The Role of Secondary Invaders in *Meloidogyne incognita* Infection

P. S. MAYOL AND G. B. BERGESON[1]

Abstract: Secondary microbial invasion of tomato plants inoculated with 6,000 *Meloidogyne incognita* larvae caused 75 and 48% weight reduction of foliage and roots, respectively. Under aseptic conditions the same number of larvae caused only 37% reduction of foliage and increased root weight by 50%.
Key Words: Meloidogyne incognita, Root-knot, Soil microflora, Tomato, Secondary invaders.

Root-knot nematode infected plants frequently undergo premature senescence as a result of root necrosis and destruction of feeder roots. Alone, this nematode is a well adapted parasite causing little, if any, necrosis in a compatible host (3). Therefore, it is assumed that secondary invaders are involved in the root deterioration phase of the disease syndrome. The purpose of the present study was to measure the pathogenic effect of normally non- or low-grade pathogenic rhizosphere microorganisms which become secondary invaders of roots attacked by *Meloidogyne incognita* (Kofoid & White) Chitwood. Consideration of the well-known synergism of this nematode and recognized plant pathogens such as *Fusarium* spp., *Phytophthora* spp. or *Pseudomonas solanacearum* E. F. Sm. was purposely avoided.

MATERIALS AND METHODS

Glass cylinders (90 × 300 mm) (Fig. 1) filled with a fine river sand were sterilized by autoclaving (2 hr at 15 psi) twice at 24 hr intervals. Sterile tomato seedlings were placed in the planting tube (Fig. 1d) which contained vermiculite, and sterile cotton was packed around the stem and sealed with a cooled paraffin-petrolatum mixture. Each unit was separately connected to a source of sterile water and Hoagland's nutrient solution. Sixteen units that passed sterility checks were placed in a controlled environment chamber at 22.5 C with a 16 hr photoperiod. Light intensity was 2,500 ft-c at bench level. Two weeks after the seedling roots had emerged from the planting tube and penetrated into the soil column, the units were

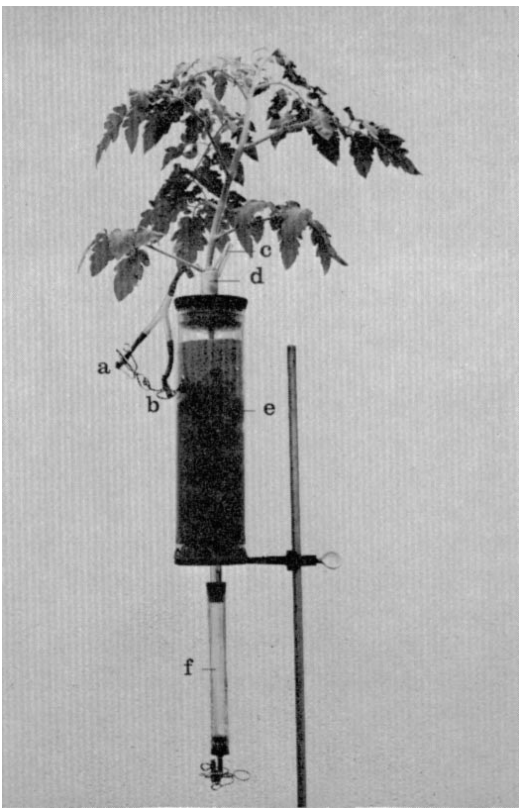

FIG. 1. Gnotobiotic root culture cylinder showing: **a.** water inlet; **b.** fertilizer inlet; **c.** air outlet and inoculation tube; **d.** planting tube; **e.** root chamber; **f.** sterility check tube.

Received for publication 20 June 1969.

[1]Botany and Plant Pathology Department, Purdue University, Lafayette, Indiana 47907. Senior author's present address: Biology Department, Stanislaus State College, Turlock, California. Portion of a Ph.D. thesis by the senior author. Journal Paper No. 3799, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

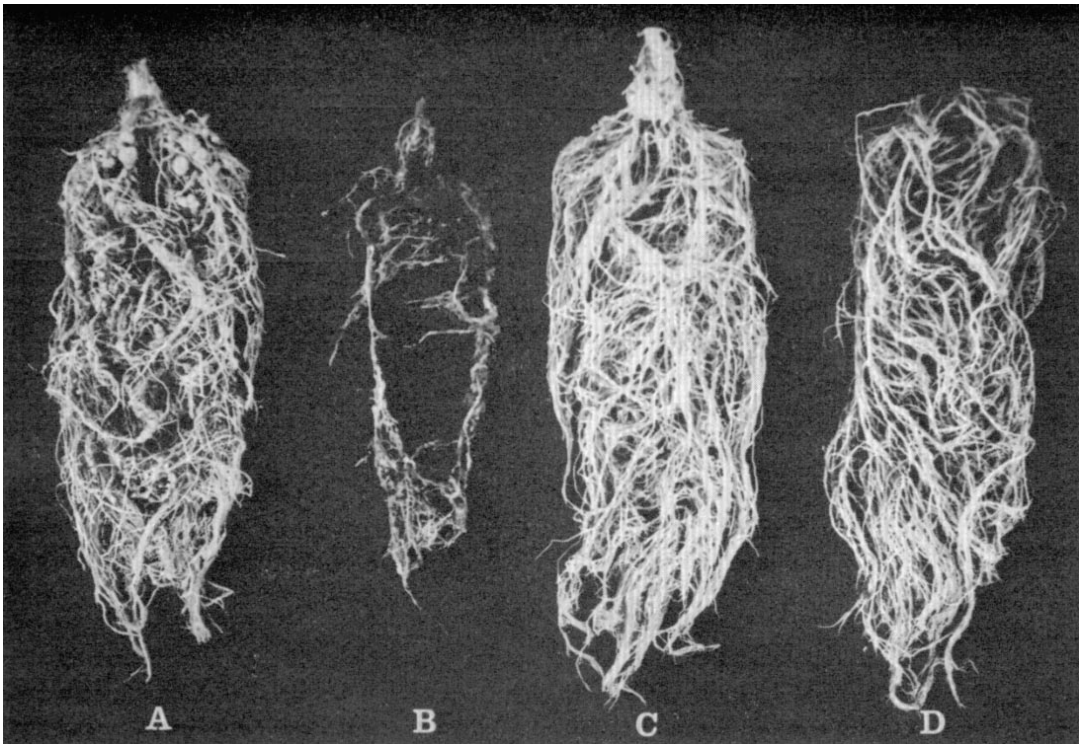

FIG. 2. Twelve-week-old tomato roots showing: **A**. plants grown under aseptic conditions with *Meloidogyne incognita*; **B**. plants grown under septic conditions with *M. incognita*; **C**. and **D**. plants grown under aseptic and septic conditions, respectively, but without *M. incognita*.

divided into lots of four and received one of the following treatments: (i) 6,000 sterile *M. incognita* larvae, (ii) 250 ml of soil suspension made by mixing 1 kg of soil with 5 liters of water. The soil came from around greenhouse tomato plants free of root disease symptoms, (iii) 6,000 nematode larvae plus 250 ml of soil suspension, and (iv) no treatment (control). At weekly intervals excess drainage that collected in the sterility check tubes (Fig. 1f) of the aseptic units was plated to nutrient agar (NA) and acidified potato dextrose agar (APDA) to check for contamination. The entire experiment was terminated as soon as any of these units showed contamination. Sterility was successfully obtained in two experiments, one for 7 weeks and the other for 12 weeks. At termination the plants were cut off at ground level, oven dried, and weighed. The roots were washed and each one divided into two equal parts; one part was oven dried and weighed and the other used to determine root surface area, number of females/g of root, and for assay of microorganisms from surface sterilized segments. Relative root surface area was determined by the titration method of Anderson (1). Females were counted by placing 1 g root sample in Jeffrey solution (10% nitric acid and 10% chromic acid w/w) 1–2 hr until root tissue cleared and the females turned black. Root galls or segments were surface sterilized 20 min in 1:10 commercial Clorox, rinsed in sterile water, transferred in two groups to 2 ml of sterile water in petri dishes, crushed, and one covered with cooled, melted NA and the other with APDA.

TABLE 1. Growth differences due to root-knot nematode infections in the absence or presence of other microorganisms.

Treatments	Dry foliage wt (g)[c,d]		Dry root wt (g)[c,d]		Nemas[e] g of root	Root surface[e,f] area
	7 week	12 week	7 week	12 week		
Nemas alone[a]	9.97	8.43	7.47	6.62*	479	20.2
Nemas & microflora[b]	4.50*	2.57**	6.50	2.37**	361	5.3**
Microflora alone	7.83	10.25	5.30	4.53		18.2
Aseptic control	9.80	12.57	4.94	4.60		18.0

[a] Nematode inoculum in 7 and 12 week experiment was 5,000 and 6,000, respectively.
[b] Microflora inoculum consisted of 250 ml of a suspension of soil from a bed of healthy tomatoes.
[c] Average of 4 replications.
[d] Data of 2 separate experiments of 7 and 12 week duration.
[e] Determined in 12 week experiment only.
[f] Relative indications of root surface area.
* Significant at .05 level.
** Significant at .01 level.

RESULTS

Foliage dry wt reduction by nematodes and soil microflora combined was significant (41%) in the 7-week experiment and highly significant (75%) in the 12-week experiment. Nematodes alone did not cause a significant reduction in either experiment. Dry root wt was not significantly affected in the 7-week experiment. In the 12-week experiment, highly significant reduction (48%) was caused by nematode and soil microflora combined, but nematodes alone caused a significant increase (50%) due to heavy galling with no root deterioration. The difference between the wt of plants receiving only microflora and those grown aseptically was comparatively small (18%), indicating the microflora added in the soil suspension did not contain pathogens capable of independently causing a statistically significant growth reduction. The root surface area was greatly reduced by the combined action of microflora and nematodes, but by no other treatment. There were 25% more females/g of root in the aseptic *vs.* septic plants; probably because of the better physical condition of the host. Bacteria were most frequently isolated from surface sterilized galls whereas fungi were quite few in number ranging from 3–10/gall *vs.* 131–455 bacteria/gall. The fungi were identified as *Trichoderma* sp., *Fusarium* sp., and *Rhizoctonia solani* Kühn. Microscopic examination of cross sections of necrotic galls showed them to be often free of fungal hyphae.

DISCUSSION

Although root-knot nematode alone may cause severe injury or death if a young seedling is exposed to a very high larval inoculum (2), in our experiments using a moderate inoculum and older seedlings, secondary microbial invaders of the galled roots were the major cause of plant growth reduction. The most likely prerequisite for secondary invasion is the modification of root cell structure and physiology by the nematode which probably increases susceptibility of the host to rhizosphere microorganism. This concept was supported by the failure of secondary invaders to spread to adjacent healthy tissue even though they had already penetrated the epidermal defense of the host. Powell and Batten (4) also showed prior infection by root-knot nematode was essential for successful root invasion by secondary fungal parasites such as *R. solani*. Since their experiments did not exclude bacteria and ours did not exclude fungi, the relative importance of fungi *vs.* bacteria as secondary invaders of galled roots will require further investigation.

The results of our experiments closely parallel those obtained in studies of club root disease in which the disease syndrome resembles that of root-knot infection. If secondary invasion of club root galls is prevented, the galls remain firm and significant growth reduction does not occur (5).

LITERATURE CITED

1. ANDERSON, H. W. 1963. Soil fumigation increases the root growth of forest nursery seedlings. Down to Earth 19:6–8.
2. BERGESON, G. B. 1968. Evaluation of factors contributing to the pathogenicity of *Meloidogyne incognita*. Phytopathology 58:49–53.
3. DROPKIN, V. H., AND R. E. WEBB. 1967. Resistance of axenic tomato seedlings to *Meloidogyne incognita acrita* and to *M. hapla*. Phytopathology 57:584–587.
4. POWELL, N. T., AND C. K. BATTEN. 1967. The influence of *Meloidogyne incognita* on *Rhizoctonia* root rot in tobacco. Phytopathology 57:826. (Abstr.)
5. FLENTJE, N. T. 1965. Pathogenesis by soil fungi, p. 225–268. In K. F. Baker and W. C. Snyder (ed.) Ecology of soil-borne pathogens prelude to biological control. Univ. California Press, Berkeley, Los Angeles. 571 p.