# Disease Complexes in Tobacco Involving Meloidogyne incognita and Certain Soil-Borne Fungi

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ABSTRACT

Flue-cured tobacco cultivar C 316, susceptible to the root knot nematode *(Meloidogyne incognita)*, was tested in combined inoculations with *M. incognita* and species of the soil-inhabiting fungi *Pythium, Curvularia, Botrytis, Aspergillus, Penicillium,* and *Trichoderma*. Treatments included inoculation with each microorganism alone, as well as inoculation with the fungi separately several weeks after inoculation with *M. incognita*. Roots showed symptoms of necrosis when subjected to *M. incognita* in combination with any one of the fungi. Necrosis was especially severe in treatments in which nematodes preceded the fungi by several weeks. None of the fungi induced disease unless *M. incognita* was present. These observations stress the importance of disease complexes in root breakdown, and emphasize the dominant role of *M. incognita* as a predisposing agent.

*Additional key words: Nicotiana tabacum.*

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There have been a number of reports dealing with complexes involving more than one pathogen on a host plant (7, 8, 10, 13). Generally, such research has been concerned with the simultaneous activity of two organisms, and plant parasitic nematodes are frequently included as one component of the interaction. Nematodes of the root knot group (*Meloidogyne* spp.) appear to be involved more often and to a greater extent than other nematodes. Several investigators have suggested that, in tobacco, this may be due to the unique effect of root knot nematodes on the host; i.e., gall formation and accompanying morphological and physiological changes in the root (3, 5, 12).

Most of the studies of disease complexes in tobacco in which *M. incognita* is the primary pathogen involve other organisms which, by themselves, are capable of behaving as primary pathogens in susceptible crops. Interactions in such cases are marked by increased disease incidence and more rapid disease development when the two pathogens occur together than when either occurs alone. However, neither pathogen is dependent upon the other for successful infection. Often, infection by *M. incognita* specifically predisposes cultivars resistant to a second pathogen so that the secondary infection becomes dominant. The black shank-root knot and *Fusarium* wilt-root knot complexes in tobacco provide examples of such interactions (9, 12).

The recognition that nematode activity has a profound influence upon the development of diseases due to other types of pathogens renews speculation about their importance when they occur along with microorganisms which cause little damage when they are present alone with a given crop. Some early investigators observed that nonspecific superinfections of root knot-infected roots by decay-producing organisms are common and change the clinical picture (1, 14). Such infections greatly increase the amount of damage. These workers presumably regarded necrosis as a part of the root knot syndrome, perhaps even the terminal phase of a severe root knot infection, because the nematode was the essential and decisive factor in the etiology of the disease. However, when the obligate nature of the root knot nematode is considered, along with the delicately balanced relationship established between host and pathogen, extensive necrosis would not seem to be a part of the disease syndrome. Conceivably, much of the damage observed in root knot nematode infections may be due, in fact, to complexes involving nematodes and other soil-inhabiting microorganisms. These latter components, apparently innocuous unless plants have been effectively predisposed, have not received appropriate attention in phytopathological research.

Recent research with *Pythium ultimum* and *Rhizoctonia solani* in tobacco has indicated the importance of such organisms (6, 11). Neither of these fungi is important on tobacco, especially after plants have progressed beyond juvenile stages, unless root knot nematodes are present. In galled roots, necrosis due to either fungus is extensive, resulting in yellowing and stunting of aboveground plant parts. Mayol & Bergeson (4) have studied similar relationships involving bacteria and root knot nematodes.

The research reported herein was designed to determine whether certain soil-inhabiting fungi, relatively unimportant in tobacco when considered alone, are involved in complexes with root knot nematodes. The fungi tested included *P. ultimum* Trow, which causes seedling damping-off disease of young tobacco transplants, but is unimportant on tobacco after plants progress beyond juvenile stages; *Curvularia trifolii* (Kauff.) Boed.; *Botrytis cinerea* Pers. ex Fries; *Aspergillus ochraceus* Wilhelm; and *Penicillium martensii* Biourge, none of which has been reported as a pathogen of tobacco; and *Trichoderma harzianum* Rifai, which is rarely considered as a pathogenic organism. A preliminary report involving *M. incognita, P. ultimum,* and *T. harzianum* has been published (6).

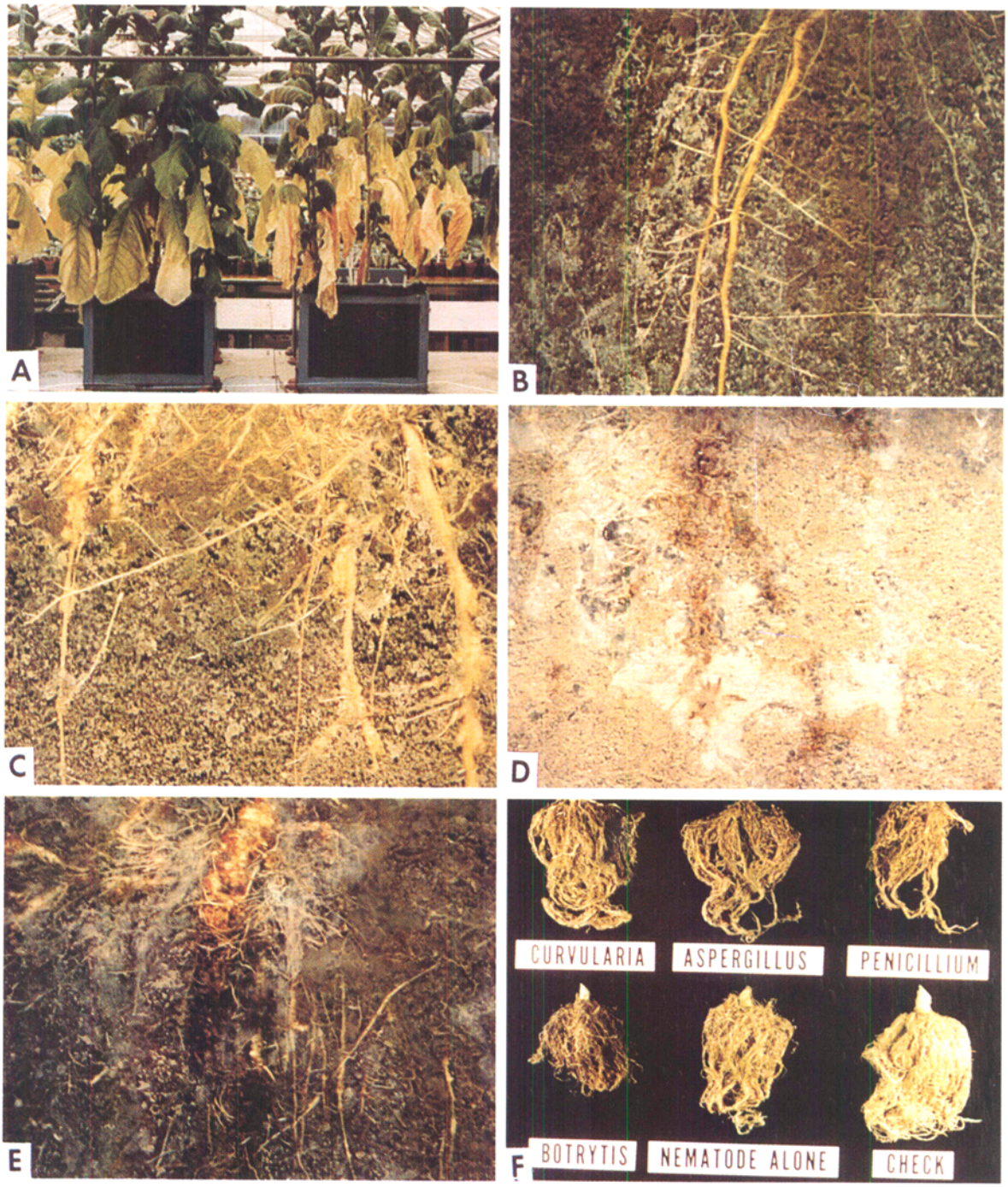


**Fig. 1.** A) Tobacco plants growing in observation boxes equipped with glass sides. **B)** Roots of noninoculated control plants of C 316 tobacco cultivar growing along glass sides of observation boxes. No necrosis developed in these roots. **C)** Roots of C 316 tobacco cultivar in observation boxes 50 days after inoculation with *Meloidogyne incognita*. Galling is extensive, but decay is lacking. **D)** Roots of C 316 tobacco cultivar in observation boxes 50 days after inoculation with *Trichoderma harzianum*. The fungus was added 4 weeks after inoculation with *M. incognita*. Extensive necrosis is evident. **E)** Roots of C 316 tobacco cultivar in observation boxes 50 days after inoculation with *Pythium ultimum*. The fungus was added 4 weeks after inoculation with *M. incognita*. Note necrosis. **F)** Roots of tobacco cultivar C 316 24 days after inoculation with various fungi. Fungal inoculum was applied 4 weeks after inoculation with *M. incognita*. Note decay of roots inoculated with any one of the fungi as compared to those exposed to the nematode alone and to the noninoculated check.

MATERIALS AND METHODS. – Experiments using *Pythium* and *Trichoderma* were run separately from those involving other fungi, and methodology was slightly different. The two types of experiments conducted are described below.

*Meloidogyne incognita with P. ultimum or T. harzianum*. – Tobacco, Coker 316, susceptible to *M. incognita*, was used as the test plant. Seedlings were grown to transplant size in 5-cm plastic pots and transferred, with soil intact, to wooden observation boxes (ca. 52 x 42 x 20 cm). Three seedlings were planted in each box. Boxes were equipped with glass sides to permit observation of disease development during the course of the experiments (Fig. 1-A). The glass was covered with black plastic except when observations were being made. Each box contained a steam-treated 2:1 soil-sand mixture, and at transplanting, 3 open-end glass inoculation tubes were placed equidistantly around each plant, with one end in contact with the plant roots. All inoculations were made through the tubes.

The experiments included the following inoculation treatments: *M. incognita* alone; *P. ultimum* alone; *T. harzianum* alone; *P. ultimum* 4 weeks after inoculation with *M. incognita; T. harzianum* 4 weeks after inoculation with *M. incognita*, and noninoculated control plants.

Populations of *M. incognita* used as inoculum were maintained on Rutgers tomato plants. At the time of nematode inoculation, egg masses were individually removed from tomato roots, and a total of 12 egg masses was added to each plant by pouring four of them through each inoculation tube. The inner walls of the tubes were rinsed with distilled water to insure that the inoculum reached the roots at the bottom of the tube.

The isolate of *P. ultimum* used was obtained from tobacco roots in North Carolina. The isolate of *T. harzianum*, identified by C. S. Hodges, Jr., Forestry Sciences Lab., Research Triangle Park, N.C., was taken from galled roots of root knot-infected tobacco plants grown in the greenhouse. For inoculum, the fungi were grown separately for 6 days on potato-dextrose agar in petri dishes. Inoculum suspensions of the separate fungi were prepared by blending the contents of one petri dish in 100 ml distilled water in a Waring Blendor for 5 sec. Plants were inoculated with 60 ml of inoculum suspension by dispensing 20 ml through each of three inoculation tubes.

Roots were observed for development of necrosis during the course of the experiments. Tests were terminated 50 days after fungal inoculations. Plant tops were discarded, roots were washed free of soil, and the extent of necrosis was recorded using the following classification system: class 0 = no necrosis; 1 = less than 10% of root system necrotic; 2 = 11-25% necrotic; 3 = 26-50% necrotic; 4=51-75% necrotic; 5 = 76-100% necrotic. Each root system was assigned one of the classes, and a disease index for each treatment was calculated using the following formula: Disease index =

$$\frac{\left[\begin{smallmatrix}\text{no. plants}\\ \text{in class } 1 \times 1\end{smallmatrix}\right] + \left[\begin{smallmatrix}\text{no. plants}\\ \text{in class } 2 \times 2\end{smallmatrix}\right] + \cdots \left[\begin{smallmatrix}\text{no. plants}\\ \text{in class } 5 \times 5\end{smallmatrix}\right]}{\text{Total no. plants in treatment} \times 5} \times 100.$$

This disease index is used in data presentation to describe relative necrosis development in the various treatments. Data were subjected to statistical analysis.

*Meloidogyne incognita with C. trifolii, B. cinerea, A. ochraceus, or Penicillium martensii*.–Plants of tobacco Coker 316 were grown to transplant size in 5-cm plastic pots and transferred, with soil intact, to 15-cm clay pots containing a methyl bromide-treated 3:1 soil-sand mixture. Inoculation tubes were placed around each plant as previously described. The following inoculation treatments were applied: *M. incognita* alone; *C. trifolii* 4 weeks after inoculation with *M. incognita; B. cinerea* 4 weeks after *M. incognita; A. ochraceus* 4 weeks after *M. incognita; P. martensii* 4 weeks after *M. incognita;* and noninoculated plants. Preliminary experiments had revealed no disease when nematodes were absent; therefore, these treatments were omitted.

Egg masses of *M. incognita* for inoculum were produced and applied through the inoculation tubes as described for the previous experiment. Isolates of *P. martensii, C. trifolii,* and *A. ochraceus* were obtained from galled root segments of tobacco plants infected with *M. incognita*. *Penicillium martensii* was identified by Dorothy Fennell, ARS Northern Research Laboratory, Peoria, Ill. The isolate of *B. cinerea* was obtained from Robert Aycock, Department of Plant Pathology, N.C. State University, Raleigh. Techniques used in fungus culture, inoculum preparation, inoculation, statistical analysis, and disease indexing were the same as those previously described for *P. ultimum* and *T. harzianum*. Plants were observed for aerial symptoms such as wilting and chlorosis during the course of the experiments. The study was terminated 24 days after fungal inoculation.

RESULTS.–*Meloidogyne incognita with P. ultimum or T. harzianum*.–Plants growing in observation boxes were observed for below- and aboveground symptoms throughout the experiments. Noninoculated control plants remained apparently healthy, and no symptoms on aboveground plant parts were observed in plants inoculated with either *P. ultimum* or *T. harzianum* alone. Plants receiving only *M. incognita* were slightly stunted, but leaves remained green.

Plants, inoculated with either *P. ultimum* or *T. harzianum* after root knot nematodes, developed symptoms aboveground 4 weeks after fungus inoculation. Plants were stunted and became chlorotic; leaves and internodes were reduced in size when compared to noninoculated plants. These symptoms were followed by permanent wilting of the leaves. Those plants exposed to *T. harzianum* after nematodes developed more severe symptoms than those receiving *P. ultimum*. In fact, some plants

inoculated with *T. harzianum* were almost dead when the test terminated.

No evidence of root damage was observed through the glass sides of the observation boxes among control plants (Fig. 1-B) or among plants inoculated with either *P. ultimum* or *T. harzianum* alone. No necrosis developed in roots of plants in these treatments. Roots which received only *M. incognita* were galled and developed slight necrosis as the experiment neared termination (Fig. 1-C). Plants in this treatment received a root disease index of 13 (Table 1).

The first necrotic symptoms were seen in roots of plants jointly inoculated with both *M. incognita* and *P. ultimum* 24 days after inoculation with the fungus. However, roots inoculated with *M. incognita* and *T. harzianum* decayed faster than those exposed to nematodes and *P. ultimum*. Among plants receiving the former treatment, all roots observable along the glass sides of the observation boxes were severely decayed 18 days after detection of the first necrotic symptom. The highest index, 71, was recorded in roots inoculated with *M. incognita* and *T. harzianum* (Table 1; Fig. 1-D). An index of 57 was assigned to those plants receiving the nematodes and *P. ultimum* (Table 1; Fig. 1-E).

*Meloidogyne incognita with C. trifolii, B. cinerea, A. ochraceus, or P. martensii.*—Some of the plants receiving only *M. incognita* developed slight root necrosis, resulting in an average disease index of 5. Plants inoculated with either *B. cinerea* or *P. martensii* after *M. incognita* developed chlorotic symptoms aboveground 2 weeks after fungus inoculation. In addition, these plants were stunted and leaves became wilted as the tests approached termination. Plants receiving *C. trifolii* or *A. ochraceus* after root knot nematodes became chlorotic, but suffered little reduction in growth.

Root symptoms of all plants jointly inoculated with *M. incognita* and either of the fungi tested were severely necrotic when experiments were terminated (Fig. 1-F). Roots receiving *B. cinerea* and *P. martensii* were damaged more than those exposed to other treatments, and were assigned average disease indexes of 75 and 65, respectively. Inoculation with *C. trifolii* and *A. ochraceus* after *M. incognita* resulted in disease indexes of 60 and 50, respectively (Table 2).

DISCUSSION.—We are becoming increasingly convinced that much damage to plants observed in nematode infections is due to disease complexes. This is certainly true in situations involving root knot nematodes in tobacco. This conclusion does not tend to lessen the economic significance of plant parasitic nematodes as plant pathogens; rather, in our view, it strengthens and emphasizes their importance. It does appear, however, that root knot nematode damage to tobacco reaches its zenith only when these pathogens are components of complexes.

TABLE 1. Root necrosis in tobacco cultivar C 316 50 days after various inoculation treatments with *Meloidogyne incognita, Pythium ultimum*, and/or *Trichoderma harzianum*

| Inoculation treatments | Avg disease index |
|---|---|
| *M. incognita* alone | 13 |
| *P. ultimum* alone | 0 |
| *T. harzianum* alone | 0 |
| *P. ultimum* 4 wk after *M. incognita* | 57 |
| *T. harzianum* 4 wk after *M. incognita* | 71 |
| Noninoculated control | 0 |

LSD .05 = 0.55. .01 = 0.76

TABLE 2. Root necrosis in tobacco cultivar C 316 24 days after inoculation with *Meloidogyne incognita* alone, and preceding inoculation with *Curvularia trifolii, Botrytis cinerea, Aspergillus ochraceus*, or *Pencillium martensii*

| Inoculation treatments | Avg disease index |
|---|---|
| *M. incognita* alone | 5 |
| *C. trifolii* 4 wk after *M. incognita* | 60 |
| *B. cinerea* 4 wk after *M. incognita* | 75 |
| *A. ochraceus* 4 wk after *M. incognita* | 50 |
| *P. martensii* 4 wk after *M. incognita* | 65 |
| Noninoculated control | 0 |

LSD .05 = 1.6. .01 = 2.2

Experiments including *M. incognita* with fungi which are not regarded as pathogens on tobacco further substantiate the importance of nematodes in disease complexes. *Curvularia trifolii, B. cinerea, A. ochraceus*, and *P. martensii* are not "tobacco pathogens" in the typical sense of this term. Furthermore, *T. harzianum* normally has no pathogenic capacity on tobacco. However, if a tobacco root system has been previously colonized by root knot nematodes, each of these fungi can invade this altered root system and induce extensive necrosis. In fact, damage by *T. harzianum* is as great under these conditions as that by a recognized pathogenic fungus such as *P. ultimum*.

These observations lead to the conclusion that root knot nematode root infections on certain hosts effectively predispose these roots to subsequent invasion by a range of other microorganisms present in the rhizosphere. Our results suggest that this is true regardless of whether the subsequent invader is capable of attacking the host under other conditions. The recent work of Mayol & Bergeson (4), emphasizing bacteria, strengthens this possibility.

The present study involves experiments with known combinations. Other organisms were not excluded after initial inoculations were made, but the treatment combinations and checks gave sound evidence of profound associative effects between the root knot nematode and certain fungi. This relates well to the discussion by Gäumann (2), who stated that in some plant diseases, "The primary pathogen not only breaks down the host's resistance to penetration but also its resistance to spread, thus making possible for the secondary parasites not only entry but also affording them, by a local change of substrate, a start for their further extension". Thus, the nematode would be regarded as a primary pathogen which predisposes the host to super-

infection by nonspecific secondary pathogens which greatly modify the disease syndrome.

Interactions focus attention upon basic aspects and terminology of disease etiology and diagnosis. According to the traditional doctrine of specific etiology, a single factor or agent is proved to be the determinant in disease causality by the application of certain prescribed requirements or standards. If the causal agent is an infectious microorganism, it must have the inherent, specific, and independent capability of producing the disease in question. This doctrine was one of the milestones in the advancement of the study of disease, and is still one of the most important principles of plant pathology. Even so, it has been known for many years that this doctrine does not provide a complete account of disease causation in all cases.

An understanding of the doctrine of predisposition has contributed to a better understanding of the problem. However, there may be a tendency to go to such extremes as to hold that disease states are the outcome of a constellation of circumstances and are rarely, if ever, the direct result of single determinant factors. Obviously, this concept does not hold true for all disease situations.

The role of secondary pathogens in disease complexes merits intensive study. Accurate disease diagnosis is obviously complicated where interactions are present, and a tendency to ascribe every particular disease syndrome to a single pathogen should be avoided. It is possible that failure of disease control practices in certain situations could be due to incomplete diagnosis of disease complexes, resulting in incomplete or inappropriate control treatment. It would seem unwise to give primary emphasis to root diseases of plants caused by a single pathogen, when under natural growing conditions, this is indeed rare.

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