Thermotactic adaptation in two foliar and two root-parasitic nematodes

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SUMMARY

Thermotaxis in agar was compared among *Ditylenchus dipsaci*, *D. phyllobius*, *Rotylenchulus reniformis*, and *Tylenchulus semipenetrans*. All species exhibited thermotactic preferenda and dispersion temperatures. In most cases, these temperatures were altered in less than 24 hours by differential temperature storage. Responses were complex and highly specific. Foliar parasites were attracted to and dispersed from much lower temperatures than root parasites.

RÉSUMÉ

Adaptation thermotactique de deux nématodes du feuillage et deux nématodes des racines

Ditylenchus dipsaci, *D. phyllobius*, *Rotylenchulus reniformis*, et *Tylenchulus semipenetrans* ont été soumis à des gradients de température, sur agar, et leurs comportements ont été comparés. Chaque espèce est attirée par une température relativement basse (température de preferendum) et évite une température plus élevée (température de dispersion). Très souvent, ces températures sont modifiées en moins de 24 heures après transfert des nématodes à une température ambiante différente. Les comportements thermiques sont compliqués et apparaissent particuliers à chaque espèce. Cependant, les parasites foliaires sont généralement attirés et dispersés par des températures plus basses que les parasites de racines.

. Many hookworm and filariform nematodes are attracted to heat, a response that likely assists penetration of vertebrate hosts (Khalil, 1922; Parker & Haley, 1960; Ronald, 1960; Gupta, 1963; McCue & Thorson, 1964; Croll & Smith, 1972; Al-Hadithi & Habash, 1979; Mok *et al.*, 1986). Aggregations at the warm ends of temperature gradients also have been observed for insect-parasitic, plant-parasitic and free living nematodes, including *Neoaplectana carpocapsae* (Byers & Poinar, 1982), *Pratylenchus penetrans*, *Tylenchorhynchus claytoni* (El-Sherif & Mai, 1969), *Ditylenchus dipsaci* (Klingler, 1972), *Aphelenchus avenae*, and *Panagrellus redivivus* (Hitcho & Thorson, 1972). A less frequently reported behavior is attraction to a preferred temperature, called the eccritic temperature (Croll, 1967; Hedgecok & Russell, 1975) or preferendum (Wallace, 1961; Rode, 1969), whereby nematodes in a cooler region move up a temperature gradient while those in a warmer region move down it. I will use the term thermotactic preferendum to distinguish this temperature from those that are optimal for other processes, such as motility and development. Thermotactic preferenda have only been described for the cod and seal parasite *Terranova decipiens* (Ronald, 1960), the bacteria feeder *Caenorhabditis elegans* (Hedgecock & Russell, 1975), the potato cyst nematode *Globodera rostochiensis* (Rode, 1969), and

Ditylenchus dipsaci (Croll, 1967). Responses of the latter three species were modified by thermal or physiological experience.

When *D. dipsaci* that had been cultured and stored for 30 days at 10", 20" or 30" were injected into tubes of sand along which a temperature gradient was maintained, they aggregated near the storage temperature (Croll, 1967). Spontaneous motility in water also was greatest at the storage temperature. Croll called this effect acclimatization. Klingler (1972) proposed the term adaptation as more appropriate. *Caenorhabditis elegans* aggregated at previous culture temperatures when dishes of nutrient agar containing nematodes were placed on a thermal gradient plate (Hedgecock & Russell, 1975). Starved nematodes dispersed from the culture temperature. The preferendum of *G. rostochiensis* in agar (Rode, 1969) was similarly shifted toward storage temperatures but, unlike *D. dipsaci*, the change was never complete and maximum motility did not occur at the preferendum.

The ecological and taxonomic diversity of the four species for which thermotactic preferenda and adaptation have been described suggest a widespread and important phenomenon. To gain more insight regarding their occurrence and ecological significance, I examined thermotactic preferenda and adaptation in two ad-

ditional root parasites, *Tylenchulus semipenetrans* and *Rotylenchulus reniformis*, and two foliar parasites, *Ditylenchus phyllobius* and *D. dipsaci* (alfalfa race).

Materials and methods

Tylenchulus semipenetrans (predominantly second-stage juveniles) and *R. reniformis* (mixed stages) were extracted from soil by Baermann funnel (16-24 hours). *Ditylenchus phyllobius* and *D. dipsaci* (predominantly fourth-stage juveniles) were extracted from foliar tissue of *Solanum elaeagnifolium* and *Medicago sativa*, respectively, that had been dried and frozen unless stated. Plant tissue was vigorously aerated in water for five hours followed by sieve separation and collection of nematodes with small debris onto a paper filter via Büchner funnel. Final cleanup was done by letting nematodes from the filter actively pass across tissue paper in a Baermann saucer. Nematode suspensions were supplemented with dilute balanced salts (4.5 mM NaCl, 0.4 mM KCl, 0.05 mM $CaCl_2$, 0.05 mM $MgCl_2$), aerated during storage, and verified to be 95 % motile before use.

Temperature gradients were generated in water agar within horizontal channels ($2 \times 3 \times 40$ mm) inside plastic containers made with thin bottoms (100 μm thick) to facilitate heat transfer (Fig. 1). Each container was clamped onto a separate aluminium alloy bar ($1.4 \times 0.4 \times 10$ cm), that served as a linear temperature gradient plate. The ends of bars were bent down around parallel copper pipes (1.5 cm diameter) at 8.3 cm increments, which were immersed in troughs of unstirred water. Water at controlled temperature was pumped through the pipes in one of two ways. Rapid concurrent

flow (2 000 ml/minute) generated identical gradients on 24 bars of a 2-m section. Slow countercurrent flow (80 ml/minute) of warm and cold water through three serially connected sections generated $1 \pm 0.25°$/cm along each bar and simultaneously a $20°$ linear drop in bar midpoint temperature along the 72 bars of the 6-m assembly. Relationships between trough water temperatures and gradients within channels were determined by measuring agar temperature ($\pm 0.01°$) with tissue implantation thermistors (YSI); in all experiments, trough water temperatures at nine points along the bar assembly were continuously monitored with thermocouples connected to a digital data logger.

Nematodes were introduced by quickly mixing 500 μl of nematode suspension ($20°$) with 500 μl 1.5 % water agar ($41°$) and immediately dispensing 250-300 μl of the mixture (100-500 nematodes) into a channel. Thus, nematodes were exposed to a temperature insult for several seconds while the mixture cooled to ambient. Only *D. phyllobius* was noticeably stunned and recovered full motility within ten minutes. To eliminate ionic shock, agar was supplemented with balanced salts identical to those of the nematode suspension. In some experiments, nematodes were dispensed uniformly along the length of the channel. In other experiments, they were dispensed uniformly along one-half of the channel while the other half was filled simultaneously with nematode-free 0.75 % water agar that had been supplemented with dilute salts. Agar temperatures equilibrated less than 2 min after placement on the bar. After exposing nematodes to desired temperatures, agar within the channel was cut in half and placed in counting dishes with a small spatula. Nematodes were then dispersed into water and counted. Results were expressed as the percentage of the total nematodes within a
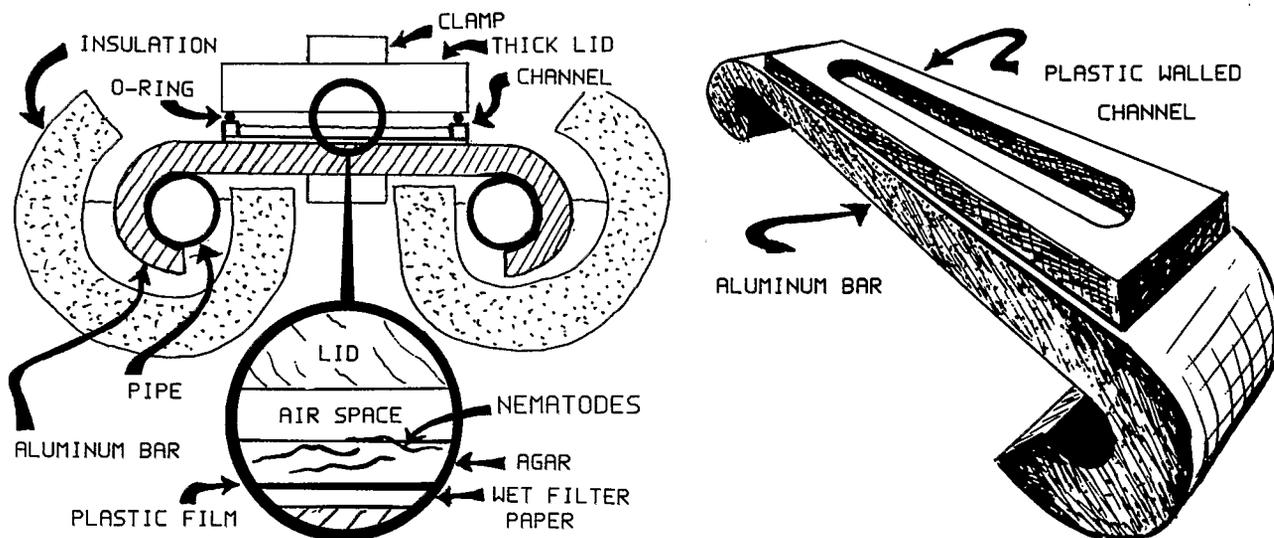

Fig. 1. Diagram of containers and gradient plate apparatus used to examine nematode thermotaxis.

channel in one half of it and were analyzed statistically after arcsine transformation.

For each species a series of experiments was done which determined *1)* optimum agar concentration for random dispersion after placing nematodes in one half of the channel, *2)* rate of isothermal random dispersion at a biologically intermediate temperature (20°) measured by counting nematodes at various time intervals (Fig. 2), *3)* rate of directional aggregation after placing nematodes throughout the channel on 0.1 and 1°/cm gradients
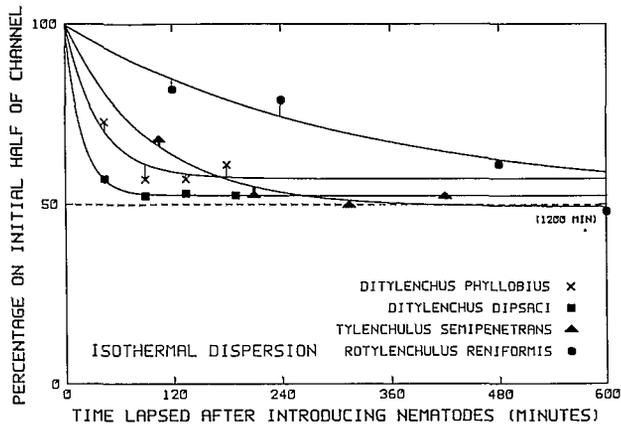

Fig. 2. Kinetics of isothermal random dispersal of four species at 20°. Each symbol represents the mean of four replicates.

centered on 20° during a time interval suitable for random isothermal dispersion of that species at 20° (Tab. 1), *4)* relative extents of random dispersion achieved after a single time interval at eight temperatures, 11-32° (Fig. 3), and *5)* direction of aggregation in response to a 1°/cm gradient centered on seven or twelve temperatures, 10-32°, after enough time for complete random dispersion at most temperatures (Figs 4-7). To
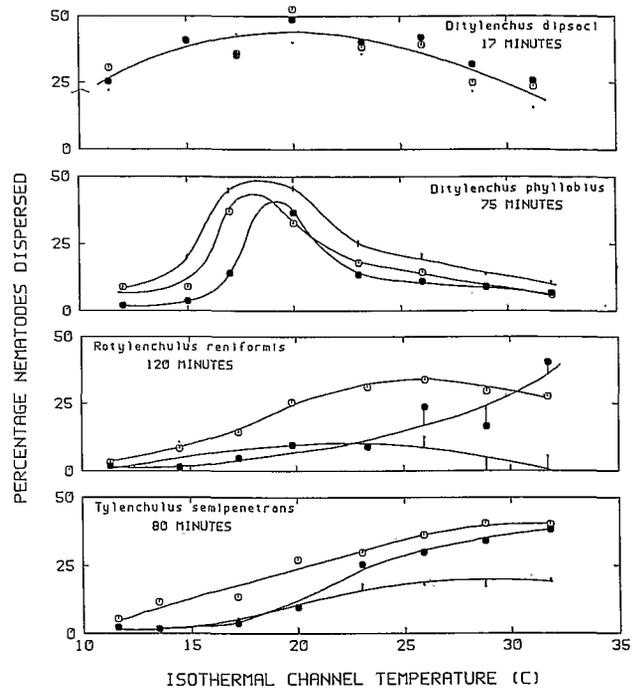

Fig. 3. Isothermal random dispersal of four species at various temperatures as affected by previous storage temperature. Previous storage temperature were 5° (small dots), 15° (open circles) and 25° (black circles) for *D. dipsaci* and *D. phyllobius* and 5° (small dots), 20° (open circles) and 30° (black circles) for *R. reniformis* and *T. semipenetrans*. Each datum is the mean of four replicates.

determine the effects of adaptation, a single nematode suspension was split into two or three parts which were stored at different temperatures for 18-24 hours before responses described as N° 4 and N° 5 above were determined. Storage temperatures were 5°, 20°, and 30°

Table 1

Initial observations of responses to temperature gradients centered on 20°
(Response : percentage of nematodes on warm end of channel)

| Species | *1°/cm* | | *0.1°/cm* | | |
	Run time (hours)	Response (%)	Run time (hours)	Response (%)	Movement toward heat
Rotylenchulus reniformis	17	82** (1)	17	70***	+
Tylenchulus semipenetrans	12	81**	12	75*	+
Ditylenchus dipsaci	1	21**	1	29**	—
Ditylenchus phyllobius	2	19*	3	28*	—

(1) Significantly different from expected value of 50 % at p = 0.05 (*), p = 0.01 (**). Method : confidence interval exclusion for 4 channels after arcsine transformation.
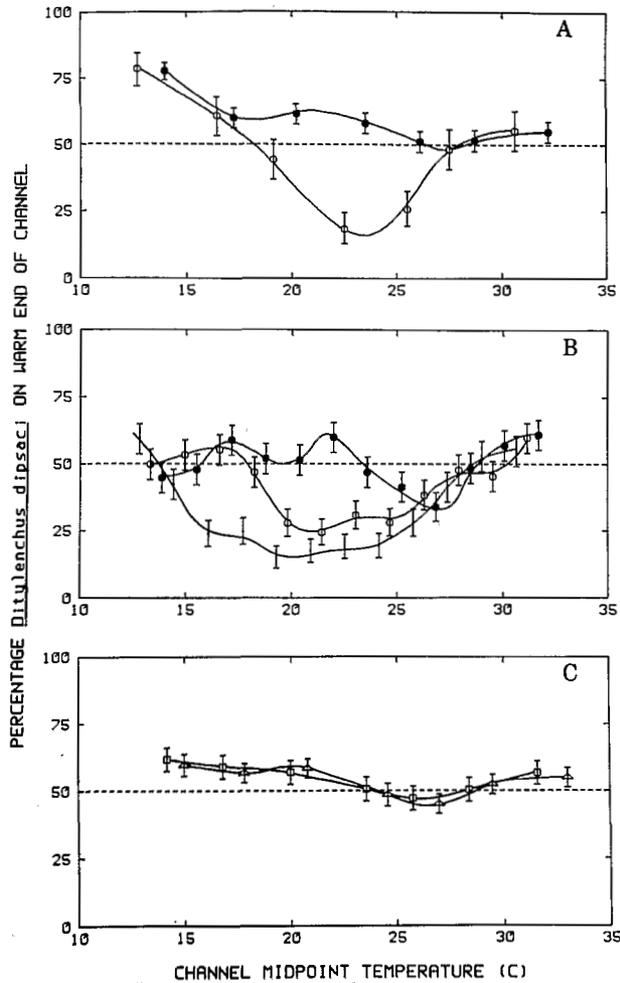
Fig. 4. Response of *D. dipsaci* after 40-60 minutes exposure to a temperature gradient of 1°/cm in assay channels with various midpoint temperatures : A : two suspensions from a single extraction examined simultaneously after overnight storage at 15° (open circles) and 25° (black circles); B : three suspensions from an additional extraction stored overnight at 15°, 25°, and at 5° (small dots); C : nematodes extracted from dried foliage of *Medicago sativa* before (triangles) and after (squares) freezing. Where there are seven data per curve, each datum represents the mean of four replicates. Where there are twelve data per curve, each datum represents the mean of two replicates. Brackets indicate confidence limits (P = 0.05) based on pooled variance for each suspension after arcsine transformation.
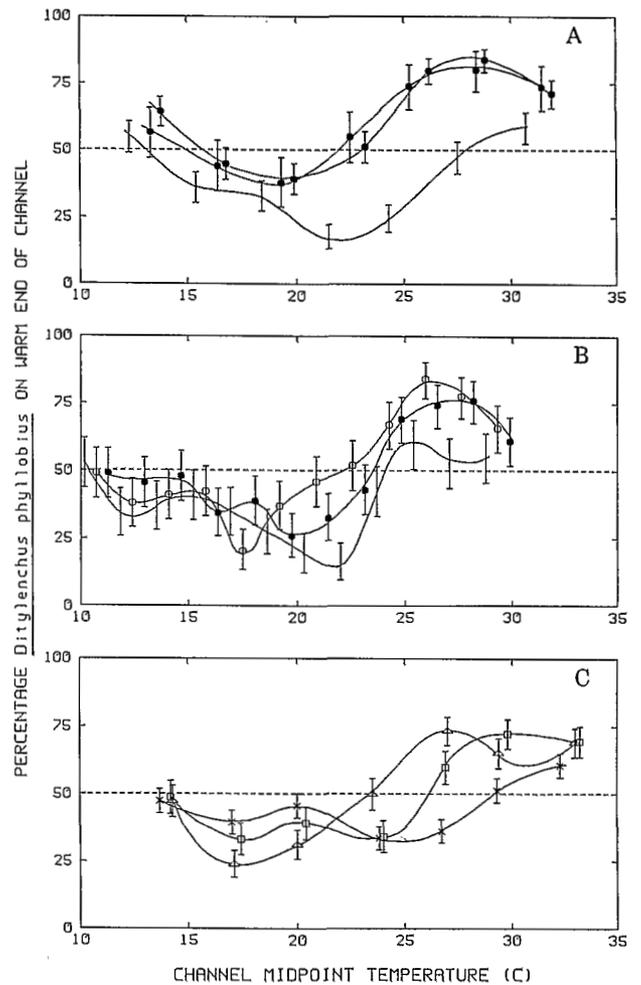
Fig. 5. Response of *Ditylenchus phyllobius* after 90-100 minutes exposure to a temperature gradient of 1°/cm in assay channels with various midpoint temperatures : A : suspensions stored overnight at 5 (small dots) and 25° (black circles); B : three suspensions from a single extraction examined simultaneously after overnight storage at 5°, 15° (open circles), and 25° (black circles); C : nematodes extracted from foliage of *Solanum elaeagnifolium* that was succulent (x's), dried (triangles), or dried and frozen (squares). Brackets indicate confidence limits (P = 0.05) based on pooled variance for each suspension after arcsine transformation.

Results

A water agar concentration of 0.75 % permitted random dispersion by all species when nematodes were placed initially in one half of each channel. At 20°, the time needed for complete dispersion varied from less than 1 h for *D. dipsaci* to more than 12 h for *R. reniformis* (Fig. 2). When gradients of 0.1 and 1°/cm were maintained along channels centered at 20° for time intervals sufficient for completion of isothermal disper-

for *R. reniformis* and *T. semipenetrans* and 5°, 15°, and 25° for *D. dipsaci* and *D. phyllobius*. In supplemental experiments, responses to temperature gradients were compared among *D. phyllobius* and *D. dipsaci* that had been extracted from plant tissue that was fresh, dried, or frozen.
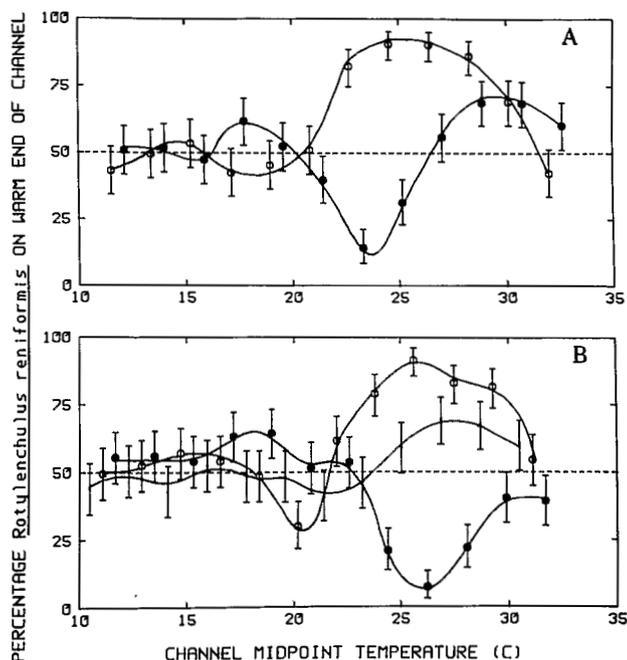
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Fig. 6. Response of *Rotylenchulus reniformis* after 210-245 minutes exposure to a temperature gradient of 1"/cm in assay channels with various midpoint temperatures : A : a suspension extracted from soil via Baermann funnel, then divided and stored overnight at 20" (open circles) and 30" (black circles); B : another suspension that was stored also at 5" (small dots). Brackets indicate confiden. 'e limits (P = 0.05) based on pooled variance for each suspension after arcsine transformation.

sion, all species responded. *Ditylenchus phyllobius* and *D. dipsaci* aggregated at the cool ends of channels whereas *R. reniformis* and *T. semipenetrans* aggregated at the warm ends (Tab. 1).

Differential temperature storage altered rates of isothermal random dispersion differently for different species (Fig. 3). Substantial dispersion by *D. phyllobius* after 75 minutes occurred in a narrow range of temperature (17-21") while dispersion of *D. dipsaci* after 17 min was almost uniformly progressed between 13" and 28". The warmer the storage temperature was, the more slowly *D. phyllobius* dispersed. This effect was not detected for *D. dipsaci*. The thermal optimum for isothermal dispersion was not shifted appreciably in the direction of the storage temperature for either species. Compared with the foliar species, thermal optima for isothermal dispersion by *R. reniformis* and *T. semipenetrans* were relatively high (above 25") even after 5" storage. Nematodes of the latter two species dispersed fastest over a wider range of temperatures after 20" storage than after storage at 5 and 30". After 30" storage, thermal optima for dispersion were near or above the highest temperature examined, 32".
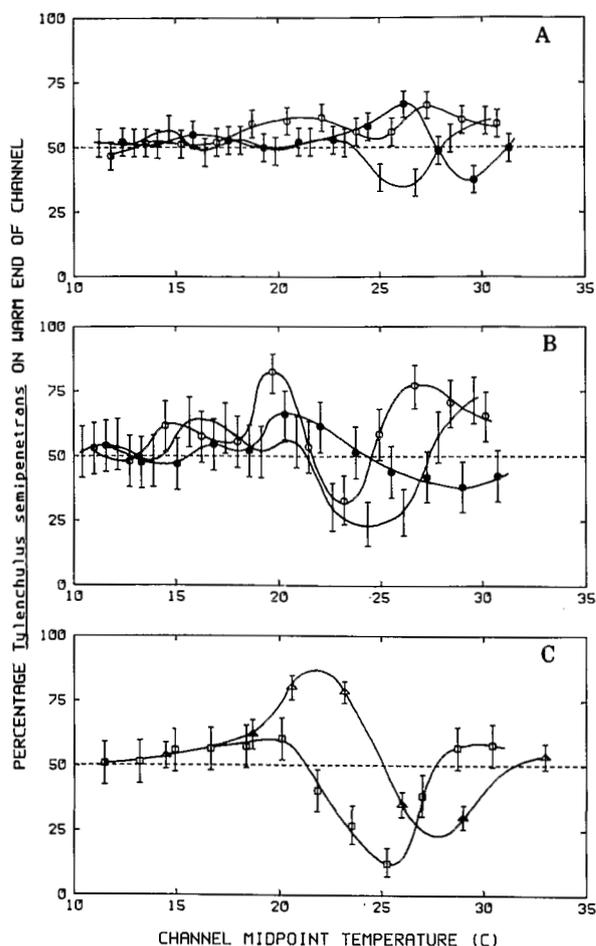
When a gradient of 1"/cm was maintained in channels

Fig. 7. Response of *Tylenchulus semipenetrans* after 150-160 minutes exposure to a temperature gradient of 1"/cm in assay channels with various midpoint temperatures : A : a suspension extracted from orchard soil in October via Baermann funnel, then split into three parts stored overnight at 5" (small dots), 20" (open circles), and 30" (black circles); B : same as A but extracted from soil in December; C : suspensions assayed the same day they were obtained from soil in July (triangles) and December (squares). December nematodes were from the same extraction as B but prior to differential temperature storage. Brackets indicate confidence limits (P = 0.05) based on pooled variance for each suspension after arcsine transformation.

at various midpoint temperatures, the effect of previous storage temperature on the direction of movement varied appreciably among species. Storage temperature strongly influenced the response of *D. dipsaci*, which exhibited preferenda of 14, 18, and 24" after being stored at 5, 15, and 25", respectively (Fig. 4). *D. dipsaci* always dispersed from 28", however, regardless of storage. Temperatures from which nematodes dispersed will be referred to as dispersion temperatures. Movement of *D.*

dipsaci up a gradient toward the preferendum was consistently less pronounced than down it; results of concurrent isothermal dispersion experiments indicated time was not a limiting factor. By comparison, storage temperature had less effect on *D. phyllobius* and a simple shift in the preferendum was not detected. Nine of ten suspensions moved toward cold over a wide range of temperatures (11-24) regardless of their storage temperature (Fig. 5).

The root parasites, *T. semipenetrans* and *R. reniformis*, had higher thermotactic preferenda than the foliar parasites (Figs 6, 7). The lowest preferenda detected were 16" for *R. reniformis* stored at 20" and *ca* 22" for *T. semipenetrans* stored at 20 and 5". Both species had high dispersion temperatures (20 to 32") that were shifted more by storage temperature than the preferendum. After 20" storage, movement toward heat above the dispersion temperature diminished near 32", suggesting a second, higher preferendum.

For all but one combination of species and storage temperatures, preferenda occured at temperatures well below corresponding thermal optima for random dispersion. The only exception was suspensions of *D. dipsaci* stored at 25" whose optimum temperature for dispersion was not determined accurately enough to make this comparison. Dispersion temperatures for *D. phyllobius* and *D. dipsaci* were 3-9" above thermal optima for random movement whereas dispersion temperatures for *T. semipenetrans* and *R. reniformis* were near or below thermal optima for movement. Drying and freezing plant tissue before extracting *D. phyllobius* or *D. dipsaci* did not alter behavioral reponses appreciably. A suspension of *Tylenchulus semipenetrans* extracted from orchard soil in July exhibited a preferendum and dispersion temperature *ca* 4" higher than a similar suspension extracted in December (Fig. 7).

Discussion

Generally, there could be discerned a low range of temperature within which nematodes tended to move toward a preferendum, separated by a dispersion temperature from a high range within which nematodes moved toward heat (Fig. 8). Aggregations toward preferenda occurred at temperatures which permitted rapid random dispersion and thus appear to have resulted from taxis rather than kinesis or trapping. Nematodes responded to gradients as small as 0.1"/cm; similar thresholds have been estimated for other species (Mok *et al.*, 1986). Effects of overnight storage at new temperatures on the thermal preferendum of *D. dipsaci* were particularly consistent.

Wallace (1961) originally reported negative thermotaxis by *D. dipsaci* and Croll (1967) found this behavior to be subject to adaptation; nematodes cultured at 10, 20, and 30" aggregated on gradients in sand near their
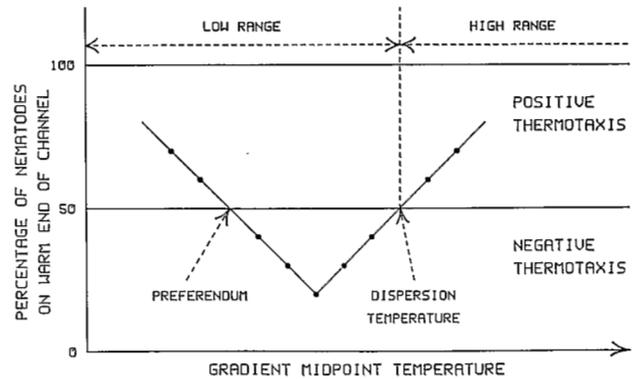

Fig. 8. General response of plant-parasitic nematodes submitted to a temperature gradient.

previous culture temperatures. When Klingler (1972) compared thermotaxis by *D. dipsaci* and *P. penetrans*, however, he observed only positive thermotaxis and interpreted his results to be inconsistent with those of Croll and Wallace. After culture in onions at 17.6", *D. dipsaci* were strongly attracted in agar to heat sources 1" above ambient not just at 8.6" ambient temperature, which Croll's results would have predicted, but also at 28.3". Thus, nematodes appeared to respond thermopositively over a wide range of temperatures regardless of storage. Positive thermotaxis at 8.6 and 28.3" was also observed for *P. penetrans* cultured at 17.6", in agreement with positive thermotaxis observed in *P. penetrans* by El-Sherif and Mai (1969). Klingler therefore proposed that technique differences somehow were responsible for differences between his and Croll's results. Although I did not examine responses at temperatures as low as 8.6", my observations of *D. dipsaci* suggest that Croll's and Klingler's results are in agreement. Klingler's nematodes would be predicted to react thermopositively at 8.6" (below the preferendum), and at 28.3" (above the dispersion temperature), but thermonegatively between 18 and 27", temperatures Klingler did not examine. An interpretation of Croll's data suggests that his nematodes also dispersed from 25-30".

Dispersion temperatures were reported previously for *C. elegans* (Hedgecock & Russell, 1975). The dispersion temperatures I observed for *D. dipsaci* and *D. phyllobius* are far enough above thermal optima for random dispersal to suspect that aggregations above dispersion temperatures may result from negative kinesis or trapping. This interpretation cannot be made for the suspensions of *R. reniformis* and *T. semipenetrans* stored overnight at 20", which dispersed from temperatures below thermal optima for random movement. *R. reniformis* stored at 20" in particular strongly aggregated thermopositively from 21-31" even though fastest isothermal dispersal occurred above 25".

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Big changes in the direction of movement on a temperature gradient after only 18-24 hours differential temperature storage is consistent with the notion that diurnal soil temperature fluctuations resulting from surface heating and cooling may be behaviorally important. Rapid behavioral adaptation was reported previously for *C. elegans*, whose responses to temperature are reversed within 2-6 hours (Hedgecok & Russell, 1975). Rode (1969) observed changes in thermal behavior of *G. rostochiensis* after five days of temperature storage. There appear to be species-dependent limits to the degree of adaptation that can occur. This is particularly apparent for *D. phyllobius* juveniles from fresh field-grown foliage of *S. elaeagnifolium*. These nematodes were extracted with water at 25° from succulent leaf galls which according to weather data were never exposed to air with dewpoint below 24°, and therefore could never have cooled below 24° by evaporation. In agar, the nematodes oriented toward a preferendum (13°) far colder than any temperature they had experienced during development.

A clear understanding of the ecological implications of thermotaxis and adaptation will require much more research in several areas. Except for *D. dipsaci*, thermotaxis by plant-parasitic, insect-parasitic, and free living nematodes has been examined only in artificial gels and should be verified in natural substrates. Also, more species need to be examined and rates of adaptation need to be related to rates of temperature change in soil and on foliar surfaces. It is noteworthy, however, that among the four species I examined, each from southwestern North America, thermal preferenda and optima of foliar parasites were much lower than those of root parasites. To invade host tissue, infectives of both foliar species move from dead foliage or from soil up moist stem surfaces after dew or rainfall when temperatures of stems and leaves are likely to be substantially reduced by evaporation. It may be instructive to carefully examine the thermal behavior of mermithid and trichostrongyle nematodes that accend plant foliage before host invasion. It probably is premature to speculate on the role that preferenda and dispersion temperatures may play in host-finding or stress avoidance by soil borne species.

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