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## A comparative study of the movement of some microphagous, plant-parasitic and animal-parasitic nematodes

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### INTRODUCTION

Most nematodes in their free-living stages, whether they feed on plants, animals or microbes, have long cylindrical bodies. This makes it likely that they will move by the same mechanism regardless of their host or habitat, but this has never been studied. The work described in this paper was done to see whether or not microphagous nematodes and those parasitic on plants and animals behave similarly when in the same environment.

### METHODS

Wave formation and forward speed of some plant and microphagous nematodes were observed by projecting pictures frame by frame from a ciné film, and by drawing the tracks of nematodes with a camera lucida and marking off the time intervals. Wave frequency was measured by counting the number of times the head moved from one position back to the same position per unit time. Body length was measured with a camera lucida after the nematodes had been straightened by heating in water at 60° C.

Methods for determining the relationship between mobility of third-stage infective larvae of *Trichostrongylus colubriiformis* and hydrostatic pressure deficiency (suction) in a sand–water medium were described by Wallace (1958*a, b*). Polythene tubes, 5 cm. long and 0.5 cm. diameter were attached to the end of sintered glass tubes and then filled with sand of particle diameter 150–250 $\mu$ . The sand was saturated with water, the tubes arranged horizontally and a known suction applied. Ten suction in twofold replication were obtained in this way to give a range of moisture contents from saturated to a stage where only small amounts of water remained at the points of contact of particles. The relationship between the moisture content of the sand and suction, and the distribution of water was previously determined from the moisture characteristic of the medium (Wallace, 1958*a*). The polythene tubes were then removed from the sintered tubes and about 150 nematodes were introduced at one end in a small drop of water. The tubes were sealed at both ends with plasticene to prevent evaporation and placed horizontally in an incubator at 24° C. for 1 day. Each tube was then cut into two, 1 cm. from the inoculation end, and the nematodes in each section counted. Mobility was expressed as the percentage of nematodes migrating more than 1 cm. in 1 day.

The method of assessing the influence of temperature on nematode activity was similar to that described by Wallace (1958*b*). The lower ends of vertical polythene

tubes 4 cm. long and 1 cm. diameter were covered with nylon gauze and filled to a depth of 1 cm. with sand of 150–250 $\mu$  diameter. Each tube dipped into water in a specimen tube so that the sand remained saturated. Four such tubes were placed in each of a series of constant temperature rooms ranging from 5 to 30° C. Nematodes were placed in about 0.5 ml. of water in the constant temperature rooms and after 1 hr. about 100 of them were pipetted on to the surface of the saturated sand. Three hours later the nematodes that had migrated down through the sand into the water and those left in the sand were counted. Activity was expressed as the percentage that migrated down through 1 cm. of saturated sand in 3 hr.

The effect of desiccation on the subsequent activity of third-stage *T. colubriiformis* larvae was studied by the method of Wallace (1962). About 100 nematodes were placed on a coverslip in a small drop of water. When the water was almost evaporated, the coverslip was suspended over a KOH solution in a specimen tube closed with a rubber bung. The concentration of the KOH was such as to give a relative humidity of 50% in the tube. Twenty-four such tubes were placed in an incubator at 24° C. and, at intervals over 32 days, the coverslips from three of the tubes were removed and immersed in water. Resistance to desiccation was expressed as the numbers of nematodes that showed undulatory movement after 24 hr. in water.

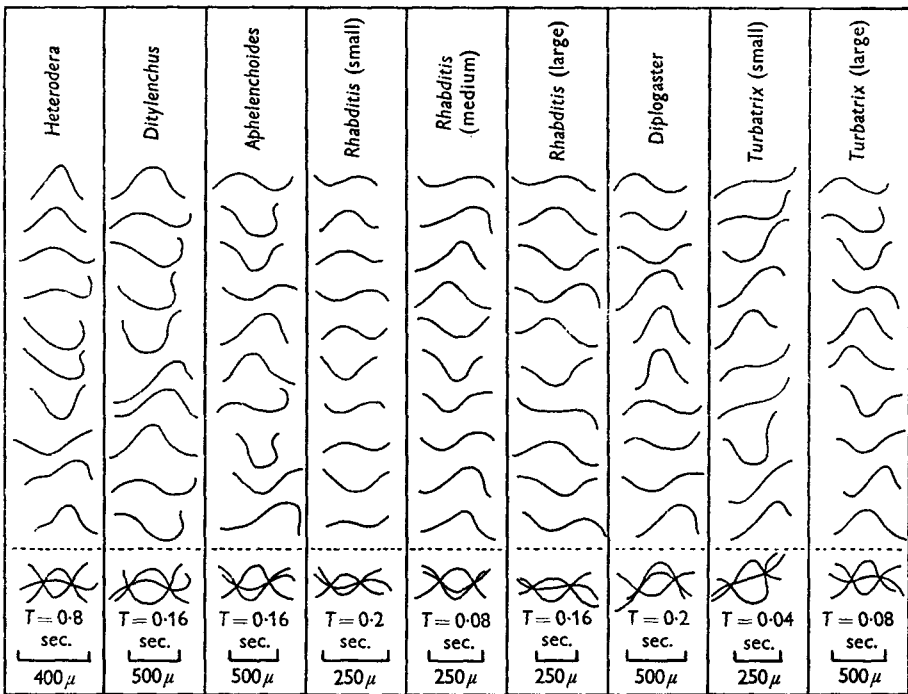
Observations on the attraction to, and aggregation around, roots of Timothy grass (*Phleum pratense*) by *T. colubriiformis* were made by two methods: (1) Petri dishes, 9 cm. in diameter, were marked on the undersurface with parallel lines (Text-fig. 8), and then filled with 0.5% water agar. Ten 4-day-old grass seedlings were placed together within the agar at one end of each of five dishes. Five other dishes received no seedlings. After 5 days, a core of agar, about 0.5 cm. diameter was removed from the centre of each of the ten dishes and replaced with cold, fluid 0.5% agar containing about 200 nematodes. The dishes were then placed in the incubator in the dark at 24° C. and were removed only for examination. After 0.5, 1.5, 2.5 and 3.5 hr., the nematodes in each sector of the agar were counted. This method resembles that of Wallace (1960) and Lavalley & Rohde (1962). (2) The roots of five grass seedlings were arranged parallel to each other on 1% agar at one side of each of five Petri dishes 5 cm. in diameter. A similar series of dishes was also set up, but the roots were previously killed by immersion in boiling water for 3 sec. The dishes were left for 16 hr. and then about 150 nematodes were placed in a line across each dish so that they were about 0.5 cm. from the root tips. At the end of 0.5, 1, 2 and 4 hr., the nematodes at the roots were counted. This technique was suggested from the experiments of Bird (1962). No attempt was made in either method to maintain sterile conditions on the agar.

## RESULTS

### *Undulatory propulsion*

The movement in deep water of the plant nematodes, *Heterodera rostochiensis*, *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi* and the microphagous nematodes *Rhabditis* sp., *Diplogaster* sp. and *Turbatrix aceti* was recorded on ciné film.

Text-fig. 1 shows sequences of pictures with the time interval between successive positions of the nematodes. The main facts that emerge from these observations are: (1) Only one asymmetrical wave is formed at a time and this usually decreases in amplitude as it passes back along the body. (2) During forward progression in most nematodes, there are two points in space at which there is little lateral movement of the body (Pl. 1, fig. 1); in the slower moving species these points are not obvious but can be seen when frames from the film are superimposed. Gray (1953) terms these two places 'nodes', and states that ceratopogonid larvae and eels also have them. Thus, wave pattern in those nematodes examined was similar

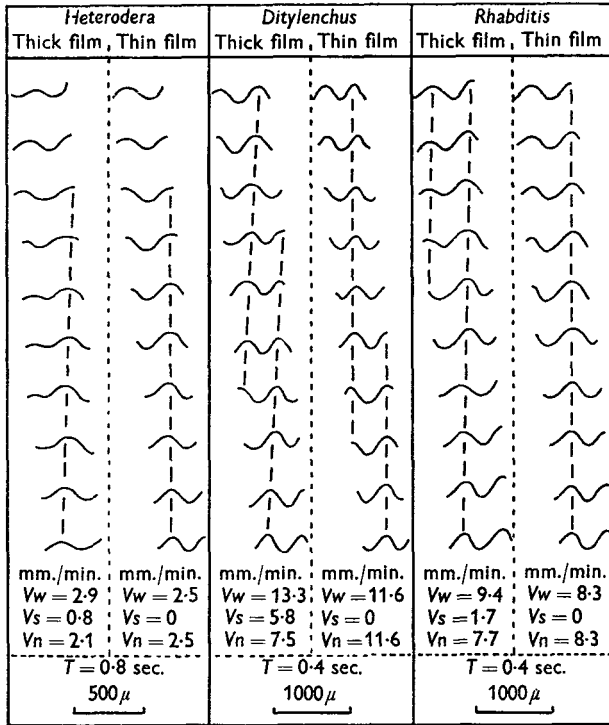


Text-fig. 1. Wave formation of plant parasitic and saprophytic nematodes in deep water. The drawings were made from ciné film at different frame intervals. The time interval between each position of the nematode, the linear scale and the position of the two nodes are shown.

and direct observations on third stage larvae of the animal nematodes *Trichostrongylus colubriformis*, *Dictyocaulus viviparus*, and *Nippostrongylus brasiliensis* showed the same general pattern. Gray's (1953) pictures of a ceratopogonid larva swimming in water closely resemble those in Text-fig. 1, so the bending waves of most undulatory organisms may be similar.

In water films as thick as the nematode's own body diameter the nematode is in close contact with the substratum and the waves are more symmetrical. In such films, movement is intermediate between swimming and crawling, because the waves slip back relative to the substratum. It is the component of motion of this wave slip, normal to its own longitudinal axis, that elicits a normal reaction

from the water and propels the nematode forward (Gray, 1953). As the film gets thinner, surface tension forces act on the nematode and increase the normal reaction between nematode and substratum (Wallace, 1959). Ventral friction also increases and the amount of wave slip decreases until, at a critical film thickness, there is no slip and succeeding parts of the nematode's body follow one another exactly. A track is left the same thickness as the nematode's body and this type of movement is termed 'crawling'. During swimming, crawling and all the intermediate stages of movement the velocity of the nematode ( $V_n$ ) equals the velocity of the



(activity) and body length of several species of microphagous, plant and animal nematodes. The results (Table 1) were based on observations of twenty individuals from each species except for the *Plectus* sp. and *Monhystera* sp., of which only ten individuals were observed. Plotting the product of body length and activity against speed for each species produced a scatter of points (Text-fig. 3). A line fitted to the points by the method of least squares had a correlation coefficient of 0.96, indicating a close linear relationship between the points and supporting the generalization that nematodes have a characteristic wave pattern that reacts in a predictable way to changes in environmental resistance. The observations also resemble previous ones on the movement of plant and microphagous nematodes in thin films (Wallace, 1958c).

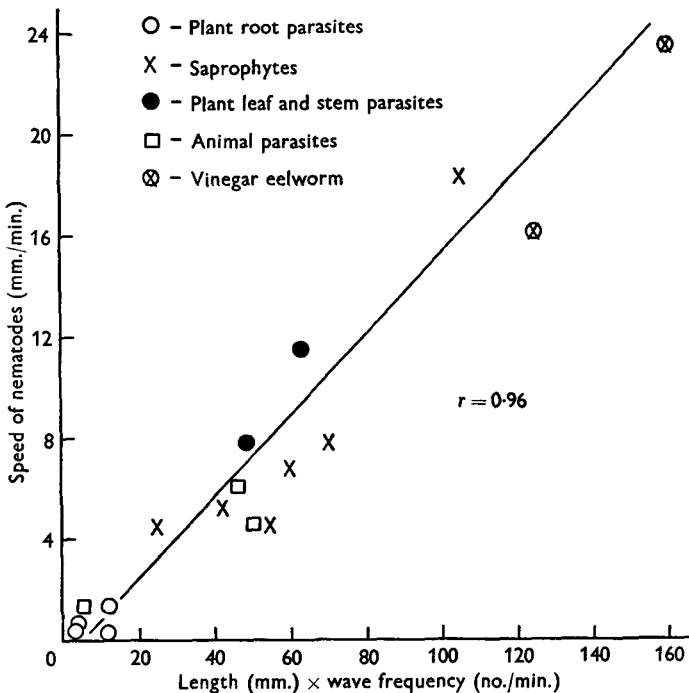
Table 1. Measurements of body length (*L*), wave frequency (*F*) and velocity (*V*) of some saprophytic, plant parasitic and animal parasitic nematodes moving in deep water at 18° C.

(Each figure is the mean of twenty observations except for *Plectus* and *Monhystera* where ten observations were made.)

	<i>L</i> (mm.)	<i>F</i> (No./min.)	<i>L</i> × <i>F</i>	<i>V</i> (mm./min.)
Microphagous nematodes				
<i>Rhabditis</i> sp. (small) larvae	0.25	100	25	4.5
<i>Rhabditis</i> sp. (medium) larvae	0.44	125	55	4.5
<i>Rhabditis</i> sp. (large) adults	0.56	125	70	7.8
<i>Diplogaster</i> sp. adults	0.82	75	62	6.8
<i>Turbatrix aceti</i> (small) larvae	0.50	250	125	16.1
<i>T. aceti</i> (large) adults	0.85	188	160	23.5
<i>Plectus</i> sp. adults	0.70	60	42	5.2
<i>Monhystera</i> sp. adults	0.71	150	107	18.2
Plant parasites				
Leaf and stem parasites				
<i>Ditylenchus dipsaci</i> (4th-stage larvae)	0.90	54	49	7.8
<i>Aphelenchoides ritzemabosi</i> adults	1.00	63	63	11.5
Root parasites				
<i>Heterodera rostochiensis</i> (2nd-stage larvae)	0.48	8.3	4.0	0.7
<i>Meloidogyne hapla</i> (2nd-stage larvae)	0.50	6.9	3.5	0.4
<i>Xiphinema diversicaudatum</i> adults	4.10	3.0	12.3	0.2
<i>Tylenchorhynchus dubius</i> adults	0.80	16	12.8	1.3
Animal parasites				
<i>Trichostrongylus colubriformis</i> (3rd-stage larvae)	0.63	73	46	6.0
<i>Nippostrongylus brasiliensis</i> (3rd-stage larvae)	0.66	76	51	4.5
<i>Dictyocaulus viviparus</i> (3rd-stage larvae)	0.30	20	6.0	1.1

A comparison of the wave pattern, wave frequency and forward speed of the plant nematode *Ditylenchus dipsaci* and the animal nematode *Trichostrongylus colubriformis* indicated further similarities. The wave characteristics of twenty individuals in each species were obtained by recording their movements in water

and on the surface of six different strengths of water agar (Text-fig. 4). In both species: (1) the angle of the body waves to the line of progression was greater on 0.625% than on 1.25% agar; (2) as agar strength increased to 10% the angle increased; (3) the number of body waves at any one time increased with increase in agar strength; and (4) speed and wave frequency decreased as agar strength increased (Text-fig. 5). The two nematode species both reacted similarly to changes in the external forces. As the water film on the surface of the agar decreased in thickness with increase in agar strength, the surface tension forces pulling the

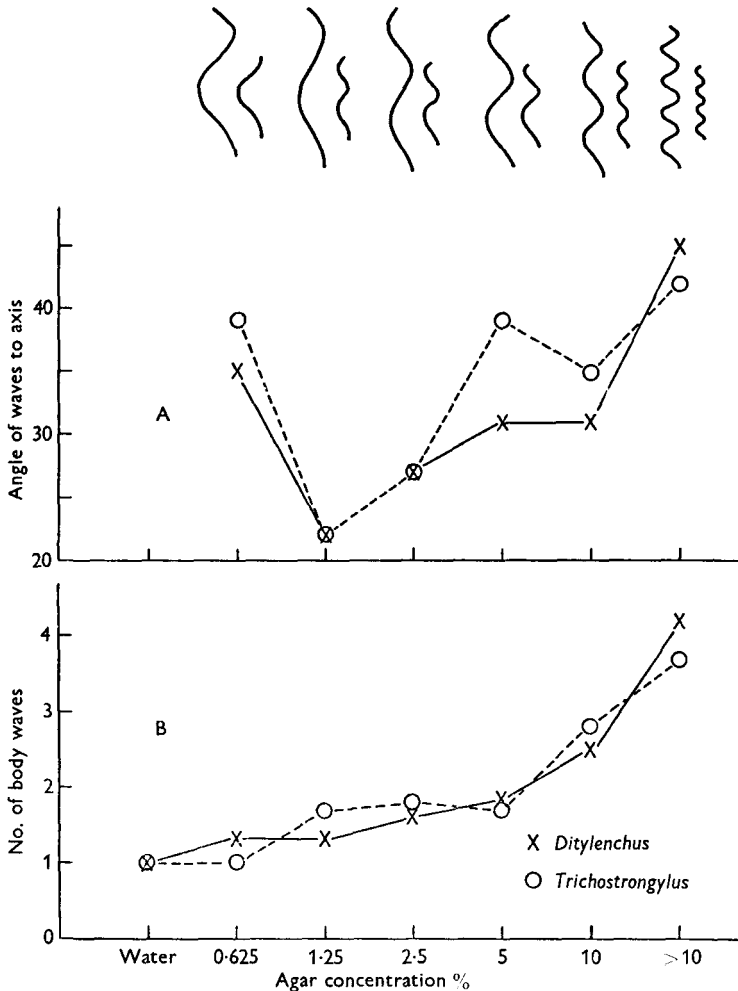


Text-fig. 3. The relationship between the speed of nematodes and the product of their length and wave frequency when moving in deep water. Each point is the mean of twenty observations. Full data are in Table 1.

nematode down on the agar increased. Consequently, resistance to forward movement was increased and the angle of the waves increased, giving greater propulsive power (Gray, 1953). The number of waves formed by the body also increased and this may have given increased propulsion. That *D. dipsaci* and *T. colubriformis* move faster in deep water and thick films than in the intermediate film thicknesses demonstrates that they are efficient swimmers, unlike the plant nematode *Heterodera schachtii* which moves fastest when crawling in thin films (Wallace, 1958b). This difference between species is related to activity; nematodes swimming in deep water, for example, must generate enough propulsive power to overcome the downward force of their own body weight. *H. schachtii* is a relatively inactive nematode and cannot swim, whereas *T. colubriformis* and *D. dipsaci* are active enough to swim upwards in thick water films.

*The influence of suction on the mobility of T. colubriformis*

Text-fig. 6 shows the relationship between mobility and suction. At low suction when the sand is saturated mobility is low, probably because of lack of aeration, and it increases with increase in suction as water is removed from the pores. At higher suction mobility decreases because the pockets of water at the points of



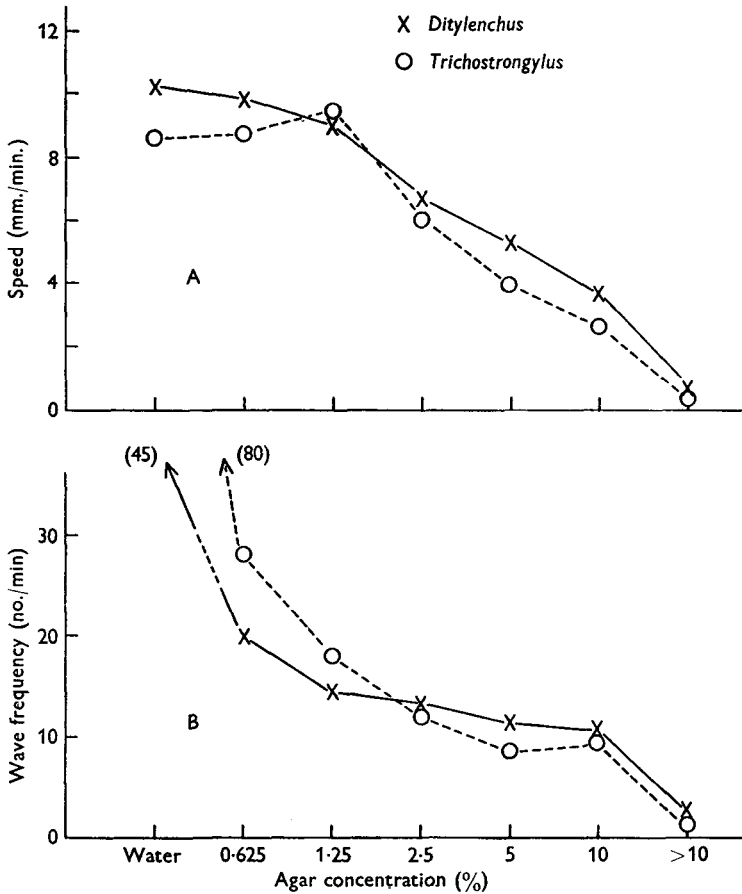
Text-fig. 4. Wave formation by *Ditylenchus dipsaci* and *Trichostrongylus colubriformis* in water and on the surface of agar of different strengths. (A) Angle of waves to the axis of progression. (B) Number of waves formed by body. Each point is the mean of twenty observations. The drawings of nematodes above the graphs show the changes in wave formation at each agar strength. The large nematode is *D. dipsaci*, the smaller one *T. colubriformis*.

contact of particles decrease in volume, and resistance to movement increases in the thin water films. Similar movement in sand at different suction was observed for ecto-parasitic and endo-parasitic plant nematodes (Wallace, 1964).



*The influence of temperature on the activity of T. colubriformis*

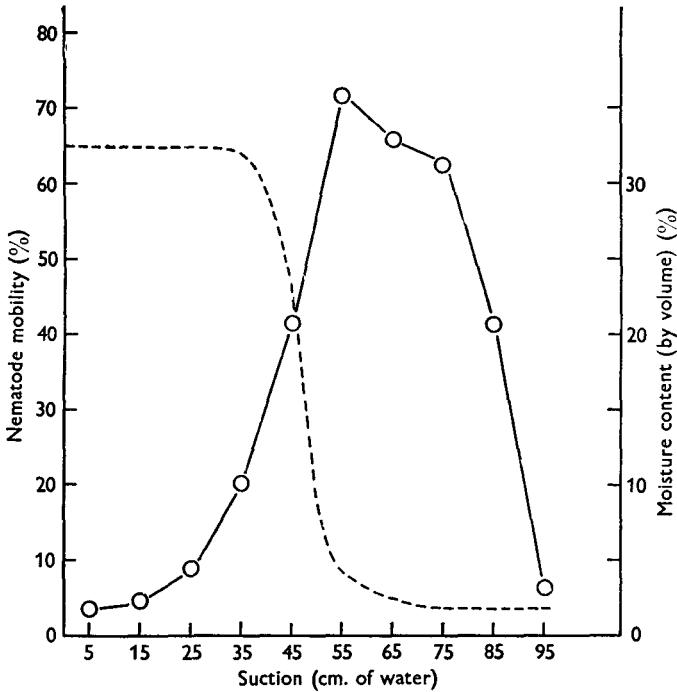
The optimum temperature for activity was about 20° C. (Text-fig. 7). Measurements on the activity of *D. dipsaci* using the same technique as that for *T. colubriformis* gave a similar result (Wallace, 1962) and support the hypothesis that the optimal temperature range for plant and animal nematodes (free-living stages) is similar (Wallace, 1961). The optimum temperature range for activity of *T. retortaeformis* larvae is 22–25° C. (Goodey, 1922).



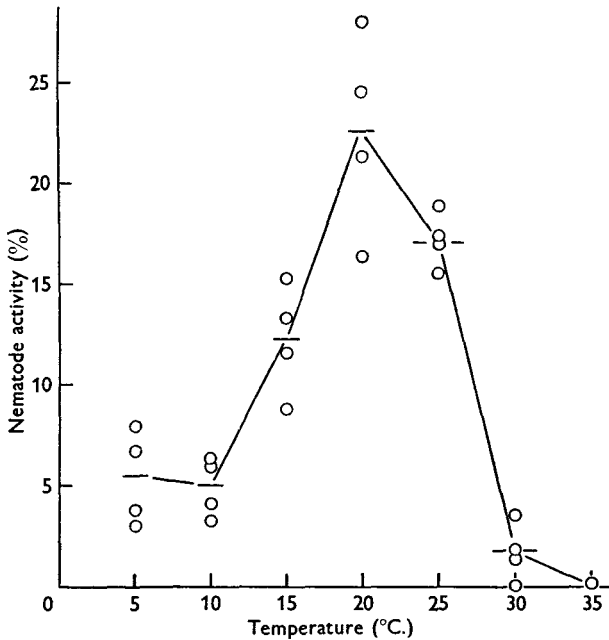
Text-fig. 5. (A) The speed and (B) wave frequency of *D. dipsaci* and *T. colubriformis* on the surface of agar at different strengths. Each point is the mean of twenty observations.

*The influence of desiccation on subsequent activity of T. colubriformis*

A previous experiment showed that *D. dipsaci* survived desiccation at 50% relative humidity and 24° C. for at least 32 days (Wallace, 1962). An identical experiment with *T. colubriformis* larvae gave similar results. At the end of 32 days all the larvae revived in water and had a characteristic undulatory motion. Infective larvae of *Trichostrongylus axei* also resist desiccation; they survived 104 days at 38° C. and 29% relative humidity (Stewart & Douglas, 1938).



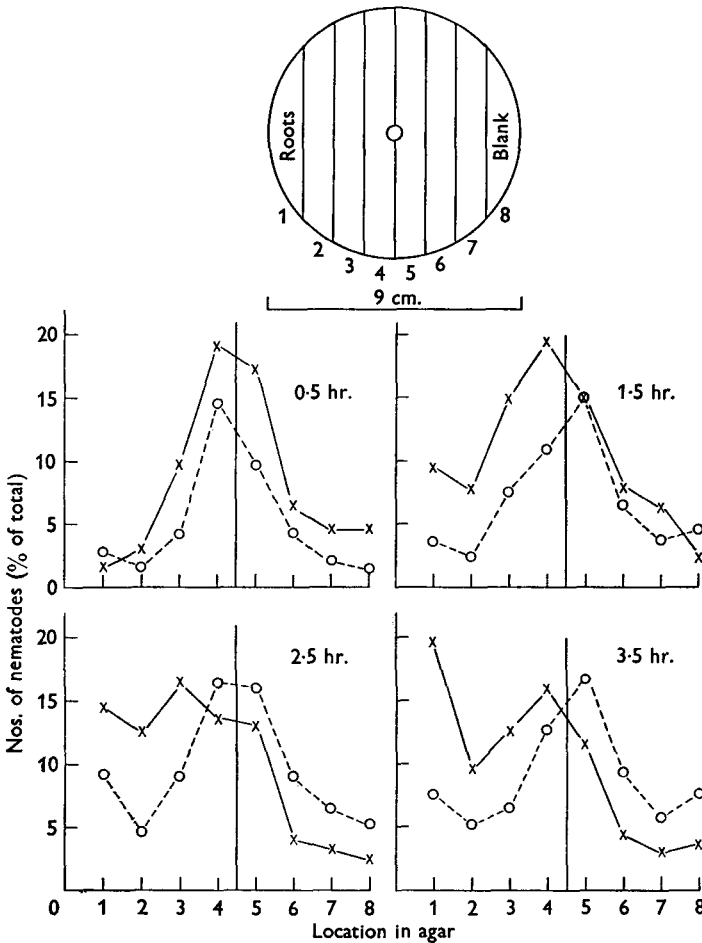
Text-fig. 6. The mobility of *T. colubriformis* in sand at different suctions. Each point is the mean of two replicates. Mobility is expressed as the percentage of nematodes migrating more than 1 cm. in 1 day. The moisture characteristic (dotted curve) gives the relationship between moisture content of the sand and suction.



Text-fig. 7. The influence of temperature on the activity of *T. colubriformis*. There were four observations at each temperature and the mean is shown as a horizontal line. Activity is expressed as the percentage of nematodes migrating downwards through 1 cm. of saturated sand in 3 hr.

*The aggregation of T. colubriformis around grass roots*

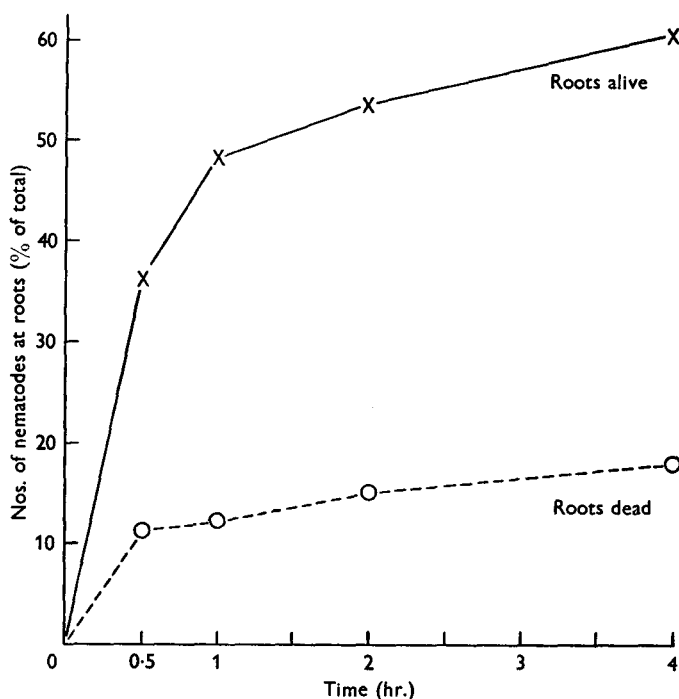
The spread of nematodes through agar from the centre of a Petri dish containing grass roots at one side, was observed at intervals over 3.5 hr. (Text-fig. 8) and compared with the spread in dishes without roots. At the end of half an hour



Text-fig. 8. The attraction of *T. colubriformis* to grass roots (*Phleum pratense*). Petri dishes were marked as indicated in the diagram at the top of the figure. Nematodes were introduced into 0.5% agar in the centre of the dishes and their distribution in the eight sectors recorded after 0.5, 1.5, 2.5 and 3.5 hr. Each point on the graph is the mean of five replicates; the continuous lines indicate nematode distribution in dishes containing roots and the discontinuous lines the distribution in dishes without roots. The vertical line in each graph shows the point where the nematodes were introduced.

there was no indication that the nematodes were moving towards the roots. However, significantly more nematodes ( $P < 0.01$ ) occurred on the side containing roots in sectors 1 and 2 than in the blank side in sectors 7 and 8 after 1.5, 2.5 and 3.5 hr. Significantly more ( $P < 0.01$ ) occurred in sectors 2 and 3 on the root side

(roots were confined to sector 1) than in sectors 6 and 7 on the blank side after 2.5 and 3.5 hr. This suggests that the nematodes moved towards the grass roots and that accumulation was not entirely due to random movement accompanied by inactivity at the root surface. In fact few nematodes occurred at the roots because of the short duration of the experiment. Thus it seems likely that the grass roots influenced the nematodes' direction of movement from a distance. In the dishes without roots nematode numbers in each half of the dish did not differ significantly.



Text-fig. 9. The aggregation of *T. colubriformis* around living and dead grass roots (*Phleum pratense*) on the surface of 1% agar over 4 hr. Each point is the mean of five replicates.

In a second experiment the nematodes aggregating around living and dead roots were counted at intervals over 4 hr. Numbers of nematodes around both types of roots increased with time, but significantly more ( $P < 0.01$ ) accumulated around the living roots (Text-fig. 9). Observations showed that the nematodes became inactive and curled up about 15 min. after reaching the roots (Pl. 1 fig. 2), so aggregation could have occurred by random movement followed by loss of activity at the root surface. Nematodes also became inactive at the dead roots but more nematodes appeared to move directly to the living than dead roots.

*T. colubriformis* is attracted to grass roots and tends to aggregate there in much the same way as do plant nematodes. However, the factors controlling the aggregation of *T. colubriformis* are unknown, for in the present experiments non-sterile roots and nematodes were used. Thus, root exudates, micro-organisms

and their excretions in the rhizosphere may have provided the attraction stimulus. Henderson & Katznelson (1961) found that more plant parasitic and other nematodes occurred around the roots of barley, oats, wheat, soyabean and peas than elsewhere in the soil. It seems likely, therefore, that the attraction stimulus is non-specific and that nematodes, whether microphagous, plant parasitic or animal parasitic tend to aggregate in the rhizosphere.

#### DISCUSSION

The hypothesis that in the free-living stages the movements of microphagous, plant parasitic and animal parasitic nematodes are similar, is not contradicted by the results described. This hypothesis needs confirmation by comparisons between more species but it is useful because it enables the ecology of the Nematoda to be considered as a whole. Thus, nematodes can be arbitrarily divided into three groups: (1) those with the largest wave frequency which are active swimmers; (2) those with intermediate range frequency which can move in thick films of water; and (3) those with the smallest wave frequency, the crawlers, which can move only in thin films of water. The three groups show clearly in Text-fig. 3 and they occupy different kinds of natural habitat. Nematodes with a length  $\times$  wave frequency (LF) more than 100 can swim upwards in deep liquids; vinegar eelworm which lives at the surface of vinegar, and *Monhystera*, which inhabits ponds and streams, are included in this group. The intermediate group with an LF value between 20 and 80 are active enough to escape from the soil and ascend stems, leaves, walls, etc.; they include the stem and leaf nematodes *Ditylenchus dipsaci* and *Aphelenchoides ritzemabosi*, the animal nematodes *Trichostrongylus colubriiformis* and *Nippostrongylus brasiliensis* and the microphagous nematodes *Rhabditis*, *Diplogaster* and *Plectus*. The third group with an LF value smaller than 20 are not sufficiently active to move in the thick water films and have therefore less chance of escaping from the soil. They include the root parasitic nematodes and the animal nematode *Dictyocaulus viviparus* which is spread on to grass by being thrown into the air from the discharging sporangia of the fungus *Pilobolus* (Robinson, Poynter & Terry, 1962). The habitat of the free-living stages of nematodes is therefore related, at least in part, to their propulsive power which is in turn determined by their size and activity.

It is apparent that animal and plant nematodes behave similarly. Thus, *D. dipsaci* and *T. colubriiformis* have similar wave patterns in water films; their mobility in sand is influenced in the same way by moisture; the temperature for maximum activity is about the same; they are both resistant to desiccation; they are attracted to and aggregate around plant roots and ascend plants above ground level. In fact, in their free-living stages, the leaf and stem nematodes more closely resemble animal nematodes like *Trichostrongylus* than they do the root parasitic nematodes which are less active and less resistant to desiccation. The ability of nematodes to resist desiccation has survival value for those species which inhabit the soil surface or move on the surface of plants above ground level. Further, although Crofton (1954) showed that the upward migration and accumulation of

infective larvae of strongyloid nematodes on grass blades probably occurred by random movement, their aggregation around plant roots may help to determine the numbers that move up the plant.

## SUMMARY

Ciné film and direct observation showed that microphagous, plant and animal nematodes have the same basic wave pattern when moving. Their speed is linearly related to the product of their length and wave frequency during unrestricted movement in deep water. The speed, wave pattern and frequency of the plant nematode *Ditylenchus dipsaci* and the animal nematode *Trichostrongylus colubrifomis* changed in a similar way in response to changes in water film thickness on the surface of agar of different concentrations. *T. colubrifomis* like *D. dipsaci* were most mobile in a sand-water medium at a suction corresponding to the stage when most of the water has drained from the pores. The optimum temperature for activity of *T. colubrifomis* and *D. dipsaci* was about 20° C. and both survived desiccation at 50% relative humidity and 24° C. for 32 days. *T. colubrifomis* was attracted to and aggregated around grass roots (*Phleum pratense*). The results suggest that the free-living stages of microphagous, plant parasitic and animal parasitic nematodes move similarly and that nematodes can be divided arbitrarily into three groups: (1) those active enough to swim in deep water; (2) those able to swim in thick water films, escape from the soil and ascend plants above ground level; and (3) those too inactive to swim, the crawlers, which are largely confined to the soil. The habitat of free-living stages of nematodes, irrespective of their host or feeding habits, is probably related to their propulsive power.

We thank Dr D. Poynter for supplying the animal parasites used.

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#### EXPLANATION OF PLATE

Fig. 1. Nodes formed by *Ditylenchus dipsaci* swimming in deep water. Successive frames from a ciné film were superimposed to give this picture.

Fig. 2. The aggregation of *Trichostrongylus colubriformis* around a grass root (*Phleum pratense*) on 1% agar. Nematodes became inactive and curled up after they had been at the root for about 15 min.

