Studies on the Host-finding Mechanisms of *Neotylenchus linfordi*[1]

J. W. KLINK, V. H. DROPKIN, AND J. E. MITCHELL[2]

Abstract: The plant-parasitic nematode, *Neotylenchus linfordi*, congregated around colonies or filtrates from mycelia of *Gliocladium roseum, Rhizoctonia solani, Pyrenochaeta terrestris* and *Chaetomium indicum*. The average time required for the nematodes to reach the fungal colonies ranged from less than 4 hr for *G. roseum* to 20 hr for *R. solani*. Nematodes first circled near the point of introduction, then moved toward the fungus or filtrate. Several methods of measuring the response of *N. linfordi* to *G. roseum* culture filtrate were evaluated. The response was strongest when the test materials were assayed on an agar disk submerged in water agar and the introduced nematodes suspended in agar in a center well midway between the test materials. Filtrates obtained from cultures of *G. roseum* incubated between 12 and 21 days in potato dextrose broth, were most active. The attractants were small thermostable molecules, soluble in methyl alcohol and unaffected by pH. A yellow pigment with properties similar to a mixture of aurantiogliocladin, rubrogliocladin, and gliorosein was shown to be one of the active materials. The response of *N. linfordi* to the *G. roseum* filtrate was not associated with any nutritive factors which would result in reproduction. *Key Words: Neotylenchus linfordi*, Host-finding, Nematode attraction, Assay technique, Fungal nematode attractants.

The mechanism by which nematodes locate host plants has long been a controversial topic among nematologists (11, 15). In 1925 Steiner (28) concluded nematodes locate host roots by means of their amphids, suggesting these were chemoreceptive organs. Linford (18) also demonstrated attraction of a number of plant pathogenic nematodes by host plants. Two opposing hypotheses are currently proposed to explain host-finding. One states that nematodes are attracted to the host by root exudates (2, 3, 13, 14, 15, 19, 24, 25, 26, 30, 31). The other maintains that nematodes locate the host root by moving about at random (1, 17, 27), and are retained there by localized rhizosphere stimuli and/or become trapped in free water in the vicinity of the root from which they are unable to escape. Support for the distant attraction hypothesis was provided by experiments in sand and under aseptic conditions on agar where larvae of *Meloidogyne hapla, M. incognita* var. *acrita, Ditylenchus dipsaci,* and *Heterodera schachtii* were shown to congregate near host roots on dialysis membranes placed as far as 10 mm away (3, 19, 33). Townshend (30) found that *Aphelenchus avenae* and *Bursaphelenchus fungivorus* were attracted to some 57 of 59 fungi representing both plant pathogens and saprophytes. He observed the majority of nematodes of both species to move directly to the fungus colonies, as far as 2 cm away, rather than at random.

Stimuli reported to attract nematodes *in vitro* include electrical potentials (4, 11), amino acids (2, 22), inorganic compounds (2, 13), and carbon dioxide (13, 14, 15). Very little is known, however, about the attractant effect of host excretions. Carbon dioxide would be concentrated around all roots; therefore, it alone could not enable nematodes to find roots of specific host plants. Nematode nervous and sensory systems are well developed and probably able to detect stimuli from some distance (5, 9, 25, 26, 32). Attraction seems to depend largely on environmental conditions and the technique of observation.

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The objectives of this study were: (i) to determine the extent of attraction of *Neotylenchus linfordi* Hechler to four fungus hosts, (ii) to develop methods to study the response of *N. linfordi* to the metabolic products of the host, (iii) to determine the properties of the attractants, and (iv) to determine whether the attractants were nutritive to this nematode.

MATERIALS AND METHODS

Monoxenic cultures of *N. linfordi* were maintained on *Pyrenochaeta terrestris* (Hansen) Gorenz *et al.* growing on potato dextrose agar (PDA) at 24 C, and transferred every two weeks to actively growing 10-day-old cultures of *P. terrestris*. Nematodes for experiment were Baermann-extracted within 12 hours of the time they were used, surface sterilized 20 min in 0.1% "Hibitane" (chlorhexidine diacetate) and washed 6 times with sterile distilled water.

Gliocladium roseum Bainier, *Chaetomium indicum* Corda, *Rhizoctonia solani* Kühn, and *P. terrestris* were maintained on PDA and transferred every two weeks. To obtain filtrates from *G. roseum*, 250 ml Erlenmeyer flasks containing 100 ml potato dextrose broth (PDB) were inoculated with two mycelial disks (12 mm diameter) from the periphery of 5- to 6-day-old cultures. The flasks were incubated in still culture at room temperature for 12 days. The used culture medium was separated by filtration through Whatman No. 2 filter paper on a Büchner funnel, sterilized by passage through a 0.22 μ Millipore filter and either used immediately or stored at –6 C.

ATTRACTION TO FUNGI: The response of *N. linfordi* to *G. roseum, R. solani, P. terrestris,* and *C. indicum* was measured by a modification of Townshend's method (30). Fifteen ml of PDA were poured in 90 × 15 mm plastic petri dishes. After solidification of the agar, disks (6 mm diameter) were cut from the periphery of one-week-old cultures on PDA, and placed in opposite quadrants of the test plates, 15 mm from the rim (Fig. 1A, B). Similar disks of uninoculated PDA were placed in the other quadrants as controls. These plates were incubated 24 hr at 24 C. Then 400–500 *N. linfordi*, including all stages, suspended in 2% water agar (WA) were introduced into an 8 mm-diam well in the center of the dish midway between the test fungus colonies. Four hr after introduction of the nematodes the agar from a 2 × 2 cm area of the WA centered at the test fungal colonies, from similar areas around the control PDA dishes, and from the remaining agar in the dishes (Fig. 1A, B) was removed and the nematodes extracted in Baermann funnels. A duplicate set of plates was similarly extracted 16 hr later. The number of nematodes recovered from these various sites was expressed as a percentage of total nematodes recovered from the dish after 24 hr extraction.

ATTRACTION TO THE CULTURE FILTRATES OF *G. ROSEUM*: Because *G. roseum* colonies attracted *N. linfordi* most strongly, this host was chosen for detailed studies of attraction exhibited by its metabolic products. Three testing techniques were compared: (i) PDB culture filtrates were mixed 1 : 1 with 2% WA and placed in glass tubes (4 mm O.D. × 15 mm long) and sealed at one end with petroleum jelly. Tubes containing the test mixture were placed in opposite quadrants of a petri dish with the open ends 2.5 cm from the center of the dish (Fig. 1C, D). Similar control tubes containing fresh PDB were similarly placed in the remaining quadrants. Fifteen ml of 1% WA were then poured over the tubes. (ii) Equal volumes (5 ml) of fungus culture filtrate and fresh PDB individually mixed with 2% WA and poured in separate petri dishes (agar incorporation). After solidification, 6 mm disks were removed and placed on the surface

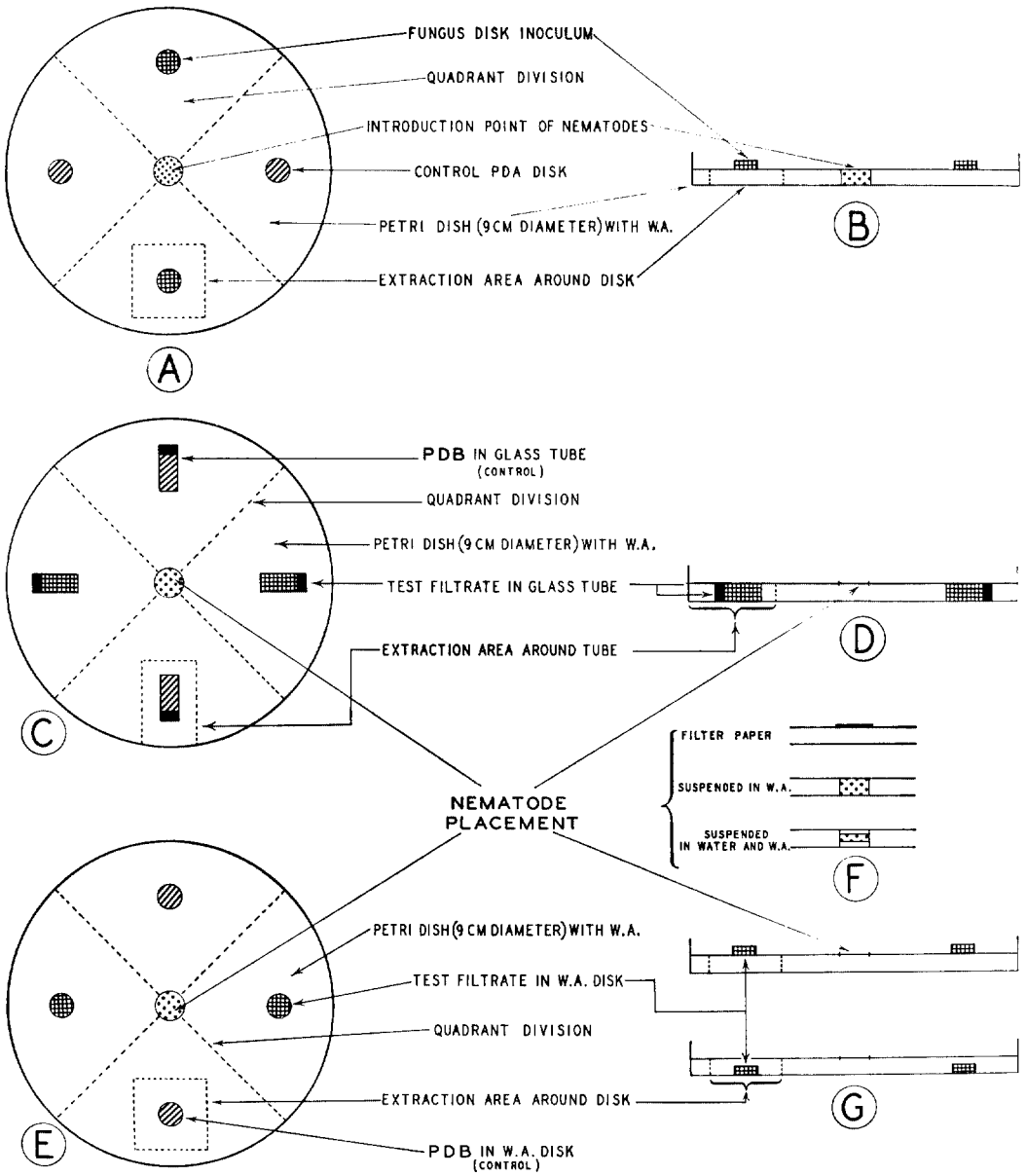

FIG. 1. Diagrams of assay methods to determine the response of *Neotylenchus linfordi* to test fungi (**A, B**) and to the culture filtrate of *Gliocladium roseum* (**C, D, E, F, G**).

of solidified 1% WA (Fig. 1G). (iii) Disks prepared as in ii were placed on the bottom of the assay plates, then covered with 15 ml of 1% WA (Fig. 1G). In both cases disks were placed 1.5 cm from the rim with 2 disks containing the filtrate in one pair of opposite quadrants, and control disks containing fresh PDB in the remaining quadrants

(Fig. 1E). Plates were incubated at 24 C for 6 hr and 400–500 nematodes were introduced in the center of the plates.

Three methods were tested for introducing the nematodes: (i) The nematodes were pipetted on 13 mm diameter filter paper disks which were then placed, nematodes down, on the WA surface (Fig. 1F); (ii) A suspension of nematodes in 2% cooled WA was used to fill an 8 mm well in the center of the test plate flush with the surrounding agar (Fig. 1F); and (iii) The bottom of the well was sealed with a drop of 2% WA, the nematodes were introduced in water, and the well filled flush with the surrounding agar with 2% WA (Fig. 1F). The response of the nematodes was determined after 4 and 20 hr incubation at 24 C. Where the test materials were assayed in glass tubes (method i), the nematodes in the glass tubes and in the direct vicinity of the tubes were determined by lifting these tubes along with a strip of WA 1 cm wide and 0.5 cm in front of the tube for Baermann extraction (Fig. 1C, D). Where the agar incorporation methods (methods ii and iii) were used, a 2 × 2 cm area centered at the test material disks were Baermann-extracted (Fig. 1E, G).

As will be noted later, the immersed agar disk method proved most reproducible and in all experiments the test materials were assayed by this method (unless otherwise stated) and incubated 6 hr at 24 C before suspensions of the nematodes in 2% WA were introduced in a center well of the test plates. The percentage of nematodes at the test material sites was determined 4 hr after introduction as described previously. The data cited are the mean values from 10 replicate plates.

PRODUCTION OF ATTRACTANTS: Erlenmeyer flasks (250 ml) containing 100 ml PDB were inoculated with *G. roseum* and incubated as previously described. At intervals of 3 days from the time of inoculation, filtrates of three cultures selected at random were collected and sterilized by filtration. The filtrates obtained at each interval were assayed against PDB controls to determine attraction of *N. linfordi*. Dilutions of filtrate of *G. roseum* cultures were assayed against comparable dilutions of PDB.

PROPERTIES OF THE ATTRACTANTS: Filtrates of 12-day cultures of *G. roseum* in PDB were used to study some of the physicochemical properties of the attractants.

To determine thermal stability, an active filtrate was autoclaved in a test tube for 20 min at 15 lbs pressure and assayed against PDB and an aliquot of nonautoclaved filtrate to determine the effect of heating on the attractivity.

To determine the pH stability of the attractants, portions of the filtrate were adjusted to pH 4, 7 or 10 with either 0.1 N HCL or 0.1 N NaOH, sterilized by Millipore filtration and incubated overnight at 24 C. The same was done for three separate portions of fresh PDB. After incubation period, the pH of each solution was readjusted to 7 and each assayed to determine attractivity.

As an indicator of the molecular size of the active compounds, the filtrate was dialyzed against double distilled water for 48 hr. Dialysate and dialyzed material were adjusted to original volumes and their attractivity for *N. linfordi* compared.

The solubility of the attractants in the filtrate of *G. roseum* at pH 7 in several organic solvents was determined. Where the solvents used were miscible with water (ethanol, methanol, acetone), the filtrate was evaporated to dryness in a rotary evaporator at 50 C and the resulting residue washed three times with equal volume of the particular solvent involved. Insoluble residue was removed by centrifugation at 15,000 g, dissolved in double distilled water and evaporated to dryness three times to eliminate the solvents. Similarly, the soluble fractions

were evaporated to dryness and redissolved in double-distilled water three times. With solvents only slightly miscible with water (n-butanol, petroleum-ether, chloroform, diethyl ether, methyl acetate, carbon tetrachloride), extractions were made in a separatory funnel. Procedures similar to those described above were used to eliminate the solvents from the soluble and insoluble fractions. The final volume of the fractions was adjusted to that of the filtrate initially extracted. The response of *N. linfordi* to the soluble and insoluble fractions was determined by assaying them against each other and separately against PDB at pH 7.

CHEMICAL NATURE OF ONE OF THE ATTRACTANTS: The attractivity of culture filtrates seemed to correlate with the presence of a deep yellow pigment. This yellow pigmented material was extracted from culture filtrates of *G. roseum* by the procedure of Pettersson (22), dissolved in 50% ethanol and the volume readjusted to that of the filtrate extracted. It was then assayed in small capillary tubes against the remainder of the filtrate and PDB, which had been evaporated and similarly taken up in 50% ethanol, to determine the relative attractivity to *N. linfordi*. The pigment was chromatographed on silica gel G using a benzene:dioxane:glacial acetic acid (90 : 25 : 4, v/v/v) solvent system. The materials separated were eluted with absolute ethanol and their UV absorption spectra determined with a Beckman DB spectrophotometer. Attractivity of the separated materials was tested at the original filtrate concentrations. To determine the preference of *N. linfordi* for the chromatographically separated materials, assay plates were divided into quadrants. Four agar disks each incorporating one of the separated materials were placed in the center of a quadrant 15 mm from the rim of the assay plate and 15 ml of 1% WA at 45 C added. After solidification, the WA was cut and removed leaving an "agar-cross" with an assay disk immersed in each side arm (Fig. 2). This was incubated 6 hr at 24 C before introduction of nemas suspended in 2% WA in an 8 mm diameter well in the center of the cross. After 2 hr the response of the nematodes to the different materials was measured by extracting the nematodes from each side arm as well and from the center portion of the agar-cross and determining the percentage of the total number of nematodes recovered from each site.

EFFECT OF *G. ROSEUM* FILTRATE ON REPRODUCTION OF *N. LINFORDI*: The effect of culture filtrate on the reproduction of *N. linfordi* was determined as follows: Single non-gravid female larvae were introduced (i) onto *G. roseum* colonies growing on 1% WA; (ii) on a 1 : 1 mixture of *G. roseum* filtrate and 2% WA; and (iii) on a 1 : 1 mixture of PDB and 2% WA. The plates were then incubated 4 weeks at 24 C and the nematodes extracted and the average number per plate and per treatment determined.

RESULTS

ATTRACTION TO COLONIES OF HOST FUNGI: Most of the nematodes had congregated around the fungal colonies within 20 hours. After 4 hr, however, 75% of *N. linfordi* were recovered from *G. roseum* and only 39% from *R. solani* colonies, while 59% and 64% of the nematodes had reached the vicinity of *C. indicum* and *P. terrestris*, respectively. Only 4% of the nematodes were recovered from the PDA sites paired with *G. roseum*, compared with 15–26% when paired with the other fungi.

Microscopic observation revealed the initial movements of *N. linfordi* to be random circling at the sites of introduction. Individual nematodes moved their heads in wide arcs. If the arc through which the head was swinging faced the fungal colony, the nematode moved in that direction. As *N.*

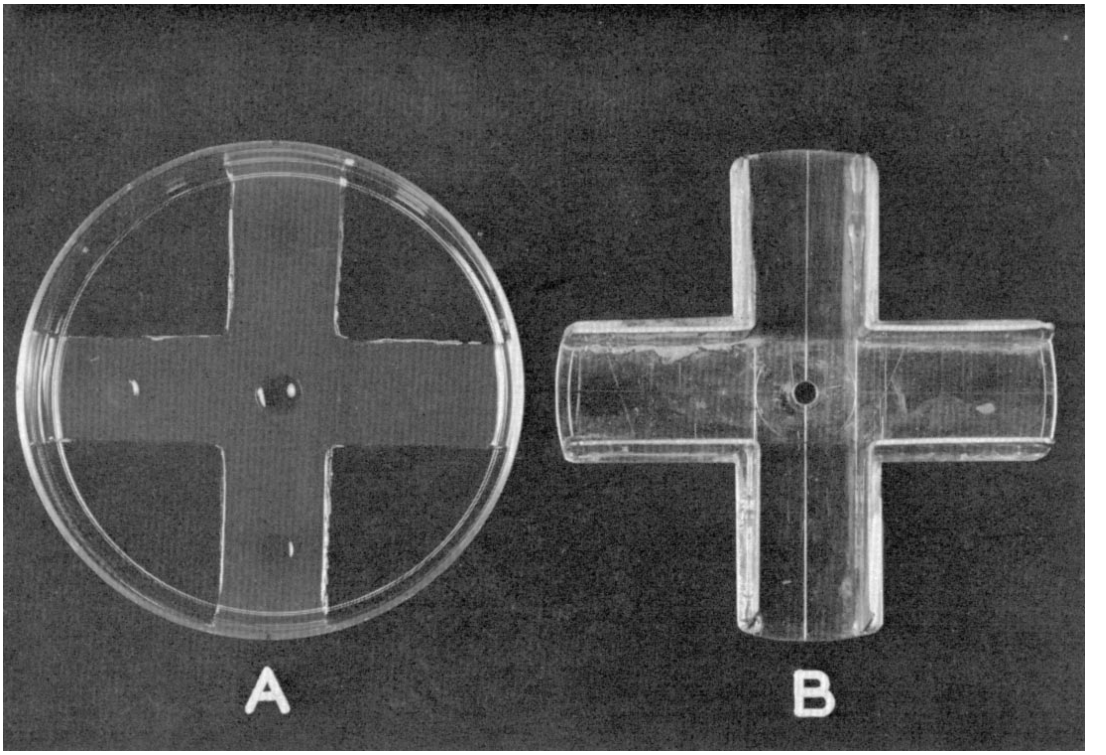

FIG. 2. (**A**) "Agar-cross" assay method to determine preferential response of *Neotylenchus linfordi* to four test materials assayed simultaneously on the separate side arms and (**B**) agar cutting tools.

linfordi approached the *G. roseum*, the arc of head movement decreased. Within 1 cm of the fungal colony, the nematodes moved rapidly to its periphery and started feeding. Feeding was not continuous as the nematodes frequently moved a short distance away from the mycelium.

ATTRACTION TO THE FILTRATE OF *G. ROSEUM*: The response of *N. linfordi* to the filtrate of *G. roseum* differed greatly depending on combinations of techniques used. The method by which the nematodes were introduced was most critical. Regardless of assay technique 70 to 86% of the nematodes introduced in 2% WA moved to *G. roseum* filtrate within 4 hr after introduction. When nematodes suspended in water were introduced in the center well and 2% WA added later, the majority of them were still in the well after 4 hr introduction but had moved away from the point of introduction by 20 hr. The nematodes introduced on filter paper disks initially appeared to move without orientation as though no stimulus was being received. The number of nematodes reaching filtrate sites increased with time when the test materials were assayed in glass tubes. Most of the nematodes entering the tubes moved up to the airspace between the petroleum jelly plug and the filtrate-agar mixture and became trapped (Fig. 3A). Only a low percentage of nematodes could be recovered from the mouth of the tubes containing PDB and none were observed to enter. When the test materials were incorporated in agar disks, the time at which the response of *N. linfordi* was determined was important. After 4 hr, 83 to 86% recovery was obtained at the

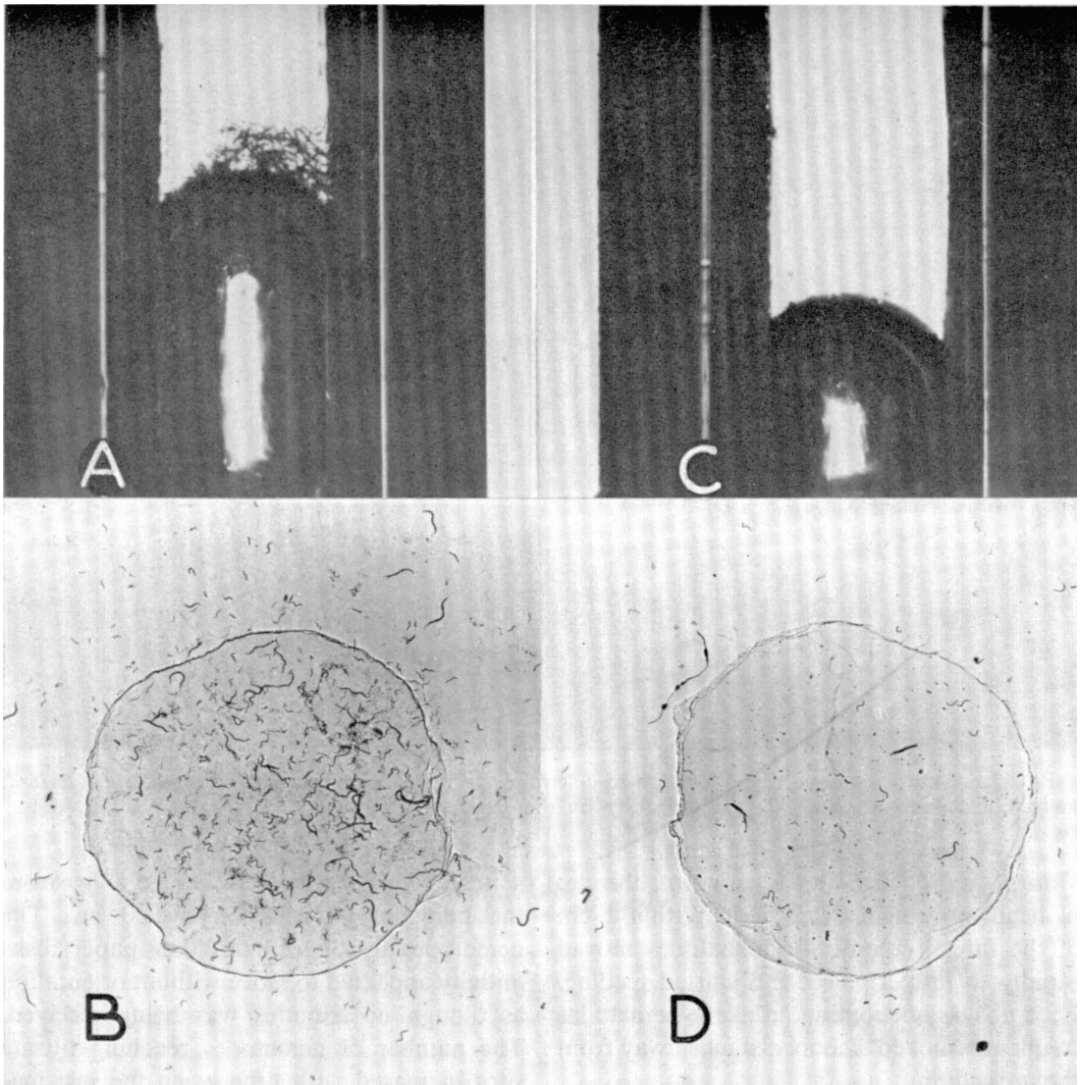

FIG. 3. *Neotylenchus linfordi* congregation in glass tube (**A**) and at disk (**B**) containing *Gliocladium roseum* filtrate compared to their controls (**C, D**) containing potato dextrose broth, respectively, 20 hr (tube) and 4 hr (disk) after introduction in the assay plates.

filtrate sites and only 2 to 4% at the PDB sites (Fig. 3B, D). After 20 hr, however, only 13 to 22% could be recovered from the filtrate sites and 2% from the PDB sites. The remaining nematodes were distributed at random outside the test material sites. The response was at a maximum 4 hr after introduction when the test materials were introduced on disks immersed in the agar of the test plate, and the nematodes were introduced by suspending them in 2% WA in a center well 6 hr later. This assay method was used routinely in subsequent work.

PRODUCTION OF ATTRACTANTS: When filtrates collected during the first 12 days of growth of *G. roseum* were assayed against

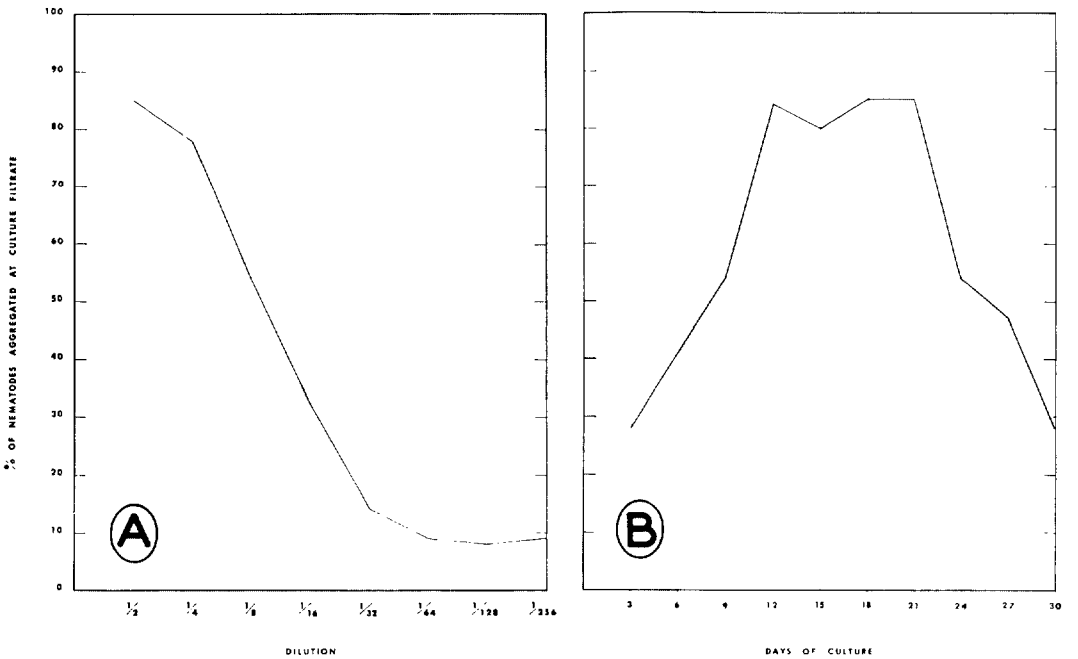

FIG. 4. The effect (**A**) of dilution and (**B**) age of *Gliocladium roseum* cultures on culture filtrate attractivity to *Neotylenchus linfordi*.

PDB, the percentage of *N. linfordi* attracted to the filtrate sites increased from 28% with the filtrate of 3-day-old cultures incubated 3 days to 84% with the filtrate of 12-day-old cultures. Over 80% of the nematodes responded to filtrates collected between 12 and 21 days of incubation. However, the response of *N. linfordi* to filtrates collected after 24 days of growth of *G. roseum* declined gradually until only 28% gathered at the filtrate assay disks (Fig. 4B). Increased attraction of nematodes to the test filtrate corresponded with a decreased movement of nematodes toward the PDB sites. Decreased nematode response to the filtrates of older cultures (24, 27 and 30 day incubation) was not accompanied, however, by increased nematode movement to PDB sites. In these cases the majority of the nematodes moved toward, but not all the way, to the site of the test filtrate.

When the filtrate of *G. roseum* cultures and uninoculated PDB were each diluted, the relative attractiveness to *N. linfordi* progressively diminished (Fig. 4A). At dilutions greater than 1/16 of the initial filtrate no response was detected.

PHYSICAL PROPERTIES: Autoclaving did not affect the attractivity of the filtrate. When either the autoclaved or non-heated filtrate was assayed against PDB, 79% of the nematodes could be recovered from each of the former and 2% from the latter. When the autoclaved filtrate was assayed against the non-autoclaved filtrate, an equal number of the nematodes was recovered from each indicating no reduction in potency with heating.

Filtrate attractivity was not altered by treatment for 24 hr at pH 4, 7 or 10. In all cases 75% of the nematodes could be re-

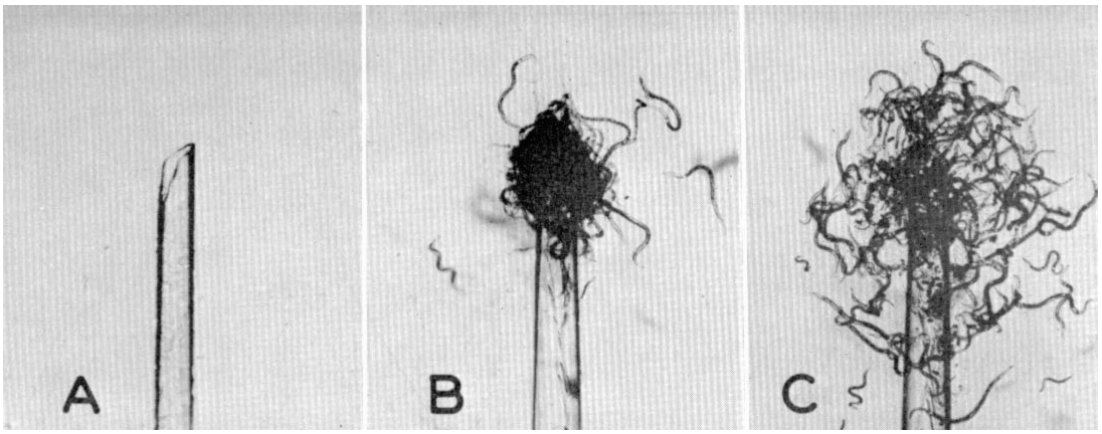

FIG. 5. Attraction of *Neotylenchus linfordi* to tube (**A**) containing the control potato dextrose broth, to tube (**B**) containing the extracted pigment of *Gliocladium roseum* 4 hr after introduction, and (**C**) the same tube 6 hr later.

covered from the filtrate sites and not more than 4% from the PDB sites.

The compounds responsible for attraction passed through a dialysis membrane. When the dialyzate was assayed against non-dialyzable fraction of the filtrate, 83% of the nematodes were recovered from the former and only 2% from the latter.

Activity of the methanol-soluble fraction of the filtrate was comparable to that of the whole filtrate. The nematodes did not respond to the methanol-insoluble fraction. When the other solvents were used and the fractions of the filtrate were assayed against each other, *N. linfordi* responded to both. The organic solvents other than methanol extracted only a part of the active material present in the filtrate.

CHEMICAL NATURE OF ONE OF THE ATTRACTANTS: The yellow pigmented fraction from *G. roseum* cultures was highly attractive when compared to PDB (Fig. 5). When assayed against the rest of the filtrate, however, equal movement to both was observed. This was not due to incomplete extraction of the pigment. It again appeared that more than one compound was present in the filtrate which could independently attract *N. linfordi*. Chromatography of the pigment dissolved in absolute ethanol resulted in the separation of four materials. The absorption maximum curve of one of these materials was at 275 mμ and of the other materials between 290 mμ and 295 mμ. Addition of a slight excess of sodium dithionite decolorized the pigment while addition of a trace of ferric sulphate returned the deep yellow color. This reversible oxidation-reduction reaction agreed with previous work on the pigment of *G. roseum* (23). The fractions separated by chromatography all actively attracted *N. linfordi*. Since the individual materials were rechromatographed several times prior to the assay, it seemed probable that the yellow pigment fraction (as a whole as well as its individual components) or an impurity that moved with the pigment were attractive to *N. linfordi*.

EFFECT OF *G. ROSEUM* FILTRATE ON THE REPRODUCTION OF *N. LINFORDI*: *N. linfordi* reproduced only when *G. roseum* mycelium was present. The average number of nematodes extracted from these plates was 1705. It was of interest, however, that nematodes added to the plates with the filtrate were still alive after 1 month while those in PDB

were dead. Thus, it appeared the attractivity of the *G. roseum* filtrate was not due to a nutritive factor sufficient to support reproduction.

DISCUSSION

While *N. linfordi* responded to all fungi tested, the degree of attractiveness as measured by the time required for the nematodes to reach the different fungal colonies varied. The majority of the nematodes could be recovered from *G. roseum* colonies 4 hr after introduction, but only after 20 hr could a comparable percentage of the total number of nematodes be recovered from the *R. solani* colonies. This response of the nematode to the four fungal species appeared to be correlated with the relative suitability of the fungi for the reproduction of the nematode as previously determined (16).

Having reached the fungus colonies, the nematodes fed intermittently and moved freely nearby indicating *N. linfordi* was not physically held. The speed of response of *N. linfordi* to the filtrate of *G. roseum* depended on the nematodes being provided a continuous solid substrate through which they could move.

The time elapsing between introduction of the test material and the determination of the nematode response was critical. The fact that the majority of the nematodes could be recovered from the filtrate sites after 4 hr but not after 20 hr was probably related to the loss of concentration gradient as the test materials diffused throughout the assay plates. The nematodes, lacking an attractant gradient, moved away from the filtrate site at random following the initial attraction (Fig. 5).

The controversy prevalent on the subject of nematode attraction may arise from the different techniques used to study this phenomenon. Sandstedt, *et al.* (27), for example, found that *Meloidogyne incognita incognita* was not attracted to tomato (*Lycopersicon esculentum* Mill.) seedlings grown in WA when nematode eggs were pipetted in a small amount of water on the surface of assay plates. On the other hand, Bird (2) found that larvae of *M. hapla* and *M. javanica* were strongly attracted to tomato root tips if they were suspended in the 1% WA of the test plates. Since all these nematode species were exposed to known hosts, it appears that the introduction of the nematodes within the agar of the test plates enhanced their ability to receive and to respond to the attractant stimuli.

The attractiveness of *G. roseum* culture filtrates appeared to parallel the development of the yellow pigment. According to Vischer (34) the yellow pigment produced by *G. roseum* consists mostly of aurantiogliocladin (2,3-dimethoxy-5,6-dimethyl-*p*-benzoquinone) with varying amounts of its corresponding quinhydrone (rubrogliocladin) and a small amount of the dihydro-derivative (gliorosein). He also reported that aurantiogliocladin showed an intense absorption band at 275 mμ and gliorosein at 295 mμ. The yellow pigment produced in cultures of *G. roseum* in PDB was shown to be one of the compounds involved in attraction and to have properties similar to those of a mixture of aurantiogliocladin, rubrogliocladin and gliorosein.

The attractiveness of filtrates of *G. roseum* cultures grown in PDB for *N. linfordi* increased up to 12 days, remained constant to 21 days, and then gradually diminished in older filtrates. The declining attractivity of *G. roseum* filtrates could have been due to the production of repellants, to the breakdown of the attractants in the older cultures, or to the fact that the sensory mechanisms of *N. linfordi* are sensitive only to a certain concentration range in a chemical gradient. Since the response of *N. linfordi* to the diluted filtrate (½) of a culture incubated 30 days was comparable to the response to the

filtrate obtained after 12 days incubation, it seems likely that the attractant concentration of the undiluted filtrate was above the range within which the nematode could detect a gradient. There is evidence of similar behavior with other nematodes. Jones (11) found *D. dipsaci* was attracted by glutamic and aspartic acids, both commonly found in root exudates, at concentrations of 1 : 100,000 but not at 1 : 1,000. Ibrahim, *et al.* (10) showed *Tylenchorynchus martini* was attracted to concentrations of 0.1 M $AlCl_3$ and 0.25 M $CdCl_2$ incubated for 6 hr in 2% WA. Higher or lower concentrations neither attracted nor repelled this nematode. Since the majority of *N. linfordi* moved only part way to the introduction point of the filtrate from 30-day cultures, it seemed logical that this nematode may have been reacting like *D. dipsaci* and *T. martini* to an excess of attractant. This was illustrated when the yellow pigment of *G. roseum* was assayed in capillary tubes and *N. linfordi* initially tried to enter the tubes but after 10 hr started to disperse. The pigment would have moved into the suspending WA producing a concentration gradient above the concentration level at which the nematodes could discriminate gradients. However, when the same tubes were placed in fresh WA they again attracted *N. linfordi.* Continuing attraction by active fungal colonies probably involves factors other than the initial attractant including those sustaining reproduction and thus would persist.

For some phytophagous insects there is conclusive evidence that they are attracted to, retained by, stimulated to feed by, and dispersed from host plants by a sequence of stimuli (29). The findings that certain plant parasitic nematodes after reaching their hosts are always associated with a particular site on the plant tends to indicate the involvement of stimuli active after the initial attraction which cause the nematode to feed and reproduce (12, 18, 21, 31). If on the other hand these ensuing stimuli are not present, the nematodes might be attracted, but would be unable to reproduce (6, 7). In some cases a non-host might produce repellent factors (30, 33).

The existence of a host-specific stimulus seems doubtful since nematodes respond to both suitable and non-suitable hosts (6, 7, 8, 20). Furthermore, *N. linfordi* responded not only to the filtrate of *G. roseum* but also to such diverse compounds as NaH_2PO_4, $(NH_4)_2CO_3$, and HCl and moved to the cathode under the influence of an electrical potential difference of 105 mV/mm (J. W. Klink, unpublished data). The fact that all motile stages of *N. linfordi* responded to the attractant stimuli indicated that the receptor sites are functional throughout the life span of this nematode. Under laboratory conditions, the response of *N. linfordi* to stimuli was dependent on both assay techniques used and concentrations of the materials tested. The techniques described should prove useful in the chemical identification of attractants and the study of the effect of drugs on nematode response. Both of these lines of investigation could contribute much to the understanding of the movement of phytoparasitic nematodes.

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