Thermotactic Response of Some Plant Parasitic Nematodes

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Abstract: Attraction of *Ditylenchus dipsaci* and *Pratylenchus penetrans* to a temperature gradient was tested. Heating wires, infrared radiations and germinating alfalfa seeds were used to create a temperature gradient as small as 0.033 C/cm in agar. *P. penetrans, D. dipsaci,* and *Tylenchorhynchus claytoni* responded to a temperature gradient of 0.033 C over a 4-cm distance from the heat source. *Trichodorus christiei* and *Xiphinema americanum* showed no response. Individuals of *P. penetrans* oriented their heads towards the heat source and moved directly towards it from a 1-cm distance within 10 min. When the heat was turned off the nematodes dispersed, but when the heat was turned on again, they reassembled. Heat from germinating alfalfa seeds, in the absence of CO_2, attracted *P. penetrans.* Carbon dioxide emanating from germinating alfalfa seeds failed to attract them in the absence of heat, even after 24 hr.

Few studies have been conducted on thermotaxis in plant parasitic nematodes. Wallace (8) reported that when *Ditylenchus dipsaci* (Kühn) Filipjev was introduced to a temperature gradient of 2 to 30 C in sand, they accumulated in higher numbers at about 10 C. Rode (6) reported a similar reaction with *Heterodera rostochiensis* (Wollenweber). Croll (1) showed that *D. dipsaci* accumulated at a temperature which corresponded to the storage temperature at which this nematode was kept for 30 days.

In the following paper data are reported on the behavior of *Pratylenchus penetrans* (Cobb) Chitwood & Oteifa and *D. dipsaci* when placed in temperature gradients in agar. Preliminary results have been published (3).

MATERIALS AND METHODS

Three methods were used to establish a small temperature gradient in 1% water agar contained in 9-cm plastic petri plates.

HEATING WIRE EXPERIMENTS: A heating wire was made of constantan wire, 4 cm long and 0.025 mm in diameter, which was coiled and embedded in plastic glue between two glass cover slips of 19 mm diameter. The diameter of the coil was about 3 to 4 mm, and its resistance was 40 to 45 ohms. The wire was connected by a copper lead to an electrical circuit consisting of rheostat, 6-volt dry cell and a voltmeter.

The wire was affixed to the outer surface of the bottom of an inverted petri plate containing nematodes evenly dispersed in a thin layer of 1% water agar. By passing a measured electrical current through the wire, the temperature under the wire was increased above ambient (22.56 C), thus creating a temperature gradient in the agar. Temperature at different locations in the agar was measured by thermocouples.

INFRARED RADIATION BEAM EXPERIMENTS: A focused infrared beam was produced by the use of a 6-volt incandescent lamp fixed in a spot-light microscope lamp and was substituted for the wire as a heat source. The current passing through the filament of the lamp was regulated by a rheostat. The beam was focused on the outer surface of an inverted petri plate by means of two converging lenses 20 cm apart. Visible light waves were filtered from the beam by means of Kodak #87 Wratten Gelatin filter. Calibration was accomplished by the use of thermocouples laid in agar contained in a petri plate. Similar plates but without heat applied were used as checks.

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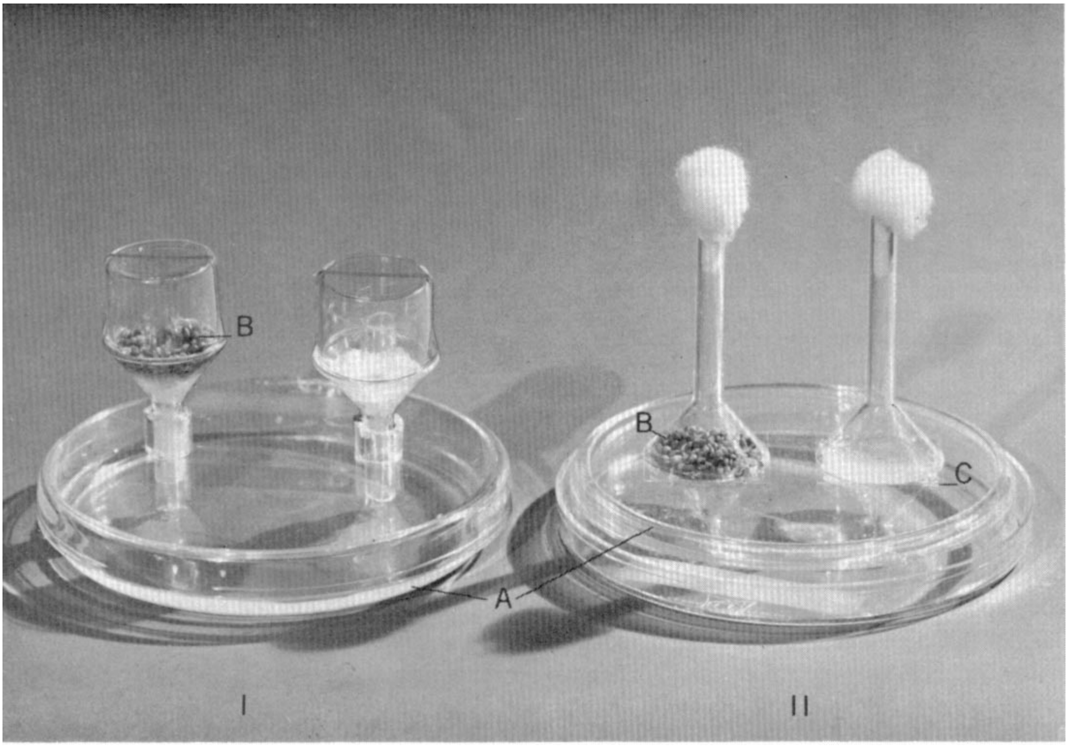

FIG. 1. The experimental set-up used to test separately the effect of CO_2 (I) and the heat (II) produced by germinating alfalfa seeds on nematode attraction, showing, A, agar containing nematodes; B, germinating alfalfa seeds; C, cover slip and thin film of water agar.

With both methods the temperature in the agar under the wire or the focal point of the beam was proportional to the current at a given voltage. Temperature gradients in the agar under the heating source stabilized within 5 min. A current of 250 mv resulted in a temperature gradient of 0.033 C between points in the agar 4 cm apart. Temperature differences were measured in Fahrenheit at 0.06, 0.25, 0.50, and 1.0 F, corresponding to 0.033, 0.14, 0.28, and 0.56 C, respectively.

HEAT SUPPLIED BY GERMINATING ALFALFA SEEDS: In this test CO_2 was separated from the heat of germination with a glass barrier and each factor was tested in the absence of the other on the attraction of *P. penetrans*. Alfalfa seeds (*Medicago sativa* L. "Du Puits") germinated for 24 hr on moist filter paper were used. Each experiment included two replicates and was repeated twice. Similar units without germinating seeds were used as checks.

Fifty germinating seeds were placed on moist filter paper in an upright funnel 2.5 cm in diameter (Fig. 1-I). Then the stem of the funnel was inserted in the cover of an inverted petri plate containing 100 nematodes in 7 ml of 1% water agar. The top of the funnel was covered with a glass container 2.5 in diameter. No temperature difference was detected under the germinating seeds.

Fifty germinating seeds were placed over a thin film of 1% water agar on a 25 mm sq glass cover slip. Then the cover slip and seeds were placed on the bottom of an inverted petri plate containing 100 nematodes suspended in 10 ml of 1% water agar. A glass funnel 2.5 cm in diameter was inverted and placed over the cover slip to prevent evaporation (Fig. 1-II). Agar temperature under the germinating seeds was 0.14 to 0.28 C higher than that under the check. The evolution of CO_2 from the germinating seeds and the accumulation of CO_2 gradient in the agar under the stem's orifice was shown by the barium hydroxide test for CO_2. A volume of 0.5 ml of 1 N barium hydroxide solution was added to 7 ml of liquid 1% water agar in a petri plate. After 3 hr a white precipitate of barium carbonate appeared under the orifice of the stem, indicating the presence of CO_2.

RESULTS

No response by the nematodes was apparent after a 3-hr exposure to a heat gradient supplied by a heating wire in which the temperature in the agar directly under the wire was 0.28 C higher than at 4 cm from the wire. After 14 hr, however, 80% of *P. penetrans* had aggregated under the wire (Fig. 2). When the wire was moved 1 cm from its previous position, the nematodes reassembled under the new position within 15 min (Fig. 3). When the wire was moved three more times, each time 1 cm from the previous position, the nematodes aggregated under the new position of the wire each time within 10–15 min. The percentage of nematodes, however, aggregating under the wire after each move of the wire was lower than that at its previous position. The number of nematodes aggregated under the wire after the fourth move was about 20% of that aggregated under the wire after the first move.

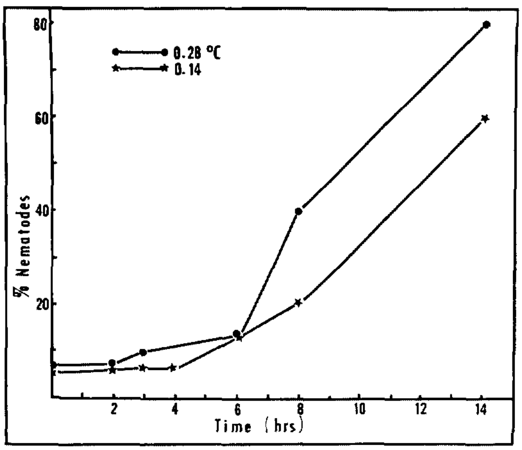

FIG. 2. Percent of *P. penetrans* that aggregated after a 14-hr period under two heating wires which raised the temperature 0.14 C and 0.28 C above that at 4 cm away.

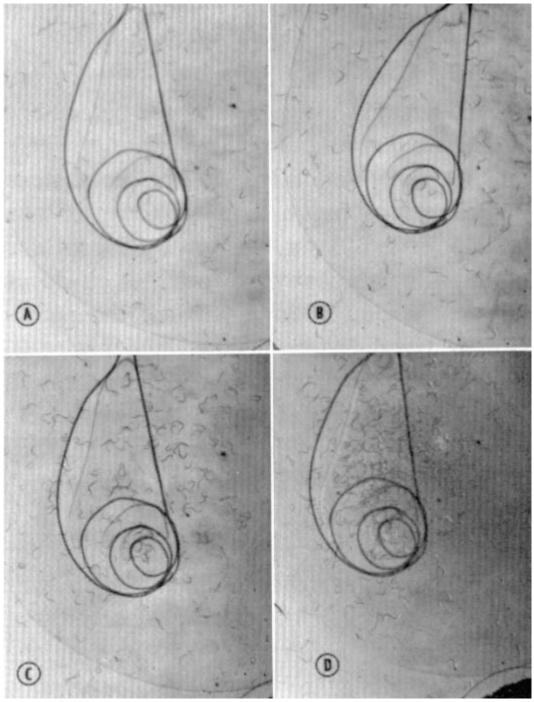

FIG. 3. Aggregation of *P. penetrans* under a heating wire supplying 0.28 C after it was moved 1 cm from its previous position, A; after 5 min, B; after 10 min, C and after 15 min, D.

Similar results were obtained when two wires were placed on the plate 2 cm apart. When the current was connected to wire A, a high percentage of the nematodes present in the plate aggregated under it within 14 hr. Then the current to wire A was disconnected and connected to wire B, and the percentage of nematodes directing their heads towards wire B was determined. The nematodes under cable A began to move towards wire B in 5 min and the heads of 82% of the moving nematodes were oriented towards wire B. Within 10 min 80% of the nematodes in the dish were located under wire B. The nematodes dispersed when the heat in both wires was turned off but when the heat was turned on again in either wire they reassembled.

D. dipsaci showed similar behavior in a separate set of experiments. The number of nematodes aggregating after 14 hr was from 65 to 70% of the total number present in each plate. The response to moving the wire was similar to that of *P. penetrans*. Similar results were obtained in three additional experiments with each nematode species.

In a second series of experiments, the behavior of both nematodes to a temperature gradient of 0.14 C over a 4-cm distance was studied. Both nematodes detected and migrated to the high temperature. Fifty percent of the nematodes aggregated under the wire within 13 hr, a longer period than when a temperature difference of 0.28 C was applied. When *P. penetrans* was given a choice between a 0.14 C and 0.28 C temperature gradient per 4 cm, nearly all of the nematodes present in the center of the plate, and 1 cm from each wire migrated to a point under the 0.28 C wire.

Further studies on the response of *P. penetrans* to temperature gradients were made using an infrared beam of radiation as a heating source. Results obtained by this method were in agreement with those obtained by using the heating wire method (Fig. 4). However, the time needed to

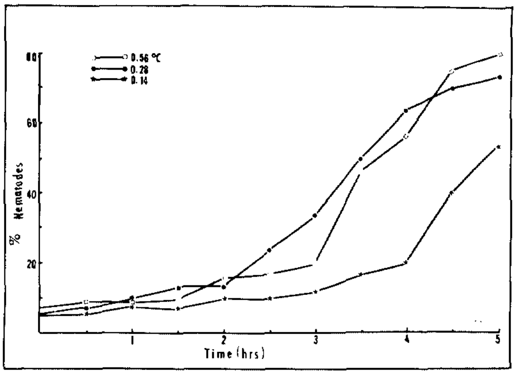

FIG. 4. Percent of *P. penetrans* that aggregated after 5 hr under three infrared beams (raising the temperature 0.14 C, 0.28 C, and 0.56 C, respectively).

obtain 70% to 80% aggregation under the heat source was about 33% of that needed when the heating wire was used. Switching off the infrared beam caused the nematodes to disperse. The nematodes followed the focal point of the beam when it was moved in 1 cm steps at 10-min intervals (Fig. 5).

When the test was performed in direct fluorescent light, the nematode response was very weak. Therefore, the following experiment was performed to test the influence of direct light on the nematode response to temperature gradients. A temperature gradient of 0.28 C per 4 cm was established by means of the infrared radiation beam. Two plates, each of which contained 100 *P. penetrans,* were placed under two separate beams of the same strength for 14 hr in the dark. During this time, nematodes aggregated under each of the two beams. Then each of the beams was moved 1 cm from its previous position in each plate. One of the plates was kept in the dark and the other was exposed to a reflected wide beam of visible light from an 8-watt fluorescent stereoscope lamp 13 cm long. The number of nematodes that migrated to the new position of the focal point of the beam in both the dark and lighted plates was determined

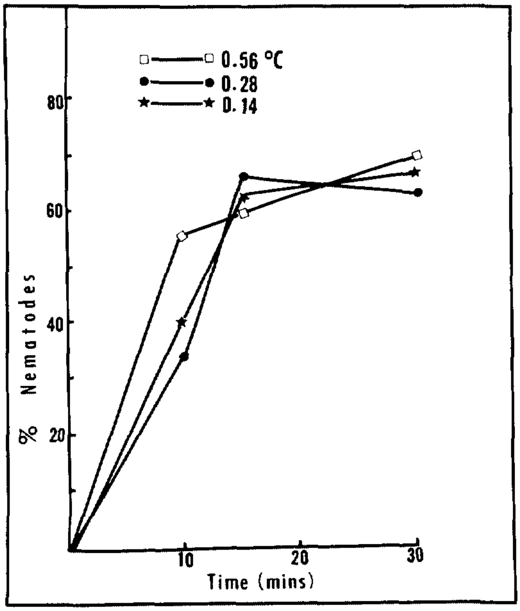

FIG. 5. Percent of *P. penetrans* that followed three infrared beams (raising the temperature 0.14 C, 0.28 C, and 0.56 C, respectively) from 1 cm distance after they had aggregated under the beam raising the temperature 0.28 C for 14 hr.

after 5, 15, 20, and 30 min. Fewer nematodes migrated to the new position of the beam when the plate was exposed to light than when the plate was in the dark (Fig. 6). In the dark plate about 80% of the nematodes migrated from the previous position to the new position after 30 min. Similar results were obtained in two additional experiments.

To test the influence of visual light alone on the attraction of *P. penetrans,* the same experimental set-up used for infrared production was used for producing a visible light beam. Wave lengths above 0.8 μ, including infrared, were eliminated from the beam by infrared filter 301. Heat energy was absorbed by a water bath placed between the light source and the nematodes. Neither temperature changes nor movement of nematodes in the agar were noted during a 14-hr period.

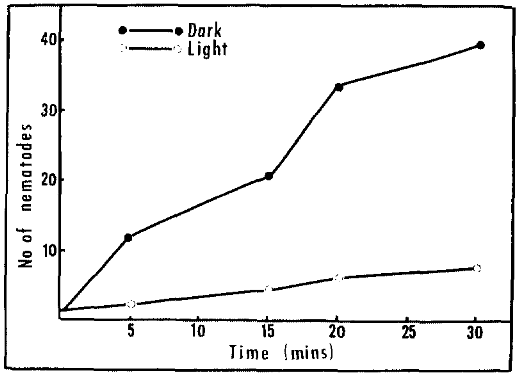

FIG. 6. Number of *P. penetrans* that aggregated under an infrared beam raising the temperature 0.28 C in light and dark.

Results obtained when heat from germinating seeds was used were in agreement with the previous results. After 16 hr 32% *P. penetrans* had migrated to a point under the germinating seeds in the absence of CO_2 compared with 7% under the check. Carbon dioxide emanating from the germinating seeds in absence of a temperature gradient failed to attract the nematode within 24 hr.

The thermotactic response of three other genera was studied. *Tylenchorhynchus claytoni* (Steiner) behaved similar to *P. penetrans* or *D. dipsaci. Trichodorus christiei* (Allen) and *Xiphinema americanum* (Cobb) showed no response to temperature gradients of 0.14 C, 0.28 C, or 0.56 C per 4 cm.

DISCUSSION

The attraction of *P. penetrans* and *D. dipsaci* to germinating seeds was reported by Edmunds and Mai (2) and Klingler (4, 5). Carbon dioxide emanating from the germinating seeds was then suggested to be a major factor responsible for such attraction. Carbon dioxide, however, is not the only by-product of the process of germination. Germinating seeds also produce a heat gradient in the surrounding medium.

The directed movement of *P. penetrans, D. dipsaci,* and *T. claytoni* along a temperature gradient under experimental conditions indicates a possible role of this phenomenon in the ecological relationships of these nematodes. The results indicate that these three species detect and migrate along a temperature gradient as small as a 0.033 C increase for each 4 cm. This temperature increase in the vicinity of a nematode may be sufficient to initiate a positive thermotactic response. Theoretically, plant roots liberate heat as a by-product of respiration. This heat along with the heat produced by soil microorganisms may set up a temperature gradient around the root system. Therefore, the positive thermotactic behavior of these nematodes may play a vital part in locating plant roots.

The attraction of *P. penetrans* to the focal point of an infrared beam of radiation may not be due to the heat produced by the beam *per se,* but rather to the electromagnetic waves in the range of infrared spectrum. In nature, plants as well as animals are constantly absorbing and emitting infrared radiation. Generally speaking, the emission of infrared radiation from plant tissues is higher than from field soil (7). Therefore, it might be concluded that a soil with fresh roots of a plant might present contrasting shades of high and low radiation. To a nematode having a means of detecting infrared radiation, the roots might be readily detectable. The mechanism of sensing these radiations by nematodes is unknown. The anatomy of the nematode sensing organs should be examined to determine whether or not they have any infrared detector configuration.

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