Simulium ochraceum* which were fed on a carrier of the Guatemalan strain of *Onchocerca volvulus* microfilariae were maintained under natural conditions at five different altitudes (350–1,500 m) within and outside of the onchocerciasis endemic zone. The results suggested the probability that these insects' chance of surviving increases with altitude. The infective stage of *O. volvulus* larvae was reached on the eighth day at Mária Santísima (alt. 650 m). At higher altitudes (1,200–1,500 m), however, no larval development beyond the late first stage occurred, except in one *S. ochraceum* female (at Guatemala City, 1,500 m) which yielded late second-stage worms on day 16. The length of the gonotrophic cycle was three or four days irrespective of altitude. Heat accumulation, calculated by means of a self-registered thermometer, suggested that the predicted period required for larvae to reach the infective stage in the vector varies considerably with altitude (4.4–28 days). The relationship between the rate of development of *O. volvulus* larvae at varying altitudes, and ambient heat accumulation, is also discussed.

INTRODUCTION

Many factors influence the transmission dynamics of onchocerciasis. Ambient temperature is one of the most important of them, affecting as it does the parasite's development in the blackfly vector as well as the latter's survival. From previous laboratory experiments, we reported the development of *Onchocerca volvulus* larvae to the third stage in *Simulium ochraceum* Walker (the principal vector of onchocerciasis in Guatemala) under simulated day and night temperatures (Takaoka *et al.*, 1981). This occurred even when night temperature dropped below 16.9°C (the predicted development zero point), although larval development-time was extended. These results suggested that the speed of *O. volvulus* larval development in field population of *S. ochraceum* might vary with heat accumulation at differing altitudes.

The present study was thus undertaken to determine the probability of survival of *S. ochraceum*, and the development of *O. volvulus* larvae within it, at selected altitudes

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where ambient heat accumulation was being monitored.

MATERIALS AND METHODS

Duplicate experiments were conducted in November and December, 1979, (*i.e.* during the early dry season — the period when workers harvesting coffee are most frequently exposed to blackfly bites). Females of *S. ochraceum* used in the project were captured between 09.00 and 12.00 hr at a coffee plantation, (Finca Mónica Ivoné) located on the southwestern slopes of Atitlán Volcano (Department of Suchitepequez).

Field procedures for the capture and maintenance of simuliids were the same as in previous work (Takaoka *et al.*, 1981). The *O. volvulus* microfilaria-carrier used in these studies was a 40 year-old man, with density of 45 mf/mg (based upon one shoulder biopsy). After collection, tubes holding individual blood-fed female blackflies were randomly divided into four (in November) or five (in December) groups, each consisting of 80–100 insects. Transportation from the collecting site to Escuintla (the lowest experimental altitude) took about two hours. Further one-and-a-half hours were required for the journey to the other locations. During this period the simuliids were held in an insulated portable cooler in which ice held the temperature to 15–20 C. Immediately upon arriving at the selected locations where the adults of *S. ochraceum* were to be maintained, all dead or moribund flies were removed. As a result, 13.1% (in November) and 10.7% (in December) of the total females were omitted. The tubes containing the remaining blackflies were packed horizontally in coverless plastic boxes, each with a capacity for 60 individuals. The boxes wrapped in damp towels were placed in a plastic bag to secure high humidity. These boxes were then aligned in wooden weather-instrument shelters (40 cm × 40 cm × 50 cm), standing one metre above the ground. Each shelter also held a hydrothermograph to record air temperature and humidity during the course of the experiment. The environmental shelters for housing the simuliids were stationed near the national highway from Escuintla to Guatemala City — at the Health Centre in Escuintla (alt. 350 m), at finca Mária Santísima (650 m), at finca Barretál (1,250 m), at finca Rincón (1,200 m) and at the laboratory, Servicio Nacional de Erradicación de la Malaria, Guatemala City (1,500 m). Human onchocerciasis and *S. ochraceum* adults were known to be present only at Mária Santísima, Barretál and Rincón. All sites were visited each day thereafter. The number of live blackflies was recorded, and dead ones, if present, were removed and preserved in the laboratory freezer (at –19 C) for later dissection.

All dead examples were dissected into head, tract and abdomen, and teased out in 0.9% saline solution using a binocular stereomicroscope. The number of larvae present was counted and the developmental stages were identified by size and morphology, following Duke (1968) and Bain (1969). Also, follicular development of the ovaries was examined to estimate the duration of the gonotrophic cycle at ambient temperatures.

Heat accumulation (Day·C) reveals the sum of daily heat accumulation (supposedly allowing the larvae of *O. volvulus* to develop in the vector). Daily heat

accumulation was directly calculated from the air temperature graphs according to the following formula: $\sum_{m=1}^{12} [(T_1 - T_0)/12]$, always providing that T_1 (the mean temperature of every two hours) exceeds T_0 (the predicted developmental zero point for *O. volvulus* larvae in *S. ochraceum*). The predicted developmental zero point and the thermal constant used in this study are 16.9 C and 45 Day·C respectively according to our previous study (Takaoka *et al.*, 1981).

RESULTS

Heat accumulation at five locations of differing altitude

Table 1 presents the temperature conditions at the five experimental stations. The temperature-fluctuation patterns together with the daily heat accumulations during four days in December are shown in Figure 1. The December-thermal pattern for Barretál was lacking due to a faulty thermograph. As was to be expected, the mean daily temperature decreased as altitudes rose, and temperatures fluctuated remarkably by day and night at all altitudes observed.

Actual heat accumulation during the November experiment is illustrated in Figure 2. At Barretál and Guatemala data were insufficient for prediction of the time required for larvae to develop into the infective stage. Heat accumulation increased linearly at Escuintla and Mária Santísima, but irregularly in Guatemala

Figure 1 The fluctuations in air temperature during four days in December at four distinct altitudes, and effective temperatures above 16.9 C for *O. volvulus* larval development in *S. ochraceum*. (Areas indicated by oblique lines represent heat accumulation; solid horizontal line represents 16.9 C (developmental zero point); dashed line represents mean air temperature).

City, due to the fact that the temperatures did not reach 16.9 C on the seventh, eighth and ninth days.

Table 2 shows the predicted periods (in days) required for larval development to the infective stage, based on the mean daily heat accumulation. Predicted

Figure 2 The heat accumulation during the course of the November experiment at four different altitudes.

Table 1 Daily temperature (C) at five localities of differing altitude in Guatemala (1979)

Place (Altitude)	Date	Mean	(Range)	Maximum		Minimum	
				Mean	(Range)	Mean	(Range)
Escuintla	Nov. 4-10	27.2	(25.7-29.5)	31.4	(30.8-32.2)	23.0	(21.8-27.0)
(350 m)	Dec. 8-20	27.1	(26.1-27.9)	33.1	(32.3-35.0)	22.6	(21.0-24.0)
Mária Santísima	Nov. 4-14	22.8	(21.8-23.3)	28.9	(26.0-30.8)	18.5	(17.0-19.6)
(650 m)	Dec. 8-21	24.2	(22.2-26.5)	30.1	(29.0-31.6)	19.6	(17.0-21.6)
Rincón							
(1200 m)	Dec. 8-21	18.6	(17.0-20.2)	26.7	(24.8-31.0)	13.1	(8.5-16.2)
Barretäl							
(1250 m)	Nov. 4-7	17.9	(17.4-18.5)	22.1	(21.4-23.0)	13.6	(13.0-14.0)
Guatemala	Nov. 4-16	17.2	(13.1-19.3)	23.0	(15.8-27.6)	12.8	(11.2-15.5)
(1500 m)	Dec. 8-21	16.8	(14.7-18.7)	23.0	(19.9-26.2)	12.7	(8.6-14.5)

Table 2 Daily heat accumulation and predicted period required for development of *O. volvulus* larvae of the third stage in *S. ochraceum*

Place and Month	Daily heat accumulation		Period for larval development in days	
	Mean	(Range)	Mean	(Range)
Escuintla				
November	9.8	(8.8–11.7)	4.6	(3.9– 5.1)
December	10.2	(9.2–11.0)	4.4	(4.1– 4.9)
Mária Santísima				
November	6.1	(4.9– 8.5)	7.4	(5.2– 9.1)
December	7.5	(5.3– 9.6)	6.0	(4.7– 8.5)
Rincón				
December	2.9	(2.0– 4.0)	15.5	(11.3–22.5)
Barretál				
November	1.8	(1.5– 2.0)	25.0	(22.5–30.0)
Guatemala				
November	1.8	(0.0– 3.7)	25.0	(12.8–)
December	1.6	(0.6– 2.6)	28.0	(17.3–75.0)

developmental periods varied considerably, with a tendency for prolongation as altitude rose.

Survival of the blackfly host

Figure 3 illustrates the survival curves for batches of female *S. ochraceum* fed on a carrier of *O. volvulus* and maintained at five different altitudes. The data suggest the probability that survival decreases with loss of altitude. In both experiments, very small number of simuliids survived beyond the predicted day when third-stage larvae should have appeared in the head. At Escuintla, 1.1% (1/90 blackflies in November) and 1.6% (1/62 blackflies in December) of the initial host population survived through the fifth day, while at Mária Santísima, 4.4% (4/90 blackflies in November) and 6.3% (4/64 blackflies in December) lived beyond the eighth and sixth day respectively. At Rincón, Barretál and Guatemala City, the only *S. ochraceum* to survive to the predicted day was one female (out of 90 experimented with at Barretál in November) which lived for 26 days.

Development of O. volvulus larvae in S. ochraceum

Figures 4 and 5 show the larval development of *O. volvulus* in *S. ochraceum* in relation to ambient heat accumulations at five differing altitudes. The mean daily heat accumulation obtained from the first four day's observation in November was used for Barretál instead of the actual one. Among 152 blackflies examined at Escuintla (350 m) no third-stage larvae were observed although two blackflies which died on the seventh day (in November) and sixth day (in December) yielded late second-stage larvae (Duke's stage K). However, at Mária Santísima (650 m), where the mean temperature was 22.8 C in November and 24.2 C in December,

Figure 3 The survival curves for *S. ochraceum* females which were fed on a carrier of *O. volvulus* microfilariae and maintained at five different altitudes.

larval development to the infective stage occurred on the eighth day (in November) and ninth day (in December), although it was somewhat asynchronous as evidenced by several stages within individual blackflies (Table 3). On the other hand, no development beyond late first stage (H) was observed in simuliids held at Barretál (1,250 m) and Rincón (1,200 m). Unfortunately, the single example that survived 26 days at Barretál did not harbour parasites. Similarly, larval development beyond stage F was not seen in Guatemala City (1,500 m), except in the case of one female which died on the 16th day in November, harbouring five late second-stage larvae (K and J).

Length of gonotrophic cycle

At all selected locations, except in Rincón, fully-grown eggs were first found in

Figure 4 The relationship between the development of *O. volvulus* larvae in *S. ochraceum* and the ambient heat accumulation at four altitudes in November. (Numbers in the figure show days after ingestion of microfilariae).

Figure 5 The relationship between the development of *O. volvulus* larvae in *S. ochraceum* and the ambient heat accumulation at five altitudes in December. (Numbers in the figure show days after ingestion of microfilariae).

Table 3 Development of *Onchocerca volvulus* in *Simulium ochraceum* which were fed on a carrier of the Guatemalan strain of *O. volvulus* microfilariae and kept under natural conditions at Mária Santísima (650 m above sea level)

Days post-infection	No. flies examined	No. flies harboring larvae	Total no. larvae	Mean no. larvae/fly (Range)	Stage of larvae (%)			
					Mf.	L ₁	L ₂	L ₃
November, 1979								
1	24	24	8,291	345.5 (20-893)	100.0	0.0	0.0	0.0
2	22	22	3,064	139.3 (7-512)	100.0	0.0	0.0	0.0
3	15	13	1,244	82.9 (0-268)	94.7	5.3	0.0	0.0
4	2	2	4	2.0 (1- 3)	50.0	50.0	0.0	0.0
5	2	2	3	1.5 (1- 2)	0.0	100.0	0.0	0.0
6	4	3	16	4.0 (0- 7)	0.0	93.7	6.0	0.0
7	5	4	13	2.6 (0- 6)	0.0	30.8	69.2	0.0
8	3	2	13	4.3 (0- 11)	0.0	0.0	23.1	76.9
9	0	—	—	— (-)	—	—	—	—
10	2	1	1	0.5 (0- 1)	0.0	0.0	0.0	100.0
11	2	2	25	12.5 (10- 15)	0.0	0.0	0.0	100.0
December, 1979								
1	25	25	8,382	335.3 (54-825)	100.0	0.0	0.0	0.0
2	14	14	1,441	102.9 (0-357)	99.9	0.1	0.0	0.0
3	9	9	1,639	182.1 (10-398)	98.8	1.2	0.0	0.0
4	9	8	40	4.4 (0- 22)	52.5	47.5	0.0	0.0
5	1	1	8	8.0 (8.0)	0.0	100.0	0.0	0.0
6	2	1	3	1.5 (0- 3)	0.0	100.0	0.0	0.0
7	1	1	4	4.0 (4.0)	0.0	0.0	100.0	0.0
8	0	—	—	— (-)	—	—	—	—
9	2	1	7	3.5 (0- 7)	0.0	0.0	42.9	57.1
10	0	—	—	— (-)	—	—	—	—
11	1	1	12	12 (12)	0.0	8.3	25.0	66.7

experimental hosts three days after ingestion of the blood-meal. Essentially, the length of gonotrophic cycle proved to be three days irrespective of altitude. The single exception was Rincón, where four days were required.

DISCUSSION

In the laboratory study (Takaoka *et al.*, 1981), it was shown that *O. volvulus* larval development in *S. ochraceum* occurred at diurnal/nocturnal temperatures where the latter were below 16.9 C (the predicted developmental zero point). In the present study, larval development in the vector species was investigated in relation to ambient heat accumulation at different altitudes (350-1,500 m). Unfortunately, the results demonstrated significantly-reduced probabilities of host-survival in the field when compared with corresponding laboratory temperatures

(Takaoka *et al.*, 1981). Increased mortality might well have been caused by high midday temperatures, since temperatures above 28 C are debilitating for *S. ochraceum* (Takaoka *et al.*, 1981 and Monroy, 1979). Under these circumstances, only a few blackflies survived to the day when larvae might have reached the infective stage. The completion of *O. volvulus* larval development in *S. ochraceum* was observed only at Mária Santísima (where mean temperatures were moderate, ranging from 21.8–26.5 C) with the developmental period being 8 or 9 days, nearly equal to that predicted by heat accumulation at this medium altitude. It is notable that the mean temperature range there coincides with that optimal for the vector's ability to transmit *O. volvulus* in the laboratory experiment (Takaoka *et al.*, 1981). On the other hand, at lower altitude (350 m) with high mean temperature (25.7–29.5 C) larvae reached only the late second stage even in females (which died on day 6 and 7) surviving beyond day 5 (*i.e.* the predicted day when larvae might be expected to develop to the infective stage at this altitude). This somewhat retarded development despite the sufficient heat accumulations might be associated with the initial cooling of females to 15–20 C during the transportation soon after the ingestion of blood meal. At higher altitude (1,200–1,500 m) no blackflies lived to the predicted critical day (15th–28th day) except one female at Barretál, and no development occurred beyond the late first stage in blackflies dying during the seventh through 13th day after ingestion of microfilariae. Therefore, it was not ascertained whether *O. volvulus* larvae could develop to the infective form in accordance with ambient heat accumulation in nature; although as shown in Figures 4 and 5, larval development (though limited to the first stage) appeared to require less heat accumulation at higher altitudes. This suggested that the first-stage larval development could occur even at temperatures below 16.9 C. The partial development to the late second stage seen in one female dying on day 16 at Guatemala City (1,500 m) seems to indicate that the extended larval development to the infective stage would take place in low percentages even at higher altitudes where night temperature drops far below the developmental zero point (about 17 C). In such cases the developmental period would probably require more than two weeks as suggested in Table 2. From the epidemiological point of view, it appears of great importance that the duration of extrinsic development of *O. volvulus* larvae in *S. ochraceum* varies considerably by altitudes (*i.e.* with temperature conditions) within endemic areas. As already reported by previous workers (Garms, 1975, Collins, 1979, and Garms and Ochoa, 1980) natural infection rates of *S. ochraceum* for third-stage larvae of *O. volvulus* in Guatemalan endemic zones were variable ranging from 0.09–3.2%. This would be probably explained partially by the different speeds of larval development in the vector simuliids and by the different longevities of the host insects within endemic areas. In this connection it should be also pointed out that the increased probability of simuliid's survival at higher altitudes may be to some extent reduced by the increased risk of exposure to natural hazards when the female oviposits and takes the subsequent blood-meals. This is because more frequent gonotrophic cycles might be repeated in the host blackfly before the larvae therein becomes infective at higher altitudes than at lower or medium ones, since the duration of one gonotrophic cycle is nearly the same — three days — even at higher altitude.

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REFERENCES

- 1) Bain, O. (1969): Morphologie des stades larvaires d'*Onchocerca volvulus* chez *Simulium damnosum* et redescription de la microfilare, Ann. Parasitol., 44, 69-81
- 2) Collins, R. C. (1979): Onchocerciasis transmission potentials of four species of Guatemalan Simuliidae, Am. J. Trop. Med. Hyg., 28, 72-75
- 3) Dalmat, H. T. (1955): The blackflies (Diptera, Simuliidae) of Guatemala and their role as vectors of onchocerciasis, Smithson. Misc. Coll., No. 125, 425 pp. (Abstracted in Rev. Appl. Entomol. B, 44, 47)
- 4) Duke, B. O. L. (1968): Studies on factors influencing the transmission of onchocerciasis. V: The stages of *Onchocerca volvulus* in wild 'forest' *Simulium damnosum*, the fate of the parasites in the fly, and the age-distribution of the biting population, Ann. Trop. Med. Parasitol., 62, 107-116
- 5) Garms, R. (1975): Observations on filarial infections and parous rates of anthropophilic blackflies in Guatemala, with reference to the transmission of *Onchocerca volvulus*, Tropenmed. Parasitol., 26, 169-182
- 6) Garms, R. and Ochoa, J. O. (1979): Further studies on the relative importance of Guatemalan blackfly species as vectors of *Onchocerca volvulus*, Tropenmed. Parasitol., 30, 120-128
- 7) Monroy, M. C. (1979): Infeccion experimental de *Simulium ochraceum* con microfilarias de *Onchocerca volvulus*, Informe de Tesis, Escuela de Biologia, Universidad de San Carlos de Guatemala, 90 pp.
- 8) Takaoka, H., Ochoa, J. O., Juarez, E. L. and Hansen, K. M. (1981): Effect of temperature on development of *Onchocerca volvulus* in *Simulium ochraceum*, and longevity of simuliid vector, J. Parasitol. (in press)

中米グアテマラの温度条件の異なる種々の標高における *Onchocerca*
volvulus 幼虫の媒介ブユ *Simulium ochraceum* 体内での発育

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中米グアテマラの標高の異なる5地点(350-1,500 m)に設置した百葉箱内で *Onchocerca volvulus* ミクロフィラリア保有者から吸血させた媒介ブユ *Simulium ochraceum* を飼育し、その後の *o. v.* 幼虫の発育と同時に設置した自記温度計より算出された有効温量との関係を調べた。標高 350 m の Escuintla では有効温量から4日目に第3期幼虫の出現が予測されたが第6日、7日目に死亡したブユ雌でもまだ第2期後期幼虫しか見られなかった。一方、標高 650 m の María Santísima ではほぼ有効温量から推測されるとおりに第8日目に第3期幼虫への発育が観察された。ここより標高の高い3地点(1,200 m, 1,250 m, 1,500 m)では、各々15日、25日、25-28日目に第3期までの幼虫発育が予測されたが飼育したブユの大半がこれらの期間内に死亡したことにより十分な観察を行いえなかった。これら3地点では、飼育開始後7-13日の間に死亡したブユ体内で見出された幼虫はまだ第1期後期までしか発育していなかった。しかしながら標高 1,500 m の Guatemala 市において飼育開始後16日目に死亡した1個体のブユから第2期後期まで発育した幼虫が見出されたことから、標高の高い場所でも非常にゆっくりとはあるが *o. v.* 幼虫の第3期までの発育は可能であることが示唆された。

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