INFLUENCE OF MELOIDOGYNE INCognITA ON NODULATION AND GROWTH OF PEA AND BLACK BEAN

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Meloidogyne incognita invaded aseptic roots of Pisum sativum and Phaseolus vulgaris and developed to adult females in about 19 days. Males were occasionally observed. Juvenile nematodes invaded nodules initiated by Rhizobium; nodules formed also on galls initiated by nematodes. M. incognita suppressed root and nodule growth. However, M. incognita stimulated the initiation of nodules which remained undeveloped. Effective nodules had more nitrogenase activity/g on plants with M. incognita than on those without nematodes. M. incognita increased the leghaemoglobin content of nodules on Pisum and decreased it on Phaseolus. All the effects of the nematode invasion were transient and 7d difference in invasion date altered the degree of effect recorded at different harvest dates.

Keywords: root knot nematode, interaction, host parasite relations, nitrogen fixation.

The development of the symbiotic association between legumes and rhizobia is of considerable agronomic importance. This symbiosis contributes much of the biologically fixed nitrogen for agriculture. Several authors have reported that Meloidogyne incognita (Kofoid & White) Chitwood interfered with nodule formation and development on leguminous crops such as Phaseolus aureus (Hussain & Seshadri, 1975; Raut, 1980), Glycine max and Pisum sativum (Barker & Hussey, 1976), Vigna sinensis (Sharma & Sethi, 1976), and Vigna unguiculata (Ali et al., 1981).

The objective of this study was to determine the effect of the sequence of M. incognita and Rhizobium spp. inoculation on nodulation on Pisum sativum cv. Scout and Phaseolus vulgaris cv. Bush Blue Lake at different sampling times.

MATERIALS AND METHODS

Plants were grown with sterile roots in 250 ml conical flasks. Seeds were surface sterilized with 10% chloros, 0.5% sodium hypochlorite for 20 min, rinsed three times with sterile distilled water and germinated on 0.5% water-agar plates. One 3-day-old seedling was transferred to each flask.
The flasks were prepared with 200 ml of graded white sand, 91% between 125 and 250 μm diameter, moistened with 30 ml of a nutrient solution (Fahraeus, 1957) with 1 mg of nitrogen added as potassium nitrate per 4 litres of solution. This moisture level was selected to provide moist sand with optimum conditions for nematode movement (Wallace, 1963). The flasks were closed with polyurethane foam bungs, sealed with aluminium foil and autoclaved at 1 bar for 15 min. The foil was removed, a seedling added to each flask and the flasks irrigated to their initial weights every day with nutrient solution. The plants were grown in a cabinet controlled at 22° and 80% relative humidity during a 15 h light period and 20° with 90% relative humidity during a 9 h dark period.

*M. incognita* egg-masses were picked from stock cultures maintained on tomato roots in the greenhouse. The egg masses were surface sterilized with 1% chloro, 0.05 sodium hypochlorite, for 1 h, placed on a 90 μm sieve in a petri dish partially filled with sterile distilled water and kept at 25°. Five hundred juveniles hatched within 4-5 d were added to each plant. The bacteria *Rhizobium* (strain RCR 1045, *R. leguminosarum*, biovar viceae for *Pisum* and RCR 3644, *leguminosarum* biovar *phaseoli* for *Phaseolus* from the Rothamsted Collection of *Rhizobium*) were grown in liquid yeast mannitol medium (Vincent, 1970) with constant shaking for 5 d and plants were inoculated with 1 ml of the liquid culture. *Rhizobium* alone, *M. incognita* alone or *Rhizobium* and *M. incognita* were introduced by pipetting the suspensions around the seedlings 7d after they were transferred to the flasks. *Rhizobium* or *M. incognita* were inoculated similarly 7 days later to half the flasks which had only *M. incognita* or *Rhizobium* on the first occasion, respectively. Each treatment and inoculated controls were replicated 10 times and arranged randomly in the growth cabinet.

Half of the experiment was harvested 26 days after the first inoculation when the first flowers were open and half after 52 days for *Phaseolus*, when the second truss of pods were filling and some plants were beginning to senesce. On each occasion fresh roots, big nodules (>1 mm diam.) taken off the roots, fresh and dry shoots and pods were weighed. Numbers of *M. incognita* females with and without egg sacs, pre-adult nematodes and small nodules (<1 mm diam.) on 0.5 g of chopped roots stained with acid fuchsin (Bridge et al., 1982) were counted using a stereo-microscope. The presence of second and third stage juvenile nematodes was noted.

The rate of nitrogenase activity in the detached nodules was determined at each harvest by using the acetylene-ethylene assay (Hardy et al., 1973) and expressed as micromoles of C2H4 formed per g of fresh weight of nodules per h. Ethylene concentration was determined with a gas chromatograph equipped with a H2-flame ionization detector after separation on a stainless steel column 20 cm × 3 mm packed with 80-100 mesh Poropak ‘N’ (Waters Associates Inc.)