

# Antagonistic Interaction Between *Meloidogyne incognita* and *Rhizobium leguminosarum* on Cowpea

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## ABSTRACT

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Greenhouse experiments and histologic investigations were done to determine interactions between cowpea root-knot nematodes (*Meloidogyne incognita*) and root-nodule bacteria (*Rhizobium leguminosarum*). Cowpea plants inoculated with both rhizobia and nematodes showed more severe nitrogen deficiency and retarded growth than plants inoculated with nematodes only or uninoculated plants. Nematode invasion reduced the numbers of nodules and inhibited nitrogen fixation by about 63% in nodular tissue. Infected nodules contained the different developmental stages of the nematode. Histologic studies indicated that nematodes developed in nodular tissue, and they were found inside vascular bundles. Many giant cells with thickened walls were produced and exhibited the morphologic characteristics of transfer cells. The nematodes did not alter the structure of nodules, but bacteriodes did not develop adjacent to nematodes. Infected nodules deteriorated earlier than uninfected ones.

divided into four groups of 10 pots (replicates).

Stock cultures of *M. incognita* (from egg masses collected from eggplant roots) were maintained on cowpeas under greenhouse conditions. We obtained inoculum by washing several heavily galled cowpea roots in tap water; the roots were then chopped, placed on a pan, and subjected to intermittent misting. Nematodes were collected by sieving the water that accumulated in the pan at 24-hr intervals. A 10-ml suspension containing 2,000 nematodes was used as inoculum, and pots were infested by

Root-knot nematodes, *Meloidogyne* spp., and other species of plant parasitic nematodes inhibit nodulation and nitrogen fixation in certain legumes (5,7). *Heterodera trifolii* (Goffart) Oostenbrink, and *Meloidogyne javanica* (Treub) Chitwood reproduced readily in nodules of white clover and altered the number of nodules (9). Soybeans inoculated with nematodes and rhizobia exhibited severe nitrogen deficiency and reduction in nodule development and total fixed nitrogen (1,2,10). In cowpea (4) and wando pea (3,12), invasion of bacterial nodules by *Acrobeloides buetschlii* and *Meloidogyne* spp. suppressed plant growth, reduced aminonitrogen content, and resulted in many giant cells without disrupting nodular tissue.

Because nitrogen fixed by *Rhizobium* spp. is usually depressed as a result of nematode invasion, this investigation was initiated to determine the influence of *M. incognita* (Kofoid and White) Chitwood on nodulation, nitrogen fixation, plant growth, and structure of nodular tissue in cowpea. Histologic studies were also done to clarify the effect of nematodes on the development and number of bacterial nodules.

## MATERIALS AND METHODS

Five seeds of cowpea, *Vigna unguiculata* (L.) Walp., were planted in steam-sterilized soil (79% sand, 8.1% silt, and 12.9% clay) in 21-cm-diameter plastic pots. Three weeks after germination, plants were thinned to two per pot. Forty pots were

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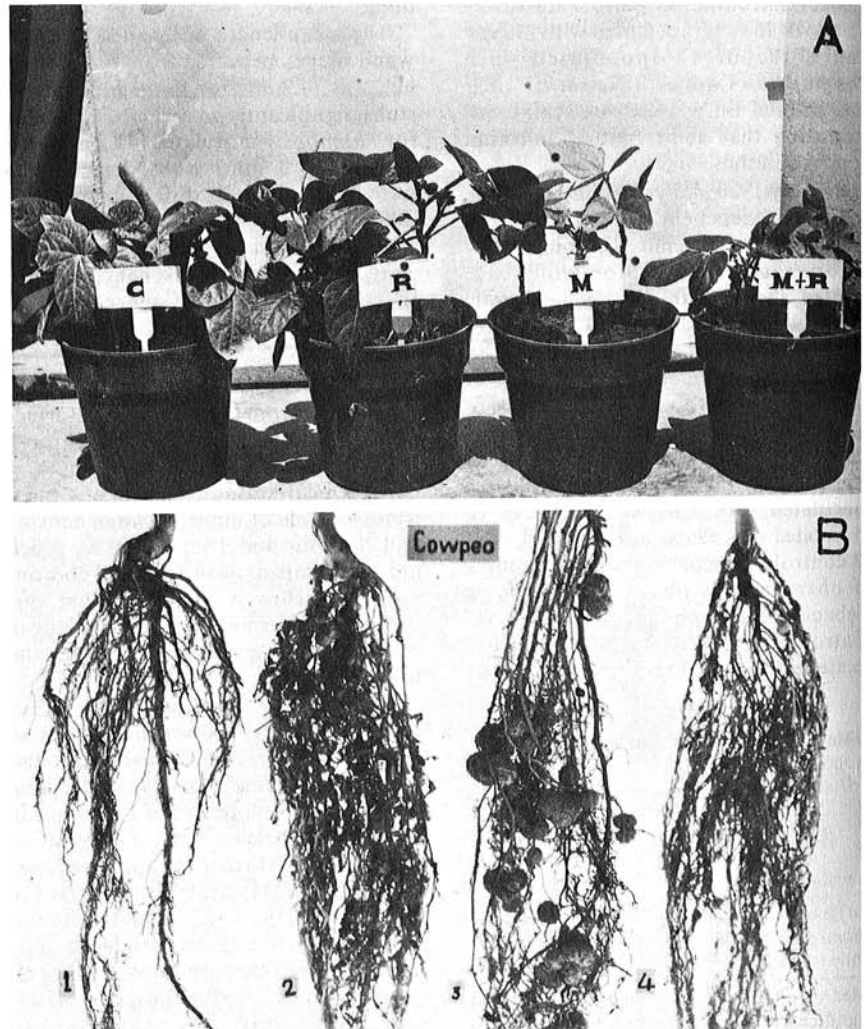
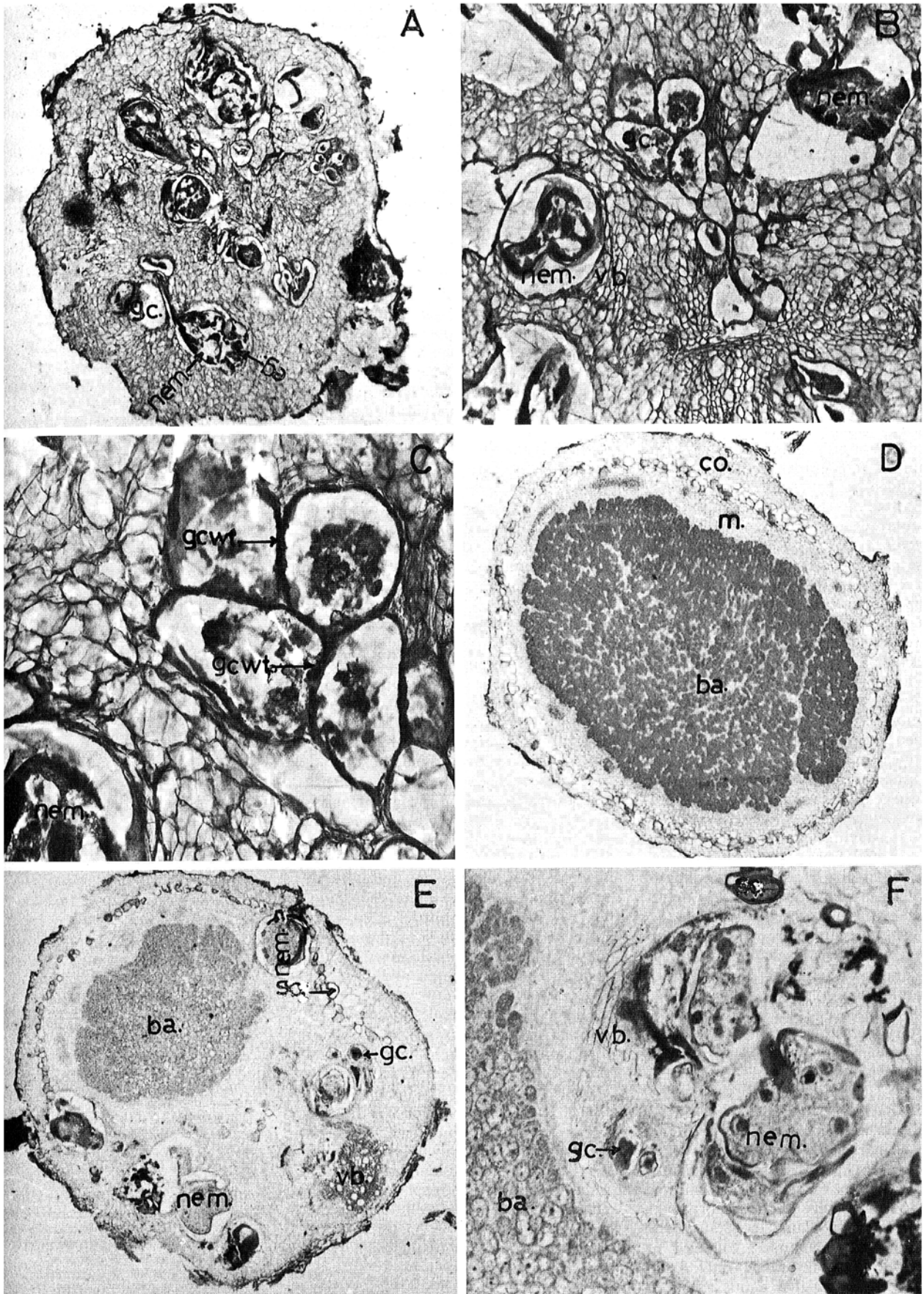


Fig. 1. Morphological changes caused by invasion of the root-knot nematode, *Meloidogyne incognita*, into roots and nodules of cowpea plants: (A) Plants: (C) free of nematodes and rhizobia, (R) inoculated with *Rhizobium leguminosarum*, (M) inoculated with nematodes, and (M + R) inoculated with nematodes and *R. leguminosarum*. (B) Roots: (1) of check plant and of plants inoculated with (2) *R. leguminosarum* and nematodes, (3) *R. leguminosarum*, and (4) nematodes.



**Fig. 2.** Tissues of cowpea: (A) Section through nodule containing *Meloidogyne incognita*. (B) Female nematodes with developed giant cells. (C) Giant cell wall thickenings induced by *M. incognita*. (D) Healthy nodule. (E) Nodule containing nematodes. (F) Portion of nodule developing on a root gall induced by *M. incognita*. ba. = bacteriodes, co. = cortex, eg. = eggs, gc. = giant cell, gcwt. = giant cell wall thickening, m. = meristem, nem. = nematode, vb. = vascular bundles, sc. = sclerenchyma.

**Table 1.** Numbers and dry weight of nodules, dry weights of shoots and nitrogen contents<sup>a</sup> of cowpea plants inoculated with *Meloidogyne incognita*, *Rhizobium leguminosarum*, or both

Treatment	Nodules/root		Plant shoot	Nitrogen fixation	
	No.	Dry weight (mg)	Dry weight (g)	%	mg/g of dry weight of tissue
Rhizobia	20.5 a	325 a	2.5 a	3.1	25.5 a
Check	...	...	1.1 b	1.3	12.8 b
Nematodes	...	...	0.7 b	1.1	11.2 bc
Nematodes and rhizobia	4.0 b	49 b	0.7 b	1.1	9.2 c

<sup>a</sup>Numbers in the same column followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

pouring inoculum into a hole about 5 cm deep and centrally located in each pot. The hole was filled with soil, and the plants were irrigated.

The bacterium, *Rhizobium leguminosarum* (strain TAL 169 from the NIFTAL project, University of Hawaii), was grown on yeast mannitol agar at 25 C. A suspension containing  $7.5 \times 10^6$  cells per milliliter was prepared from a 10-day-old culture of *R. leguminosarum*, and 10 ml was added per pot as described above.

Plants in the first group were inoculated with newly hatched *M. incognita* at a rate of 2,000 larvae per pot, those in the second group were inoculated with *R. leguminosarum*, those in the third group were inoculated with rhizobia and nematodes simultaneously, and those in the fourth group were left untreated as checks. Plants were grown in a greenhouse with ambient temperature of  $31 \text{ C} \pm 1 \text{ C}$ , watered with Hoagland's nutrient solution (6) minus N or with tap water as needed, and harvested 60 days after treatment.

Plants at harvest were uprooted, washed gently, examined, weighed, dried, and reweighed. Records were taken of nitrogen-fixing capacity per plant, number and fresh weight of bacterial nodules, and dry weights of plant shoots. Nitrogen content of shoots was determined by the micro-Kjeldahl method (8). For histologic studies, roots with nodules were fixed in formalin alcohol acetic acid, dehydrated with ethanol, and embedded in paraffin wax (9). Cross and longitudinal sections, 18  $\mu\text{m}$  thick, were cut with a rotary microtome, stained with eosin-fast green, and mounted in Canada balsam (9).

## RESULTS

Plants in the different treatments showed marked differences in growth and appearance (Fig. 1). Rhizobia-inoculated plants were the most vigorous, followed by the check plants. Reduction in growth was greatest in plants inoculated with rhizobia and nematodes, followed by those infected by nematodes only. Roots of rhizobia-inoculated plants exhibited conspicuously large nodules that involved nearly the whole root. Roots inoculated with nematodes alone had smaller galls than those inoculated with nematodes

and rhizobia. Roots of plants inoculated with nematodes and rhizobia had a mixture of nematode galls and nodules. Roots of untreated plants were normal and free of galls and nodules (Fig. 1B).

Numbers and dry weights of nodules, dry weights of shoots, and nitrogen fixation of plants varied significantly among different treatments (Table 1). Dry weights of shoots and nitrogen fixation of plants inoculated with rhizobia were approximately 2.5 and 2 times greater, respectively, than those of untreated plants. Plants inoculated with nematodes and rhizobia showed the lowest amounts of fixed nitrogen and dry weights of shoots.

These results indicate that *M. incognita* developed and reproduced in nodular tissue of cowpea. The numbers of nodules, dry weights of nodules, dry weights of shoots, and fixed nitrogen per plant of plants treated with nematodes and rhizobia were 80.95, 84.92, 72.98 and 87.85% lower, respectively, than those of plants treated with rhizobia alone.

Microscopic examination revealed egg masses, larvae, and mature females in the nodules. Heavily infected nodules were soft and dark brown. Histologic studies indicated that *M. incognita* penetrated the cortex of root tissues and induced formation of many giant cells (Fig. 2A,B). Bacterial nodules also were infected with nematode larvae where they matured, inhibited nodule function, and produced giant cells in the nodular tissue (Fig. 2E). Compared with healthy nodules (Fig. 2D), tissues of nematode-infected nodules were not disrupted or disorganized, although nematode and giant cells developed normally and occupied the vascular bundles of nodular tissue. The walls of giant cells in nodular tissue frequently contained thickened areas (Fig. 2C). Heavily infected nodules (Fig. 2E) showed considerable reduction in bacteriodes. Hypertrophy and hyperplasia of the cortical cells of infected nodules surrounded the bacterial tissue, and nodules were gradually transformed into nematode galls.

## DISCUSSION

An interesting and unexpected result was the poor growth and reduced amount of nitrogen that plants inoculated with

rhizobia and nematodes showed, compared with those inoculated with nematodes only. The presence of nematodes apparently prevented rhizobia from supplying the plants with nitrogen. Rhizobia strains in leguminous hosts may lose their capability for nitrogen fixation, especially when the hosts are infected by certain pathogens (12).

The rhizobial strain used in this study had nitrogen fixing capability as can be seen by the dry weights of shoots and nitrogen contents of plants inoculated with rhizobia. When *M. incognita* entered the association, the numbers and dry weights of nodules were altered, nodulation was inhibited, and the rhizobia lost most or all of their nitrogen fixing capability. Similar results have been reported in soybean (2,3,5,7,11).

Mature females of *M. incognita* found in the vascular bundles of nodular tissue produced many giant cells without disrupting that tissue. This indicated that the parasite developed and reproduced in nodular tissue of cowpea. *M. javanica* and *H. trifolii* that reproduced in nodular tissue of white clover altered the number of nodules only by suppressing root growth and did not disrupt nodular tissue (10).

The loss of the beneficial effects of nodules may be due to nematodes feeding on the bacteriodes and inducing formation of cavities in the central portion of nodules (13). In our study, *M. incognita* was usually restricted to the vascular bundles, and no cavities were observed. Bacteriodes could be inhibited by secretion of hydrolytic and oxidative enzymes or growth regulators by the nematodes.

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