A Disease Complex of Okra and Tomato Involving the Nematode, Meloidogyne incognita, and the Soil-Inhabiting Fungus, Rhizoctonia solani

J. K. Golden and S. D. Van Gundy

Research Associate and Professor of Nematology and Professor of Plant Pathology, Departments of Nematology and Plant Pathology, respectively, University of California, Riverside, California 92502.

Current address of senior author is Biochemicals Department, E. I. duPont deNemours and Co., Inc., Atlanta, Georgia 30326.

Accepted for publication 2 July 1974.

ABSTRACT

Galled okra and tomato roots infected with *Meloidogyne incognita* in the field and in the greenhouse were highly susceptible to infection by *Rhizoctonia solani*. Root decay by the fungus occurred 4-5 weeks after nematode infection. Fungal sclerotia were formed only on nematode gall tissues. The fungus penetrated either directly or through ruptures in the root created by the mature female nematode. *Rhizoctonia solani* colonized nematode giant cells and root xylem cells. Vascular discoloration occurred both in roots and stem, however no fungus was isolated from stems. Prepenetration studies using cellophane membranes indicated that the fungus was specifically attracted to nematode gall tissue and that sclerotia were selectively formed on nematode galls. During development of the nematode in okra, the levels of total carbon and nitrogen were higher than in control roots.

Phytopathology 65:265-273

Additional key words: Fungus-nematode interactions, root-knot nematode.

It has been known for some time that soil-borne fungi interact with nematodes to promote root decay and necrosis. Steiner (12) observed necrosis in connection with root-knot nematode (*Meloidogyne*) infection, and described smooth galls as "healthy galls" contrasted to those that had become necrotic. Necrosis and cracking of roots by nematode activity permitted invasion by secondary agents. Clayton et al. (4) first noted that tobacco growth may not be visibly affected during the early stages of the root-knot disease development, when nematode galls are firm and smooth. The plant symptoms of wilting and growth retardation were associated with root decay, the later phase of the disease. They postulated that the decay phase commenced when nematode egg masses ruptured the epidermis of the roots, which provided a means of entry for root-rotting organisms.

Root-knot nematodes have been shown to predispose tobacco plants to severe damage from *Pythium ultimum* and *Rhizoctonia solani* (1, 7). Except for the seedling stage, these fungi are minor pathogens on tobacco. However, if seedling plants or plants approaching maturity had been previously invaded by *Meloidogyne incognita*, both *P. ultimum* and *R. solani* were capable of invading roots and causing rapid necrosis. No decay developed when either fungus was applied to roots in the absence of nematodes, and little damage resulted when one of the fungi and nematodes were added simultaneously. There was no necrosis in tobacco varieties resistant to root-knot nematodes.

The interaction of *M. incognita* and *P. ultimum* in varying combinations has been studied under gnotobiotic conditions (7). These experiments confirmed previous findings that necrosis occurred only when the fungus followed the nematode by 4 weeks. Cultivars differed in the number of nematodes necessary to induce predisposition of tobacco roots to *P. ultimum*.

Fungal penetration of galled and nongalled areas of nematode-infected roots, as well as nematode-free roots, have been studied from initial penetration through colonization (8). There was no appreciable fungal invasion of roots which were free of nematodes, but galled and nongalled regions of roots infected by *M. incognita* were readily invaded by *P. ultimum* and colonization was virtually complete within 6 days. No evidence of preference was shown by *P. ultimum* in areas proximal to the nematode.

The root-knot nematode-*R. solani* complex in tobacco has also been studied histopathologically (1). The *M. incognita*-susceptible tobacco cultivar, Dixie Bright 101, exhibited extensive fungal colonization in treatments in which the nematodes were allowed to become established for 21 days before fungal inoculation. Vigorous hyphae encircled the nematode and were concentrated within the locus of nematode infection. The fungus did not invade N.C. 95, a *M. incognita*-resistant tobacco cultivar.

The results of experiments with root-knot nematodes in combination with nonaggressive pathogens on tobacco led to research with *M. incognita* and certain other soil-borne fungi that are not regarded as pathogens of that crop (10). *Curvularia trifolii, Botrytis cinerea, Aspergillus ochraceus, Penicillium martensii,* and *Trichoderma harzianum* have not been reported as pathogens of tobacco, although some have been identified as causal agents of disease of other plants. Roots infected with *M. incognita* for 3-4 weeks prior to being inoculated with any one of the above fungi, were invaded by the fungus and extensive root decay occurred. In fact, root destruction by any of these fungi in the presence of *M. incognita* is as great as that previously demonstrated for *Pythium* or *Rhizoctonia* under similar conditions. This suggests that predisposition of plant roots by root-knot nematodes is physiological in nature.

The problem under investigation was a severe root rot of okra and tomato infected with *M. incognita* occurring in irrigated soil in Riverside, California. The purposes of this investigation were to determine the chronological sequence of events leading to decay of *M. incognita*-infected okra and tomato roots in the field, and to elucidate the mechanism(s) by which *M. incognita* predisposed okra and tomato roots to secondary invasion by soil-borne fungi.

MATERIALS AND METHODS.—*Field studies.*—A

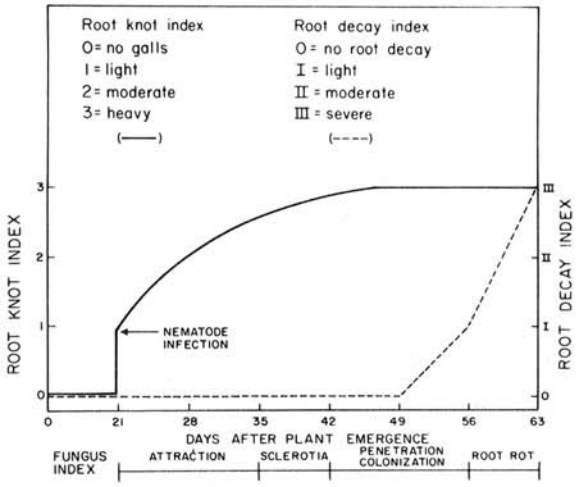


Fig. 1. The chronological sequence of events leading to a severe root rot of okra infected with *Meloidogyne incognita* and *Rhizoctonia solani*.

field soil known to be infested with the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, and possessing a population of soil-borne microorganisms that caused a severe root rot and a rapid decline of okra and tomato in 1968 was selected for soil fumigation trials in 1969. The field was divided into 24 (7.31 m × 6.62 m) plots. Methyl bromide (MB) was applied to eight plots under plastic tarps at the rate of 488 kg/hectare (ha) (1 lb/100 ft^2) to control all soil microflora and fauna. Ethylene dibromide (EDB) was applied to eight plots at the rate of 56 liters/ha (6 gal/acre) with a tractor-drawn applicator to control root-knot nematodes with minor effects on the natural microflora. The remaining eight plots containing natural populations of microflora and fauna were left as untreated controls. All plots were planted to okra (cultivar Clemson Spineless). The two border rows around each plot were planted with 4-week-old tomato seedlings (cultivar Rutgers). Okra and tomato roots selected at random were harvested at weekly intervals over a 6-week period, beginning 3 weeks after emergence of the okra seedlings, and 2 weeks after transplanting the tomato seedlings. Roots were washed free of soil, examined, and rated at each sampling date for root decay and galling. Representative roots from each treatment were cut into sections approximately 3 cm in length, passed through ten serial washings of sterile distilled water, and then plated on water agar, cornmeal agar and potato-dextrose agar (PDA). The various fungi growing from root pieces on the different agars were hyphal-tip transferred to PDA plates for identification. Stem sections from diseased and healthy plants were surface sterilized in 10% sodium hypochlorite solution and plated on water and cornmeal agars. Representative root specimens for histopathological studies were fixed in Karpechenko's solution (11) for 48 hours, washed in running water for 6 hours, dehydrated in alcohol, cleared in toluene, and infiltrated and embedded in Fisher's Tissuemat (melting point 56 C). Sections 10-μm thick were cut on a rotary microtome, affixed to gelatin-coated glass slides, stained with safranin for 24 hours and counterstained with fast green for 5 seconds.

Okra roots to be used for carbon-nitrogen analysis were collected and dried in an oven at 60 C for 48 hours. Dried root samples were ground in a Wiley mill to pass through a 240-μm (60-mesh) screen. Total carbon content of root samples was determined gravimetrically by a wet combustion method (2). Total nitrogen was determined by a semimicro-Kjeldahl method (2).

Pathogenicity studies.—Greenhouse studies were conducted to establish the pathogenicity of *Rhizoctonia solani* Kühn, the predominate primary fungus which attacked *M. incognita*-infected okra and tomato roots in the field. The okra cultivar Clemson Spineless and the tomato cultivar Pixie Hybrid were used as host plants in all tests. Okra and tomato plants were grown in 15.2-cm (6-inch) diameter clay pots containing a steam-sterilized sandy loam soil. The 5-week-old okra and tomato seedlings were inoculated with the following treatments: *M. incognita* alone, *R. solani* alone, *M. incognita* + *R. solani*, and untreated controls. The treatments were replicated 20 times. The nematode inoculum was collected daily from *M. incognita*-infected tomato roots held in a mist chamber. Ten thousand freshly hatched second-stage larvae were washed and injected directly into soil around roots of okra and tomato plants using an automatic syringe equipped with a canula. *Rhizoctonia solani* was cultured on potato-dextrose broth contained in 9-cm-diameter petri plates, and incubated for 5 days at 25 C. The inoculum was prepared by blending the hyphal mats for 30 seconds with 100 ml of distilled water in a rotary blender. Each plant was inoculated by pouring 100 ml of the mycelial suspension onto the soil surface and covering it with steam-sterilized sandy loam soil.

The tests were conducted in the greenhouse where the temperature fluctuated from a daily low of 26 C to a daily high of 31 C. Plants were fertilized weekly with double-strength Hoagland's solution (5). Three plants from each treatment were harvested at weekly intervals over a period of 6 weeks following inoculation. Roots were harvested, washed, and indexed for root decay and galling. Representative root pieces 2 cm in length were passed through ten serial washings of sterile distilled water, and plated on water agar and PDA. Fungi growing from the root pieces were hyphal-tipped to PDA plates for identification. Representative root specimens from each treatment were collected each week over the 6-week period, and processed for histopathological study as described previously.

Prepenetration studies.—A modification of the cellophane-bag technique (6) was used to study the prepenetration response of *R. solani* to stimuli originating from *M. incognita*-infected and control roots in situ. This technique allowed diffusable substances to pass through the membrane, but physically separated the fungus and host. Three-week-old tomato seedlings were transplanted into steam-sterilized sandy loam soil contained in root observation boxes equipped with removable glass and wooden sides. The observation boxes were tilted to encourage root growth along the glass sides. When sufficient roots had developed along the glass sides, the sides were removed and the roots were inoculated with 10,000 freshly hatched *M. incognita* larvae. The larvae were distributed along the root surfaces with a 10-ml pipette. Two weeks after nematode inoculation, the glass sides were removed and moist

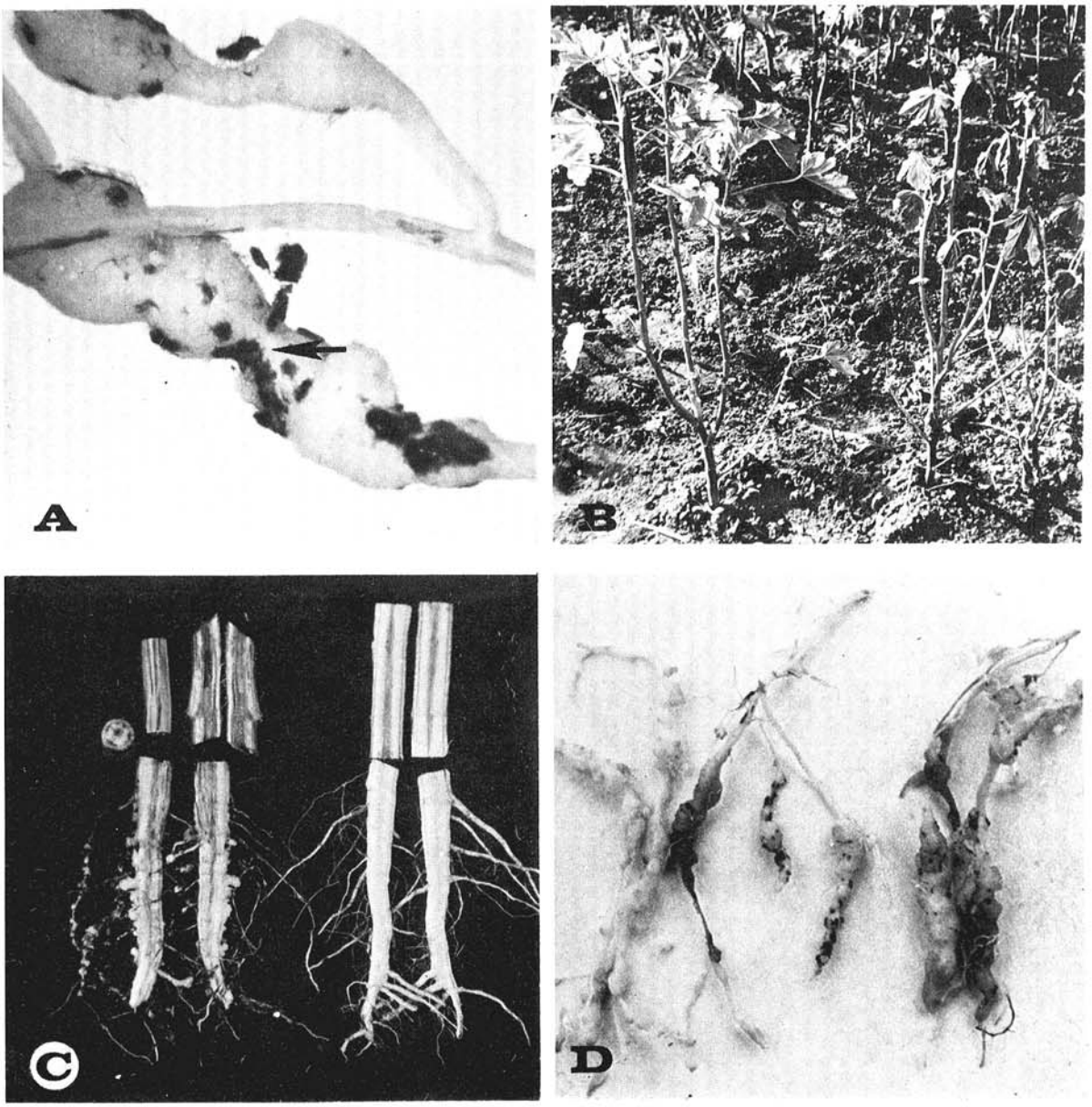
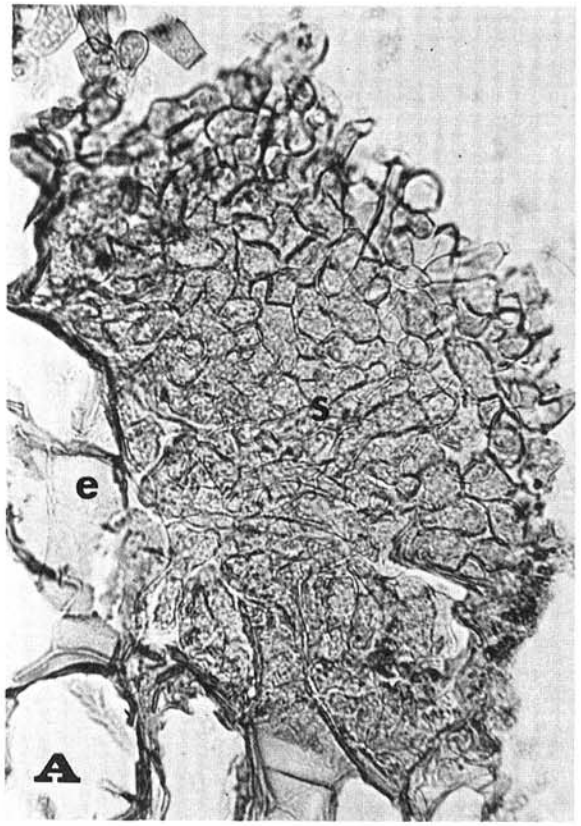
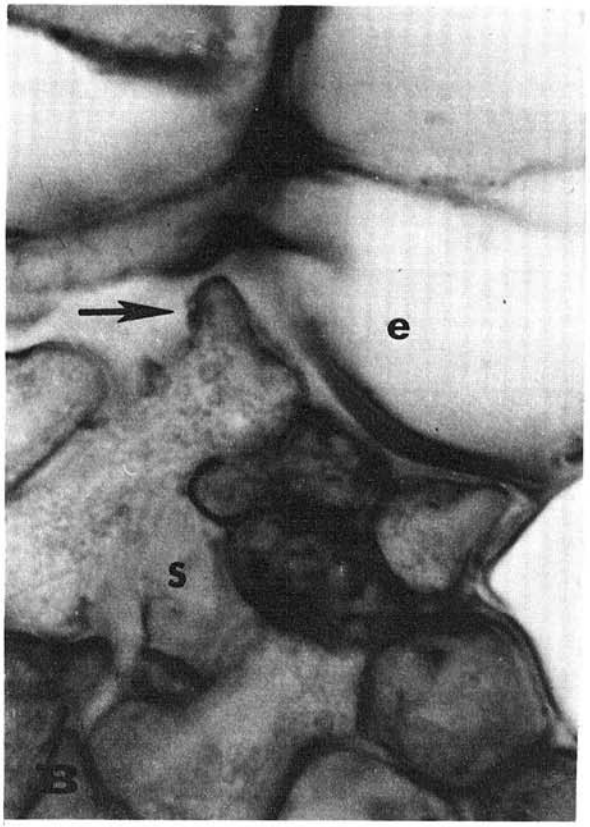
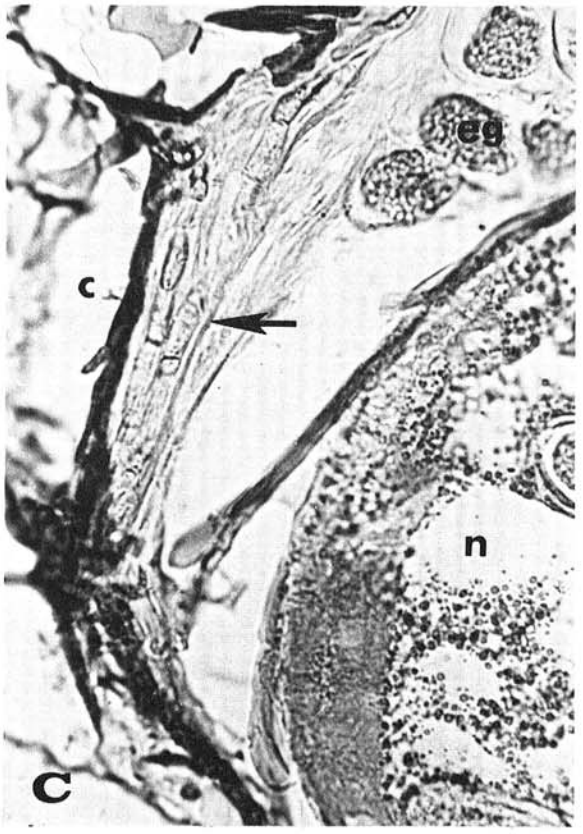


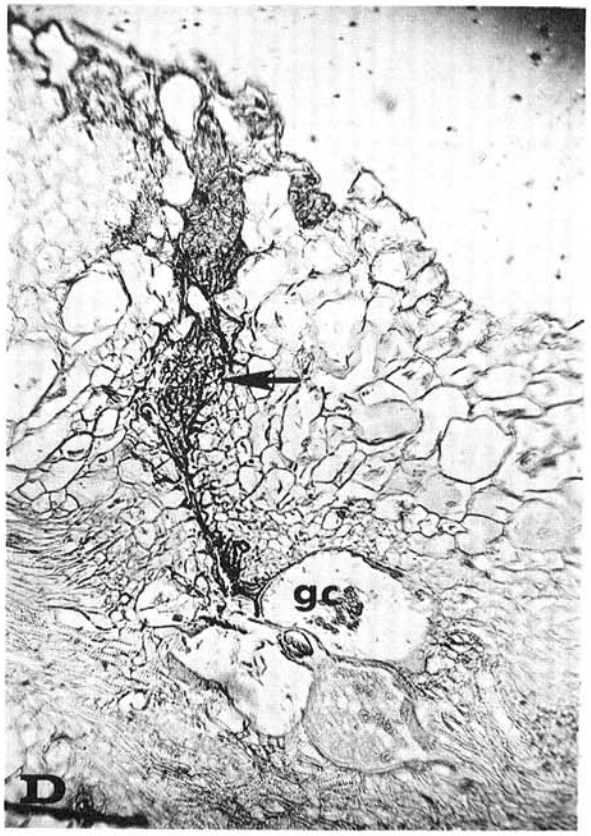
Fig. 2-(A to D). Okra roots infected with *Rhizoctonia solani* and *Meloidogyne incognita*. **A)** Five-week-old galls with massive sclerotia (arrow) and egg masses on root surface; **B)** Plants showing permanent wilting and leaf drop with only main stems remaining in nontreated plots as compared with healthy plants in plots treated with methyl bromide; **C)** The vascular system of both roots and stems of field-grown okra 6 weeks after nematode infection were brown (left) when compared with roots grown in methyl bromide treated soil (right); **D)** *Meloidogyne incognita*- and *R. solani*-infected okra roots 6 weeks after nematode infection showing severe rot.

cellophane membranes were placed on the surface of the soil containing the exposed galled roots as well as on the surface of the soil containing control roots. The cellophane inserts had been cut from sheets of moisture-proof cellophane, 25 μm thick, boiled in distilled water 30 minutes to remove any coating material, and sterilized in the autoclave. Circular 7-mm diameter plugs of *R. solani* mycelium grown on potato-dextrose broth were washed thoroughly to remove any remaining nutrients. Ten plugs were distributed randomly over the top of the cellophane membrane in each root observation box. The plugs on the cellophane membranes were covered with clear plastic sheets to prevent drying. The removable glass and wooden sides were replaced and the plants and fungus allowed to grow for 5 days. The tops of the membranes containing *R. solani* were stained in situ by atomizing with a neutral red solution followed by a gentle water rinse. The cellophane membranes were then removed and studied in detail under a stereomicroscope. More detailed microscopic studies of responses of *R. solani* to stimuli originating from *M. incognita*-infected and control roots were made in transparent root observation boxes. Each


s
e
A


e
s
B


eg
c
n
C


gc
D

unit consisted of a clear plastic shoe box in one end of which a hole was cut to accommodate a 250-ml plastic tissue culture flask. The flask had two holes drilled in the bottom and three small holes in one side and one small hole in the other side. Two-week-old tomato seedlings were transplanted into each flask filled with steam-sterilized vermiculite. The flasks were buried about 5 cm in steam-sterilized vermiculite in metal nursery flats. The vermiculite was kept moist with daily waterings using single-strength Hoagland's solution. When the roots extended through the holes in the bottom of the flasks, the flasks were gently removed, taking care not to damage the small roots. The roots were rinsed thoroughly with sterile glass-distilled water and the flasks were placed in the openings in the end of the observation boxes. The flasks were secured in the observation boxes with Daps caulking rope. The root observation boxes contained 1.0 liter of water agar as support medium for the roots under study. A single hole on the bottom of each flask served as a drain hole. This technique permitted continuous observation of *R. solani* response toward roots without mineral contamination from the Hoagland's solution. Roots grew out onto the surface of the water agar within 4-5 days, and were inoculated by pipetting 5,000 freshly hatched, sterilized *M. incognita* larvae onto the surface of the water agar. Water and Hoagland's solution were added to each culture flask daily.

Sterile second-stage larvae of *M. incognita* were obtained from handpicked egg masses treated in a solution of 2% NaOCl for 2 minutes and hatched in sterile distilled water. Freshly hatched larvae were collected daily by decanting through a sterilized 44-μm (325-mesh) screen. The larvae were rinsed from the screen into a 50-ml conical screw cap test tube with sterile water and allowed to stand for 1 hour. The excess liquid was withdrawn and the test tube filled to within 2.54 cm of the top with sterilizing solution composed of 0.008 g aretan, 0.06 g aureomycin, 0.2 g streptomycin, and 0.05 g hibitane diacetate dissolved in 400 ml of sterile distilled water. The sterilant liquid was removed, and the nematodes were then rinsed with sterile distilled water. One-ml portions of the final solution was transferred to nutrient agar plates to check for contamination. The nematodes were transferred to an Erlenmeyer flask containing sterile distilled water and stored under common refrigeration for 24 hours before use, which provided time to observe the nutrient agar plates for incidence of contamination. Only larvae free of contamination were used for inoculum.

Galls were evident on the tomato roots growing on the surface of the water agar 4 days after nematode inoculation. Two weeks after nematode infection, boiled and autoclaved cellophane membranes were laid on the surface of the water agar over galled and control roots. Six circular plugs 7 mm in diameter of *R. solani* mycelium grown on PDA and washed thoroughly were distributed over the top of the cellophane membranes. Tops were fitted onto the plastic root observation boxes and the boxes were covered with aluminum foil to exclude light from the roots. The observation boxes were maintained in the greenhouse. Fungus growth over the membranes was observed daily using a stereomicroscope, and five days after fungal inoculation, the membranes were stained with neutral red in situ.

RESULTS.—*Field studies.*—Okra and tomato plants grown in MB- and EDB-treated soil were free of root decay and galls. The plants remained vigorous throughout the 6-week sampling period. Roots of both okra and tomato plants grown in untreated areas of the field were heavily galled by *M. incognita*. Root decay symptoms appeared 28 days after the first observation of galls. *Rhizoctonia solani* was first isolated from galled roots 1 week after initial gall formation was observed. The chronological sequence of nematode infection, galling, and the onset of root rotting is summarized in Fig. 1.

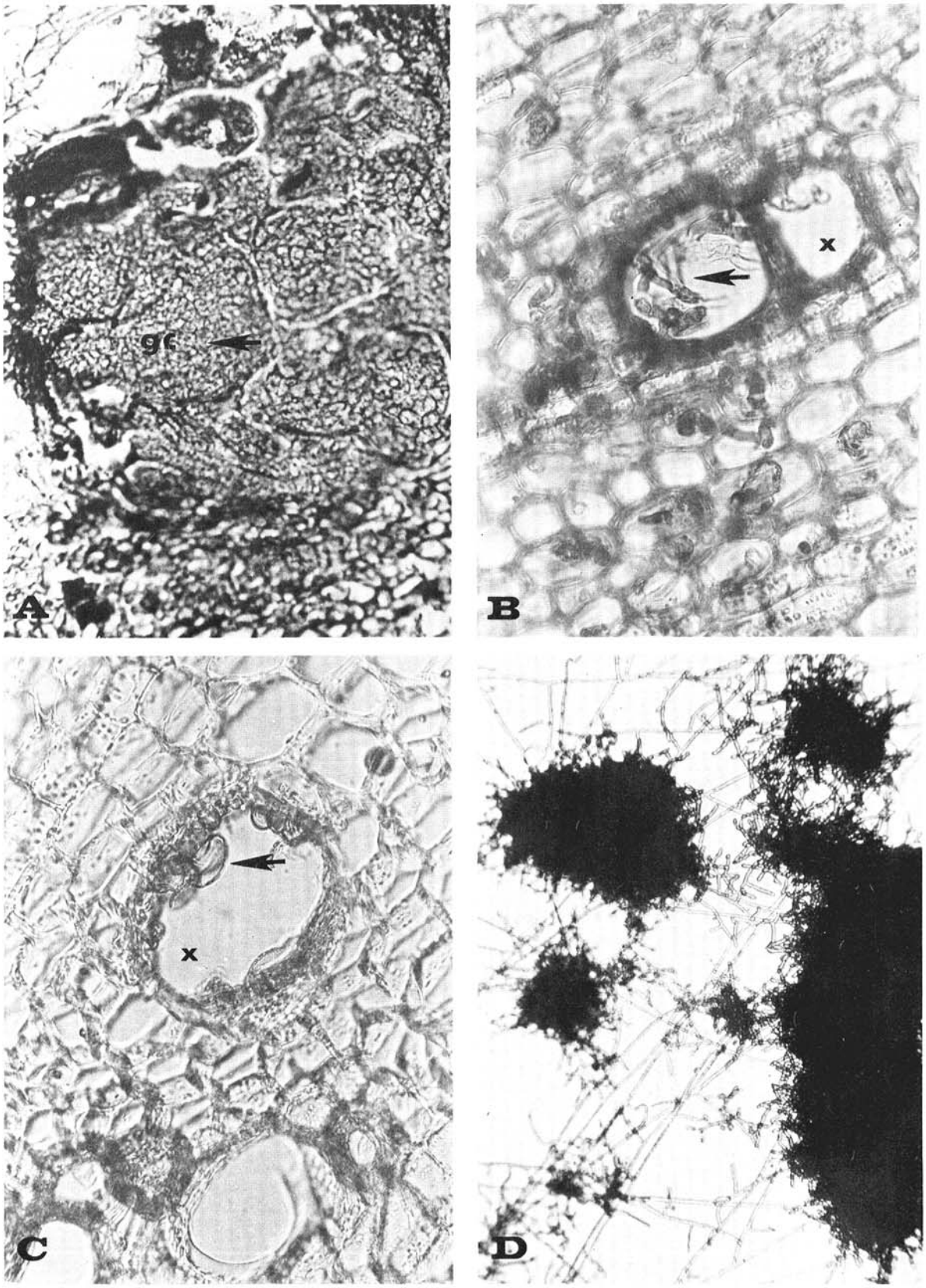
Nematode-induced giant cells were present in the roots 1 week after infection. Twenty-one days following nematode infection, numerous black sclerotia of *R. solani* were observed on the surface of the galls while nongalled portions of the same roots were free of sclerotia (Fig. 2-A).

Four weeks following nematode infection, both okra and tomato plants were stunted. Although the lower leaves of okra were chlorotic, roots were free of decay. Sclerotia of *R. solani* were larger and more numerous on gall surfaces than in previous collections, but nongalled portions of these roots were free of sclerotia. Swollen root-knot nematode females were present, but had not ruptured the cortex and epidermis of the root, and no eggs were present.

Five weeks following nematode infection, the sclerotial mass had increased and brown lesions were present on galls around the sclerotia. Nongalled portions of nematode-infected roots were still sound and contained no sclerotia. The first nematode egg masses were visible on the root surface. Lower leaves of the okra plants were chlorotic, and some leaves had dropped prematurely. Both the okra and tomato plants wilted during the day, but regained turgor during the night.

During the 6 weeks after nematode invasion, permanent wilting and leaf drop occurred in okra. Only the erect main stems remained as compared with the entire plant in the MB treatment (Fig. 2-B). Vascular browning of the main portion of the root and aboveground portions of the stem was also seen (Fig. 2-C). Roots of the plants were severely rotted with the rot extending to nongalled portions of the roots (Fig. 2-D). Isolations from the rotting roots yielded *R. solani,* plus *Thielaviopsis basicola, Fusarium* spp., *Aspergillus* spp., *Alternaria* spp., and *Trichoderma* spp. Fungal isolations from brown vascular tissues within the main tap root yielded pure cultures of *R. solani,* but no fungi were recovered from stems. All okra and tomato plants

Fig. 3-(**A to D**). Host-parasite interactions of *Rhizoctonia solani* and *Meloidogyne incognita*. **A**) Sclerotia (s) firmly attached to the epidermis (e) of a 4-week-old gall; **B**) Barrel-shaped sclerotium cells (s) adjacent to the epidermis (e) of 4-week-old galls, illustrating germination and infection pegs (arrow); **C**) Histological section of 5-week-old root gall showing that hyphae (arrow) entered roots through openings created by the female nematode (n) as she ruptured the cortex and epidermis during egg (eg) production; **D**) Fungus (arrow) penetration illustrating a marked tropic intercellular growth pattern through the cortex toward nematode induced giant cells (gc) located within the vascular cylinder of roots.


gc
A
x
B
x
C
D

growing in nontreated plots were dead approximately 7 weeks following invasion by root-knot nematodes.

Pathogenicity studies.—Greenhouse pathogenicity tests conducted to evaluate *M. incognita* and *R. solani* both alone, and in combination on okra and tomato plants produced the same etiological sequence of events found in the field. *Meloidogyne incognita* predisposed roots to *R. solani,* which resulted in a severe root rot and subsequent plant death. Okra and tomato plants inoculated with either *R. solani* or *M. incognita* alone were free of root decay for the entire 6-week study. Roots of plants inoculated with *M. incognita* were heavily galled, and egg masses were first visible during the fifth week of infection. Three weeks after nematode and fungus inoculation, black sclerotia of *R. solani* were visible on nematode-induced galls, while nongalled portions of the same root system were free of sclerotia. Roots of plants inoculated with *R. solani* alone were free of sclerotium formation. Roots of both okra and tomato grown in soil which received both *M. incognita* and *R. solani* inoculum were heavily galled and were free of root decay up to the fourth week of infection.

Five weeks after inoculation, distinct brown lesions were observed only on the galls of plants inoculated with both *M. incognita* and *R. solani.* The ungalled portions of the same roots were sound. Lower leaves of plants were chlorotic and suffered premature leaf drop. Egg masses on root surfaces were visible for the first time.

Histopathology.—Stained 2-week-old giant cells were filled with dense cytoplasm and possessed numerous swollen nuclei. The nuclei were lobed and contained large amounts of chromatin, much of which was localized in the lobes. Nucleoli were also enlarged. Roots stained with neutral red had fungus growing superficially over the surface of the galls, but histological procedures revealed no evidence of fungal infection.

Sclerotia were superficial on the gall surfaces at 3 weeks, and no infection of the underlying epidermal cells had occurred. The giant cells were similar to 2-week-old giant cells.

Sclerotia were firmly attached to the gall surfaces in sections prepared from 4-week-old galls (Fig. 3-A). Barrel-shaped cells of the sclerotia located within the immediate area of the galled root epidermis had germinated and formed infection pegs (Fig. 3-B). From the infection pegs, the fungus penetrated roots in two ways: intercellularly at the boundary between two epidermal cells and directly into epidermal cells. Inside the root, the fungus gave rise to a primary hypha (3) which continued its growth, primarily intercellularly. A septum was formed a short distance from the tip of the primary hypha. The primary hyphae appeared to function in pushing cells apart during intercellular growth of the fungus, sometimes penetrating cells directly. The intercellular growth of the fungus induced changes in the host in advance of the hyphae which resembled the separation of cell walls by enzymatic action. Giant cells contained numerous lobed nuclei and densely stained

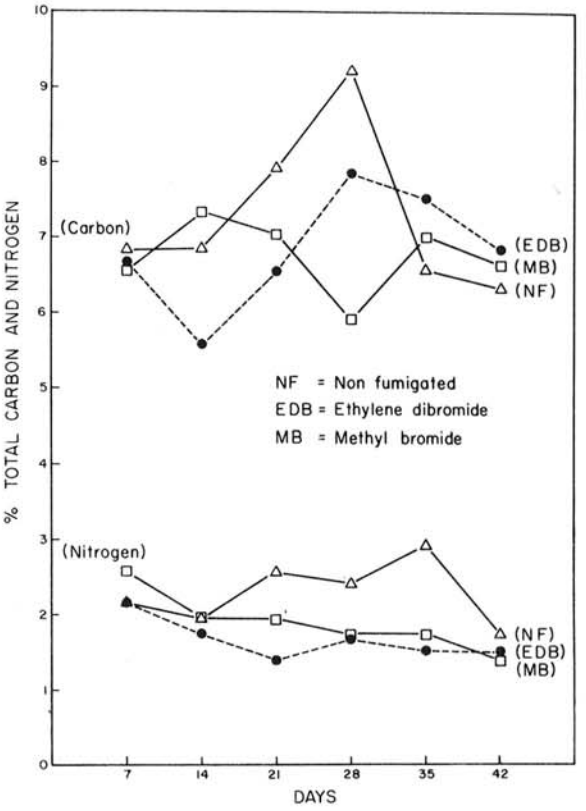


Fig. 5. Carbon and nitrogen levels within okra roots grown in ethylene dibromide treated, methyl bromide treated and nontreated field soil containing root knot nematodes. Roots were analyzed at 7 day intervals for a total of 42 days following nematode infection.

cytoplasm during this period of nematode development. Histological sections prepared from 5-week-old galled roots showed that *R. solani* also entered the roots through the opening created by the swollen female nematode as she ruptured the cortex and epidermis during egg production (Fig. 3-C). The fungus grew rapidly through the cavity between the female and the surrounding cortical cells. *Rhizoctonia solani* that had penetrated galls directly from sclerotia on the surface of galls showed a marked trophic intercellular growth pattern through the cortex of galled roots toward the nematode-induced giant cells located within the vascular cylinder of roots (Fig. 3-D). Root-knot-induced giant cells within these roots did not have an affinity for cytoplasmic stains and the nuclei were reduced to only clumps of nuclear material. Physiological changes appeared in these cells prior to physical contact by the fungus. Presumably the giant cells now served as reservoirs of breakdown products. Many of the cortical cells located within the intercellular path of fungal growth became colonized and filled with fungal hyphae. Sections prepared from nematode-infected roots

Fig. 4-(A to D). Host-parasite interactions of *Rhizoctonia solani* and *Meloidogyne incognita.* **A)** Six-week-old gall showing extensive colonization of giant cells (gc) by the fungus. **B)** Xylem vessels (x) from the tap root of nematode infected plants showing fungus mycelium within them; **C)** Xylem vessels (x) contained a brown mucilage material and numerous tyloses (arrow); **D)** Various stages of sclerotium formation by *R. solani* on the surface of cellophane membranes opposite nematode galls.

during a 5-6-week period following nematode invasion, indicated that *R. solani* had colonized giant cells extensively (Fig. 4-A). The giant cells were completely destroyed by the fungus within 2 or 3 days leaving only fragmentary remains of old giant cell walls and fragments of fungal mycelium. *Rhizoctonia solani* also colonized surrounding xylem elements of the root after the initial attack on the giant cells, and the xylem elements were filled with hyphae. Histological sections of the main tap root with vascular browning symptoms showed the mycelium of *R. solani* within vessels and surrounding vascular cells (Fig. 4-B). Sections prepared from stems with vascular discoloration showed no evidence of fungal mycelium. However, the xylem vessels were filled with a brown mucilaginous material and numerous tyloses (Fig. 4-C). There was no evidence of fungal mycelium in aboveground stem tissues. Giant cells within roots infected with *M. incognita* alone had degenerated, leaving only the remains of a few nuclei.

Prepenetration studies.—*Rhizoctonia solani* formed black sclerotia on the surface of cellophane membranes directly opposite galls induced by *M. incognita,* while ungalled portions of nematode-infected and control roots remained free of sclerotia. Cellophane membranes stained with neutral red showed sclerotia in various stages of formation (Fig. 4-D). Branches of hyphae and chains of monilioid cells formed a cluster on the surface of the cellophane membranes and gave rise to sclerotia directly opposite the galls. Monilioid cells were not formed, either on cellophane membranes opposite ungalled portions of nematode-infected roots, or on membranes opposite control roots.

In studies utilizing the clear plastic root observation boxes containing water agar as the experimental root supporting medium, *R. solani* formed sclerotia on cellophane membranes directly opposite galled roots, while cellophane membranes opposite ungalled portions of these same roots and control roots were free of sclerotia. Sclerotial cell initials (=monilioid cells) were broader and shorter than ordinary hyphal cells and proliferated by irregular branching and interwinding to form loosely constructed, undifferentiated sclerotia. Young sclerotia were first white, then turned darker with maturity. The sclerotia were globose, with irregular raised surfaces.

Carbon and nitrogen analysis.—The total nitrogen content of nematode-infected okra roots growing in nonfumigated soil increased to levels higher than those found in roots of plants growing in EDB- and MB-treated soil 21 days following nematode infection (Fig. 5). Highest levels of nitrogen were present in nematode-infected roots 35 days after infection, but an abrupt drop in nitrogen occurred 42 days after infection. The nitrogen concentration of the plants grown in EDB- and MB-treated plots remained constant during the entire experimental period.

The carbon content of nematode-infected okra roots grown in nonfumigated soil increased to levels higher than those found in roots growing in EDB- and MB-treated soil. Peak levels of carbon occurred in roots 28 days following nematode infection. An abrupt decrease in carbon occurred 35 days following nematode infection. The carbon concentration in nematode-infected roots continued to decrease in the sixth week following nematode infection.

DISCUSSION.—This study focused on the etiology of a fungus root rot in root-knot-nematode-infected okra and tomato roots. This disease complex is widespread in the warm irrigated soils of California, and similar field observations on tobacco have been reported from North Carolina (1). Powell and Batten (9) also found that a variety of secondary plant-invading fungi were implicated in this disease complex, which economically may be the most important fungus-nematode complex. Fortunately, control of this disease complex is accomplished through the control of one component, the root-knot nematode. The most important components of the specific complex involved in this study were the fungus, *R. solani,* and the nematode, *M. incognita.* Roots of plants grown in the field treated with EDB, or in pots of steam-sterilized soil inoculated with *R. solani* alone or *M. incognita* alone were always free of any root decay. Plants in untreated field soil or in sterilized soil inoculated with both organisms, developed a root rot in about 42 days. If the nematode preceded the fungus by 3 weeks, the root rot was more severe and appeared within 14-21 days. Prior to root rot development, *R. solani* demonstrated a marked preference for root galls on nematode infected roots. The fungus sclerotia were formed only on galls. Nongalled portions of nematode-infected roots remained free of initial fungal attack. Similar observations have been reported by Batten and Powell (1). *R. solani* colonization of the nematode-induced giant cells resulted in their destruction. The fungus grew into surrounding xylem elements, which caused them to become plugged with tyloses. Plants infected with both pathogens wilted severely followed by complete defoliation, severe root rot, and plant death.

Rhizoctonia solani responded to stimuli which originated from *M. incognita*-infected roots and passed through semipermeable cellophane membranes, by forming sclerotia on the surface of the membranes directly opposite galls. The results of these studies also suggested that the mechanism by which nematode attack predisposed roots to secondary invasion by *R. solani* was an indirect one. It is hypothesized that the leakage of nutrients from the root was responsible for attracting the fungus to the galls, and for initiating sclerotium formation.

Chemical analysis of roots during the course of the disease did not reveal any major shifts in C/N ratios in root tissues, except at 35 days. The levels of both carbon and nitrogen, were higher in nematode-infected roots than in control roots during the early stages of development of the nematode.

LITERATURE CITED

1. BATTEN, C. K., and N. T. POWELL. 1971. The Rhizoctonia-Meloidogyne disease complex in flue-cured tobacco. J. Nematol. 3:164-169.
2. BLACK, C. A. (ed.) 1965. Pages 1171-1175 *in* Methods of soil analysis, Part 2. American Society of Agronomy, Madison, Wisconsin.
3. CHRISTOU, T. 1962. Penetration and host-parasite relationships of Rhizoctonia solani in the bean plant. Phytopathology 52:381-389.

4. CLAYTON, E. E., T. W. GRAHAM, F. A. TODD, J. G. GAINES, and F. A. CLARK. 1958. Resistance to the root-knot disease of tobacco. Tob. Sci. 2:53-63.
5. HOAGLAND, D. R., and D. I. ARNON. 1950. The water-culture method for growing plants without soil. Calif. Agric. Exp. Stn. Circ. 347. 32 p.
6. KERR, A. 1956. Some interactions between plant roots and pathogenic soil fungi. Aust. J. Biol. Sci. 9:45-52.
7. MELENDEZ, P. L., and N. T. POWELL. 1970. The Pythium-root knot nematode complex in flue-cured tobacco. Phytopathology 60:1303 (Abstr.).
8. MELENDEZ, P. L., and N. T. POWELL. 1970. Histological studies of the Pythium-root knot nematode complex in tobacco. Phytopathology 60:1303 (Abstr.).
9. POWELL, N. T., and C. K. BATTEN. 1969. Complexes in tobacco involving Meloidogyne incognita, Fusarium oxysporum f. sp. nicotianae, and Alternaria tenuis. Phytopathology 59:1044 (Abstr.).
10. POWELL, N. T., P. L. MELENDEZ, and C. K. BATTEN. 1971. Disease complexes in tobacco involving Meloidogyne incognita and certain soil-borne fungi. Phytopathology 61:1332-1337.
11. RAWLINS, T. E. 1933. Phytopathological and botanical research methods. John Wiley & Sons, New York. 156 p.
12. STEINER, G. 1942. Plant nematodes the growers should know. Proc. Soil Sci. Soc., Florida IV-B:72-117.