IN VITRO STUDIES OF HOST-PARASITE RELATIONSHIPS
OF SOME PLANT-PARASITIC NEMATODES 1)

BY

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The feeding habits and parasitism of ectoparasitic nematodes of several genera were simultaneously studied in vitro. This method also was used to assay resistance of thirteen plants to attack by several nematode species. Hemicycliophora similis, Trichodorus christiei and Tylenchorhynchus claytoni fed on most of the thirteen plants tested. Feeding in vitro is indicative of the parasitic habit of the nematode under natural conditions. No significant difference was observed between accumulation of H. similis around roots of host and non-host seedlings. Feeding by two species of Tylenchus is described.

The host-parasite relationship in plant-parasitic nematodes has been a subject of study by a number of workers. The objectives of the present work were to 1) show that in many cases parasitism and feeding habits of several nematodes could be demonstrated simultaneously on a given host, 2) observe if an indication of host resistance and susceptibility could be achieved in in vitro tests, 3) evaluate the relative accumulation of nematodes to the roots of host and non-host plants and 4) study the symptomatology associated with feeding of nematodes on various plants in vitro.

MATERIALS AND METHODS

Feeding was studied by placing a mixture of 200-500 nematodes in petri plates which contained 3-7 day old seedlings grown from seed in 1% water agar. All seeds, with the exception of alfalfa, were sterilized in 0.1% HgCl2 for five minutes, washed with sterile distilled water and then transferred to sterile agar. Alfalfa seeds were scarified with conc. H2SO4, washed with sterile water, then transferred to agar. Plants with thick rooted seedlings (i.e., buttercup squash, corn, pea and bean) were assayed but as the roots were too opaque and broke the agar, no observations could be made. The following hosts were finally used: broccoli (Brassica oleracea L.v. botrytis), cauliflower (Brassica oleracea L.v. botrytis), cabbage (Brassica oleracea L.v. capitata), kohlrabi (Brassica oleracea L.v. gongylodes), radish (Raphanus sativus L.), carrot (Daucus carota L. sp. sativus), dill (Anethum graveolens L.), tomato (Lycopersicon esculentum Mill.), lettuce (Lactuca sativa L.), beet (Beta vulgaris L.), alfalfa (Medicago sativa L.), rye grass (Lolium perenne L.) and marigold (Tagetes sp.).

Nematode mixtures were extracted from three soil locations by Seinhorst's

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elutriation method (1956) followed by Christie & Perry's modification of the Baermann technique (1951). One location was very rich in *Hemicycliophora similis* Thorne, often with good populations of *Trichodorus christiei* Allen, *Tylenchus bryophilus* Steiner and *T. agricola* de Man. The second location had a high population of *Helicotylenchus erythrinae* (Zimmerman) Golden and *Tylenchorhynchus claytoni* Steiner with *H. similis*, *T. bryophilus* and *T. agricola*. The third location yielded a high population of *Tetylenchus jocutus* Thorne with some *H. similis* and *H. erythrinae*. The nematodes were concentrated in a single drop of water (Zuckerman, 1960a) transferred by pipette to a plate which contained several seedlings, and pressed into the agar by use of dental pulp canal file.

Other stylet bearing forms, *Atylenchus decalineatus* Cobb, *Ecphyadophora tenuissima* de Man, *Tylencholaimus proximus* Thorne and *Dorylaimus* spp. were also observed in the mixtures. These nematodes were never present in large numbers and were never seen to feed.

For the study of relative accumulation around host and non-host roots, hand-picked specimens of *H. similis* were used. These nematodes were sterilized following the procedure of Peacock (1959). A hole about 1 cm diameter was cut under aseptic conditions in the agar equidistant from the root tips of the two different seedlings (rye grass and marigold or rye grass and lettuce). Fresh 1% water agar was poured into this hole and when the agar cooled and almost solidified, 60 *Hemicycliophora* were transferred into it.

Petri plates were inverted and the feeding observed up to 400×. Observations were made at 24-hour intervals up to 3 weeks after inoculation, except study of symptoms associated with feeding which were of 5 weeks duration. The mode, location and duration of feeding of nematodes were noted. For the relative accumulation studies the number of *H. similis* specimens reaching the roots were noted.

The plant hosts were classified as good, average and non-hosts for each species, according to the number of nematodes found feeding at any one time within 72 hours of inoculation. When more than four *Hemicycliophora* or more than two nematodes of any of the other genera fed simultaneously, the plant was designated as a good host. When no feeding was observed the plant was labelled as non-host. Plants on which numbers feeding were intermediate to those given above were designated as average hosts.

Stylet penetration and esophageal movement or movement of the valve in the median esophageal bulb was taken as being evidence of feeding, except for *T. bryophilus* where only stylet penetration could be observed.

**RESULTS**

The hosts on which the nematodes fed are given in Table I. *Hemicycliophora similis* fed on eleven of the thirteen plants near the root tip, mostly between the meristematic region and the region of differentiation, occasionally in the region of elongation, for 6-48 hours. The nematodes did not feed on lettuce