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RESPONSES TO TEMPERATURE IN NEMATODES
I. MECHANISM OF HEAT TOLERANCE
IN *HELICOTYLENCHUS DIHYSTERA*

by

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Temperature is an important factor in relation to the geographical distribution of nematode species. Bird and Wallace (1965) observed that *Meloidogyne hapla* is more prevalent in cooler climates whereas *M. javanica* frequently occurs in hotter regions and has a higher optimum temperature for survival than *M. hapla*. El-Sherif and Mai (1968, 1969) found some species to react within heat gradients whereas others did not. Recently Azmi and Jairajpuri (1977) found that sudden changes in temperature resulted in posture changes of *Helicotylenchus indicus* due to abnormal neuro-muscular activity. Further experiments, which are reported here, have been undertaken to examine the effect of temperature on the activity, mortality and postural changes and locomotion of *H. dihyстера* (Cobb) Sher.

MATERIALS AND METHODS

H. dihyстера were extracted using a modified Baermann funnel technique from soil obtained from around the roots of *Cenchrus ciliaris* L. at Jhansi. The nematodes were cultured in pots on the same host and these were extracted for the experiments.

The rate of activity of *H. dihyстера* at different temperatures was examined by placing about 50 individuals in each of eight 2.5 cm diameter petri dishes filled with 10 ml of tap water. The nematodes

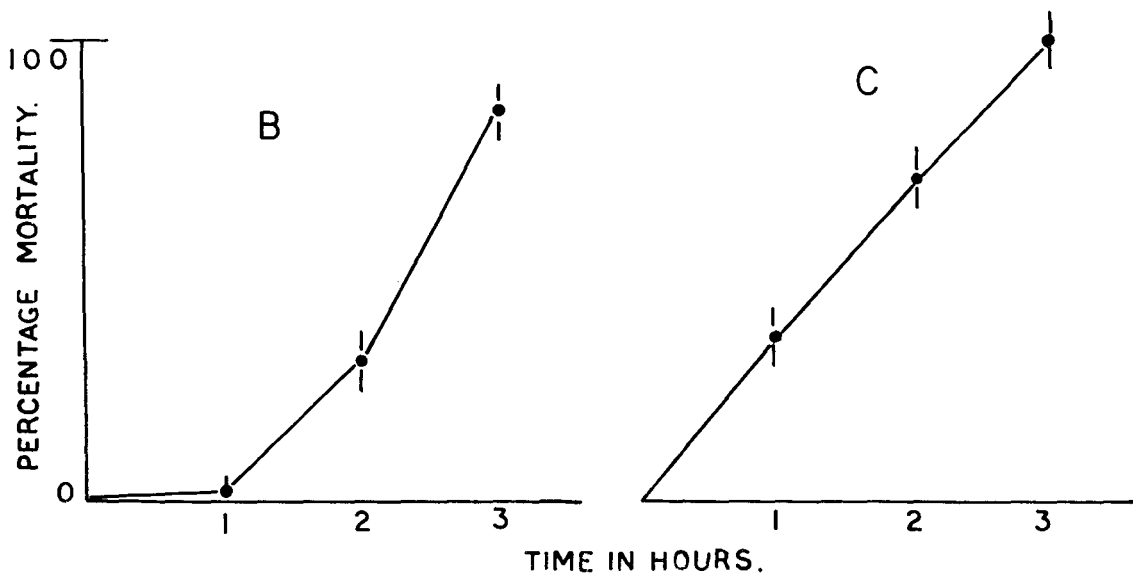
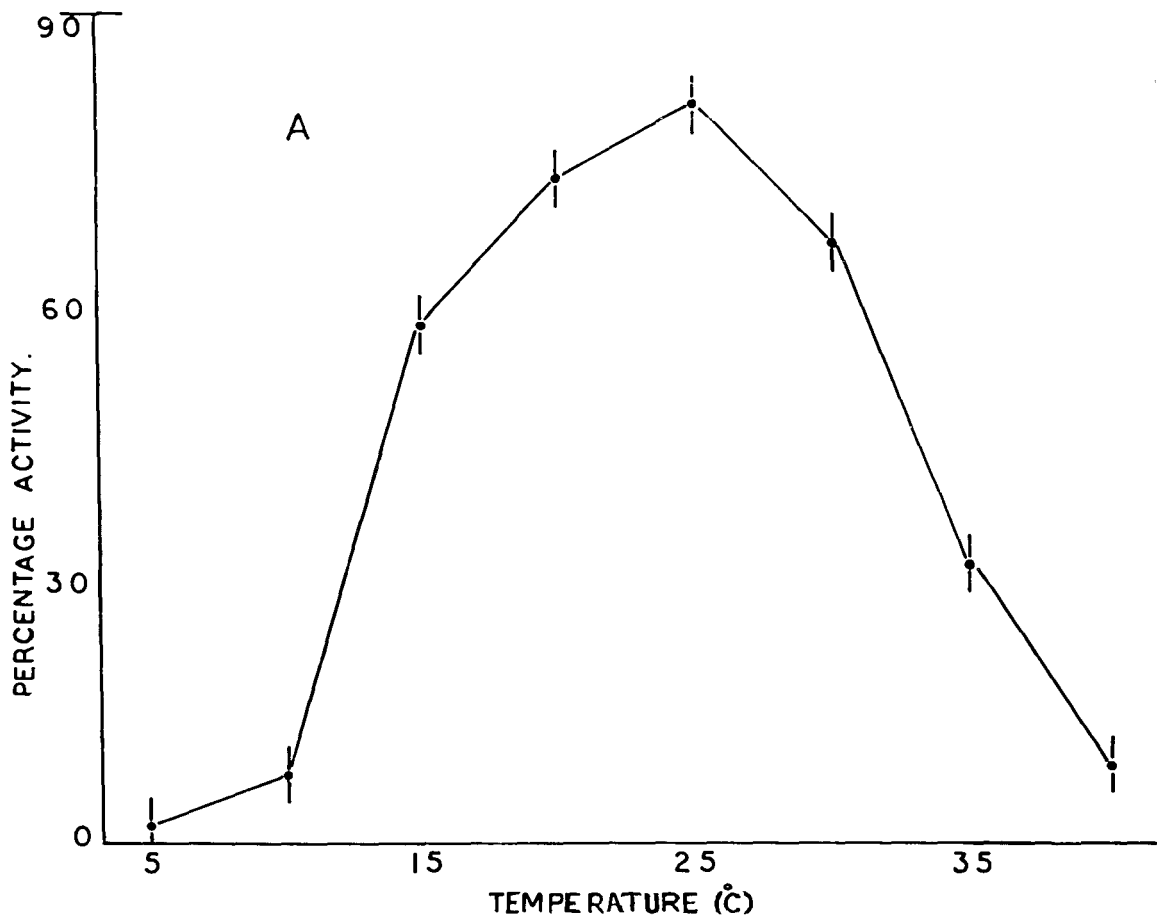


Fig. 1 - A - Percentage activity of *Helicotylenchus dihystrera* exposed for two hours at different temperatures, B and C - Percentage mortality at 0°C and 45°C exposure for different time intervals.

were maintained at 25°C for two hours before the individual dishes were subjected for two hours to temperature ranging from 5° to 40°C at 5° intervals. Then the number of active nematodes in each dish were counted. The experiment was replicated 10 times.

In another experiment 50 *H. dihystra* were placed in Petri dishes which were then kept either at 0°C or at 45°C and the number of dead individuals counted after 1, 2 and 3 hours. There were 10 replicates of each treatment.

In a third experiment 10 individuals were placed in tap water in cavity slides. The cavity was covered with a coverslip and sealed with vaseline. Six slides were kept at either 0° or 45°C for 20, 40, 60, 80, 100 or 120 minutes. The number of active nematodes were counted for each treatment and their postures were drawn with the help of a camera lucida. These observations were repeated after the slides had been kept for 5, 10, 15, 20 and 25 min. at 25°C, considered the normal temperature for activity. The posture of individual nematodes at the different treatments were numerically identified from the following ratios of measurements:

A = L/l (L = total length; l = length of the spiral along the axis).

B = l/X (X = distance between head and tail extremities).

C = V or D maximum/V or D minimum (V and D = distance between lines parallel to the axis of the spirals: Fig 1B).

Seven postures of *H. dihystra* generally were recognised (Fig. 1B) and the frequency of these in the different treatments was counted. Each experimental treatment was replicated 5 times with 100 nematodes observed in each treatment.

Studies on locomotion were made at 20°, 25° and 30°C on nematodes in 1% water-agar in petri dishes. The tracks formed by the nematodes in the agar were recorded after response to the different temperature regimes for 24 hours. Using a stop watch velocity along the axis (Va) and along the track (Vt) were estimated.

RESULTS AND DISCUSSION

Maximum activity was observed at temperatures between 20° and 30°C and activity was very much decreased between 5° and 10°C and between 35° and 40°C (Fig. 2A).

Table I - Posture changes in *Helicotylenchus dihystra* under treatment of low lethal temperature (0°C) followed by normal temperature (25°C).

Temperature °C	Exposition time	% o f n e m a t o d e						
		Wavy	C-shaped	Single spiral	Double spiral	S-shaped	Double coil	Complex
0	20 min	0	14	29	30	7	12	8
25	5 min	40	28	4	1	5	6	16
	10 min	100	0	0	0	0	0	0
0	40 min	0	35	25	40	0	0	0
25	5 min	23	15	8	30	0	1	23
	10 min	30	30	5	5	20	10	0
	15 min	100	0	0	0	0	0	0
0	60 min	5	14	15	45	0	0	21
25	5 min	0	36	7	34	0	14	7
	10 min	14	8	21	8	14	7	28
	15 min	75	25	0	0	0	0	0
	20 min	100	0	0	0	0	0	0
0	80 min	6	35	22	15	7	1	14
25	5 min	0	14	28	28	14	7	9
	10 min	0	14	40	28	13	0	5
	15 min	3	10	30	21	26	7	3
	20 min	100	0	0	0	0	0	0
0	100 min	14	31	21	28	14	2	0
25	5 min	0	24	0	24	24	8	32
	10 min	75	25	0	0	0	0	0
	15 min	90	10	0	0	0	0	0
	20 min	100	0	0	0	0	0	0
0	120 min	7	29	21	14	10	6	14
25	5 min	0	25	25	50	0	0	0
	10 min	0	25	25	50	0	0	0
	15 min	0	25	25	50	0	0	0
	20 min	0	25	25	50	0	0	0

Mortality increased with times of exposure at 0° (Fig. 2B) or 45°C (Fig. 2C) and after 3 hours most of the nematodes were dead.

It was observed that *H. dihystra* moved with a simple wave posture, whereas when stationary it assumed a flat spiral posture. Other postures observed represent transitional phases.

Lower values of 'A' indicate that the axial length of the spiral is high and is correlated with a greater relaxation of the body muscles (open spirals). Higher values of 'B' indicate that the head and tail are closer together. The 'C' values indicate the degree of coiling and spiralling of the body.

Tables I and II give percentage of various postures assumed by nematodes when treated with low and high lethal temperatures for different time intervals separately and then placed at 25°C. Treatments at a low lethal temperature, i.e. 0°C, produced sudden contraction of the body muscles and the nematode assumed its normal active state within 10 to 20 minutes, whereas after treatment at a high lethal temperature (45°C) for 60 min. treated nematodes assumed their normal active state within 25 min. Nematodes in other treatments died. Nematodes exposed to low lethal temperatures and then to 25°C assumed maximum activity quickly compared with those exposed to high lethal temperatures (Fig. 1A).

In Fig. 3 the value of 'A', and 'C' are plotted in triangular form which shows that nematodes exposed to low lethal temperatures for 10 min. assumed their active state (a) after 10 min. by decreasing their 'Z' and 'B' values and increasing 'C' value. After 40 min. treated nematodes come to their normal state after 15 min. first by decreasing the 'A' value then by decreasing 'B' and increasing 'C' values, whereas nematodes treated for 60, 80, and 100 min. assumed their active state after 20 min. and the changes in their 'A', 'B' and 'C' values were slower. Nematodes treated for 120 min. failed to recover and remained completely relaxed (r). After treatment at the high « lethal » temperature for 20 min. the nematodes assumed their active state after 20 min. first by increasing the 'C' value and then by decreasing 'A', 'B' and increasing 'C' values, whereas nematodes treated for 40 and 60 min. regained their normal active state after 25 min. by increasing the 'C' value. After 80 min. treatment some decrease in the values of 'A' and 'B' and increase in the 'C' value were recorded but the nematodes failed to regain their active state. No postural changes were observed after 100 and 120 min. treatments.

Table II - Posture changes in *Helicotylenchus dihystra* under treatment of high lethal temperature (45°C) followed by normal temperature (25°C).

Temperature °C	Exposition time	% o f n e m a t o d e						
		Weavey	C-shaped	Single spiral	Double spiral	S-shaped	Double coil	Complex
45	20 min	0	25	25	50	0	0	0
25	5 min	3	15	10	15	10	7	40
	10 min	10	10	7	8	20	15	30
	15 min	50	0	0	0	5	5	40
	20 min	100	0	0	0	0	0	0
45	40 min	0	20	30	50	0	0	0
25	5 min	0	20	25	50	0	0	5
	10 min	20	25	20	30	0	5	5
	15 min	24	10	20	25	5	6	10
	20 min	50	5	6	9	8	18	7
	25 min	75	2	4	0	10	5	4
45	60 min	0	20	30	50	0	0	0
25	5 min	0	35	25	35	0	5	0
	10 min	0	45	26	29	0	0	0
	15 min	8	21	25	25	9	7	5
	20 min	40	10	20	25	0	0	5
	25 min	60	0	20	10	0	5	5
	45	80 min	0	2	40	30	0	3
25	5 min	0	17	45	35	0	0	3
	10 min	0	20	50	30	0	0	0
	15 min	0	15	50	35	0	0	0
	20 min	0	15	50	35	0	0	0
	25 min	0	15	50	35	0	0	0
	45	100 min	0	20	60	20	0	0
25	5 min	0	20	60	20	0	0	0
	10 min	0	20	60	20	0	0	0
	15 min	0	20	60	20	0	0	0
	20 min	0	20	60	20	0	0	0
	25 min	0	20	60	20	0	0	0
	45	120 min	0	15	50	35	0	0
25	5 min	0	15	50	35	0	0	0
	10 min	0	15	50	35	0	0	0
	15 min	0	15	50	35	0	0	0
	20 min	0	15	50	35	0	0	0
	25 min	0	15	50	35	0	0	0

Both the velocities « V_a » and « V_t » were higher at 25° than at 20° and 30°C. The lowest rate of locomotion was found at 30°C, indicating that an increase in temperature reduces locomotion more than a decrease in temperature (Fig. 4). Most of the tracks consisted of arcs, formed by muscular contraction of one side of the body. The radius R of the arcs and its subtending angle A' at the centre (of the circle) were measured and their mean values were calculated. In general, R decreased as the temperature decreased from the optimum (Fig. 4).

The numbers of crossings along the tracks at 20°C were greater than at 25 and 30°C, showing that at lower temperatures nematodes more frequently change their direction of locomotion (Fig. 4).

Gray and Lissmann (1964) explained the mechanism by which nematodes increase their propulsive forces to overcome increasing resistance to locomotion by the environment and the manner of changes which occur in the wave characteristics along the locomotory tracks. According to these authors, during locomotion the body of the nematode transmits a wave which starts at the anterior end and passes to the posterior end. The formation of waves is a result of alternate contraction and relaxation of dorsal and ventral somatic muscles. It has also been calculated that the velocity of the nematode along the axis $V_a = N\lambda$ (N - representing wave frequency and λ wave length), and the pitch of the wave θ is directly proportional to the ratio of amplitude a and wave length, λ , i.e. (a/λ). The number of waves per body length (η) is inversally proportional to the angle formed by the wave at the point of amplitude (A) and the amplitude a is inversally proportional to A (cf. Wallace, 1969).

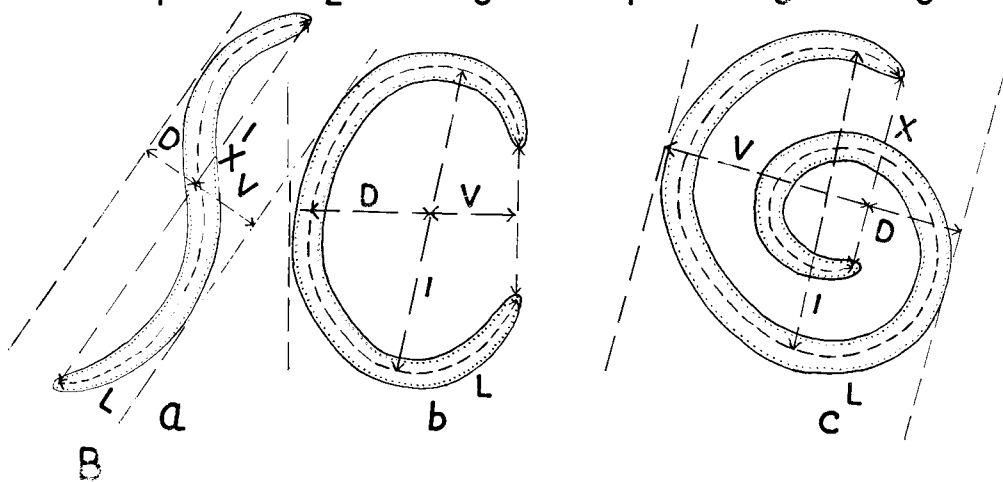
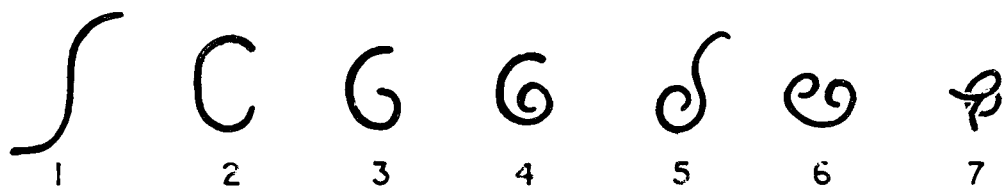
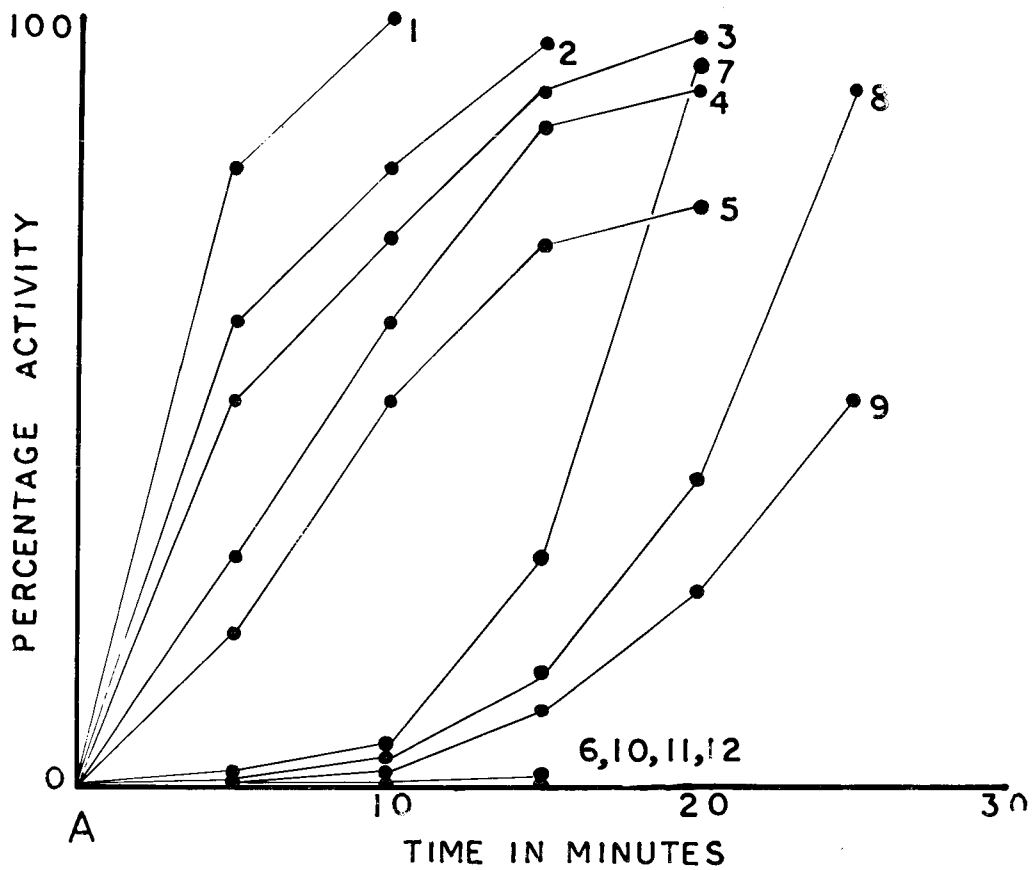
On the basis of these facts it can be deduced that to decrease their velocity nematodes must decrease their wave frequency (N) or increase the wave pitch (θ) or the number of waves per body length (η) or a combination of these properties.

At 20°C the value of N is lower and η is higher than at 25°C indicating that the nematodes decrease their velocity by decreasing N , and η . Whereas at 30°C N is lower, a , a/λ and η is higher than at 25°C showing thereby that the velocity decreases at 30°C by decreasing N , and by increasing wave pitch θ and η .

Fig. 2 - A - Percentage activity of *H. dihystra* treated at 0°C (1 to 6) and 45°C (7 to 12) and then kept at 25°C for different time intervals.

0°C treatment was given for, 20 (1), 40 (2), 60 (3), 80 (4), 100 (5), and 120 (6) min.; 45°C treatment was given for, 20 (7), 40 (8), 60 (9), 80 (10), 100 (11) and 120 (12) min. respectively

B - 1 to 7, different types of postures assumed by the nematodes, wavy (1), C-shaped (2), single spiral (3), double spiral (4), S-shaped (5), double coil (6) and complex postures (7), a-c shows measurements taken for the analysis of postures, length of the animal (L), length of the spiral along the axis (I), distance between the head and the tail (X), distance between the lines drawn parallel to the axis of the spirals (V and D).



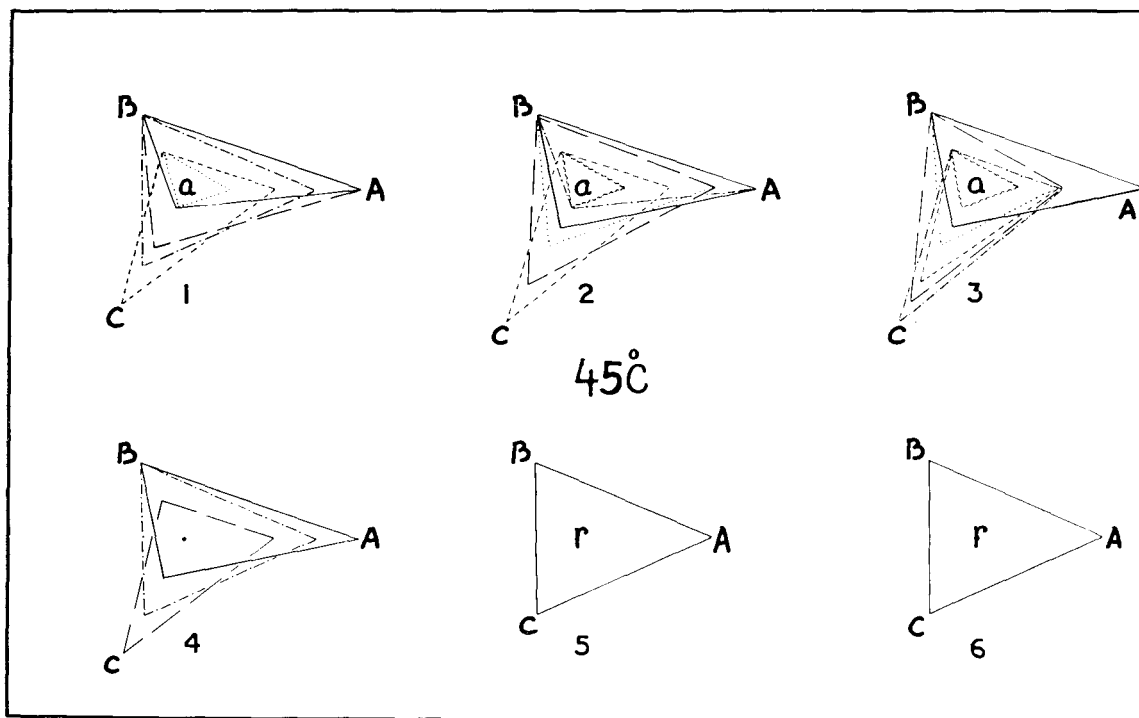
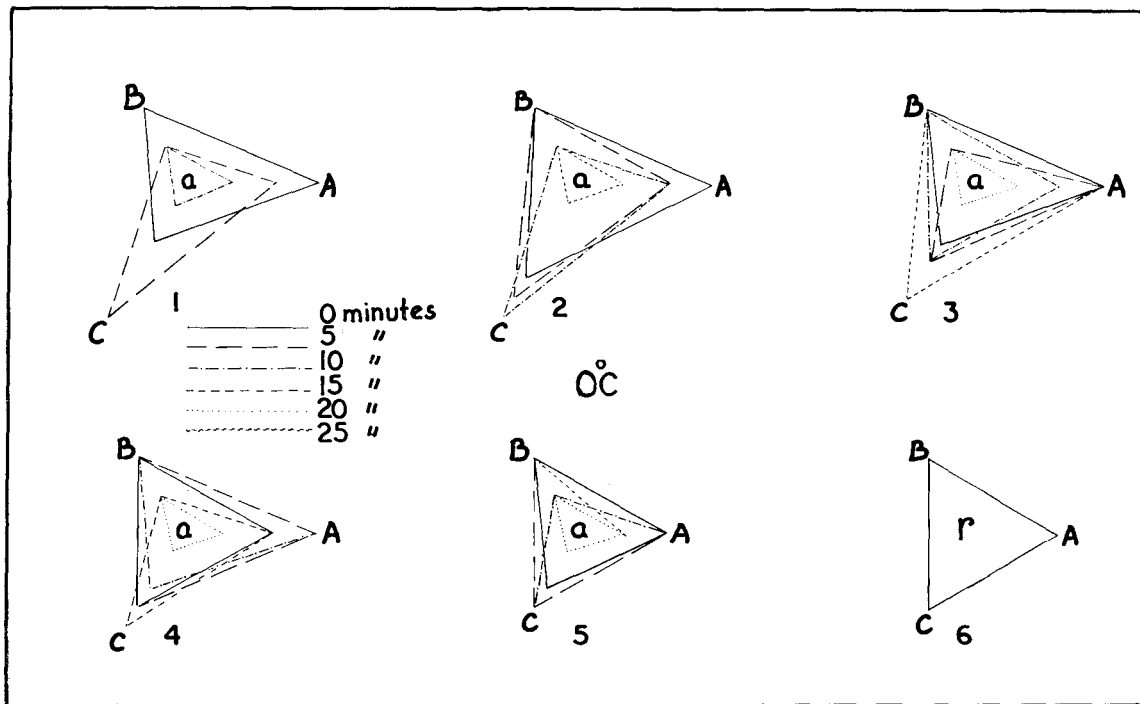


Fig. 3 - Triangular representations of the changes in the ratios of 'A', 'B' and 'C' in *H. dihystra* in different time intervals, treated for different time intervals at 0°C and 45°C; for 20 min. (1), for 40 min. (2), for 60 min. (3), for 80 min. (4), for 100 min. (5), for 120 min. (6).

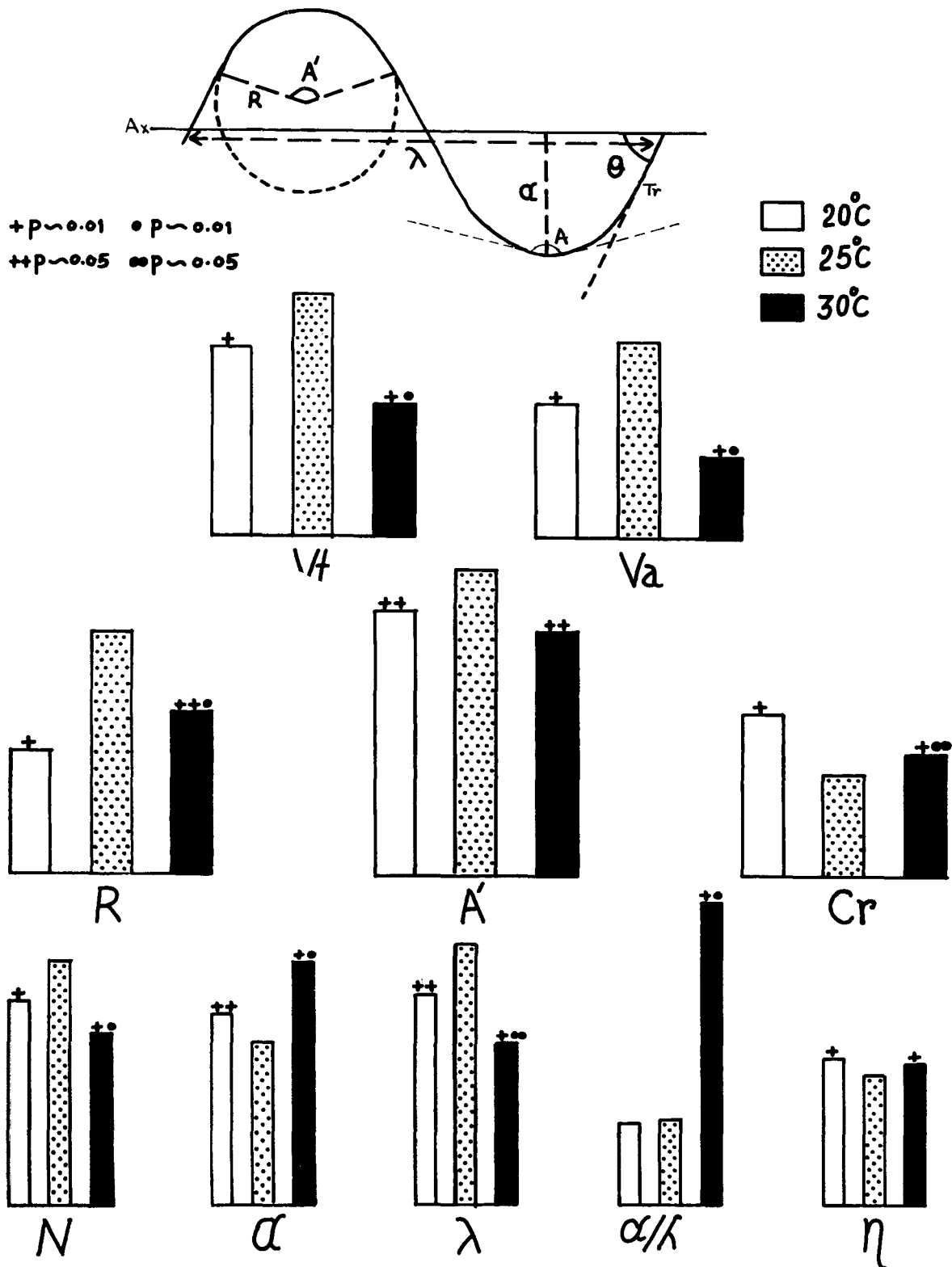


Fig. 4 - Velocity, track and wave characteristics of *H. dihystra* at 20, 25 and 30°C, velocity along the axis (V_a), velocity along the track (V_t), radius of the circle formed by the wave (R) and its subtending angle (A'), number of crossings per track (Cr), wave frequency (wave per min.) (N), amplitude (α), wave length (λ), wave per body length (η), wave pitch (θ), axis (Ax), track (Tr).

CONCLUSION

The above observations suggest that the nematode (*H. dihystra*) can tolerate low temperature by gradually decreasing its activity, coiling themselves, decreasing their velocity of locomotion and showing more muscular contraction, whereas at high temperature they show low rates of activity, high muscular relaxation and decreased velocity of locomotion. The tolerance at low temperature is greater than at high temperatures. It was also found that the nematodes showed less tissue injury at low than at high temperatures.

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S U M M A R Y

Experiments were conducted to study the mechanism of heat tolerance in *Helicotylenchus dihystra* (Cobb) Sher. Low and high temperatures were tolerated by decreasing their activity but mortality was greater at high temperatures. Low temperature tolerance is achieved by increased muscle contraction and high temperature tolerance by increased muscle relaxation. The mechanism of decreasing the velocity of locomotion at low temperature is different from that at high temperature. There was less tissue injury at low than at high temperature.

R I A S S U N T O

Risposta dei nematodi alla temperatura.

I. *Meccanismo di tolleranza al calore da parte di Helicotylenchus dihystra.*

Helicotylenchus dihystra (Cobb) Sher è stato in grado di tollerare alte e basse temperature, diminuendo la sua attività. Tuttavia il tasso di mortalità è stato più elevato ad alte temperature. La tolleranza nei confronti di basse temperature è raggiunta aumentando la contrazione muscolare e quella nei confronti di alte temperature con un maggior rilassamento dei muscoli stessi. Sono state osservate alterazioni dei tessuti di minore entità negli esemplari esposti a basse temperature che non in quelli esposti a temperature più alte.

LITERATURE CITED

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