The *Rhizoctonia-Meloidogyne* Disease Complex in Flue-cured Tobacco[1]

COLIN K. BATTEN AND N. T. POWELL[2]

Abstract: If *Meloidogyne incognita* preceded *Rhizoctonia solani* by 10 days or 21 days in roots of greenhouse-grown tobacco plants, root rot was more extensive than when the nematode and fungus were introduced either simultaneously or separately or when *R. solani* was added after artificial wounding. Histological examination of galled roots 72 days after inoculation with *R. solani* revealed extensive fungal colonization in the root-knot susceptible cultivar 'Dixie Bright 101' when *M. incognita* preceded *R. solani* by 21 days. *R. solani*, normally nonpathogenic on mature tobacco roots, may cause severe losses when present with well-established root-knot nematode infections. *Key Words:* Interaction, Predisposition, *Meloidogyne incognita*, *Rhizoctonia solani*, Root-knot, Histology.

In disease complexes the combination of pathogens may cause economic losses exceeding those caused by them individually (3, 7, 8, 9, 10, 11, 13). Reynolds and Hanson (12) noted that increased damping-off of cotton caused by *Rhizoctonia solani* (Kühn) was correlated with increased root-knot nematode population. Taylor and Wyllie (14) reported an association of either *Meloidogyne javanica* or *M. hapla* and *R. solani* reduced soybean emergence. Brodie and Cooper (1) showed that susceptibility of cotton seedlings to *R. solani* continued longer in plants infected with *Rotylenchulus reniformis*, *M. arenaria*, *M. hapla*, or *M. incognita*.

R. solani is commonly found in tobacco field soils. Damage to tobacco from this fungus generally appears as damping-off and bed-rotting of seedlings or sporadic "soreshin" of seedlings and older plants. Economic damage by *R. solani* in mature tobacco has not been demonstrated. In preliminary experiments, however, we isolated it from field-grown plants infected with *Meloidogyne* spp. Powell (9) reported that other soil-inhabiting, facultative parasitic fungi (*Trichoderma* sp., *Curvularia* sp., *Botrytis* sp., *Penicillium* sp., *Aspergillus* sp., and *Pythium* sp.) also cause severe decay in *M. incognita*-infected tobacco roots.

The objectives of the study reported here were to determine: (i) the influence of *M. incognita* on root decay by *R. solani* in flue-cured tobacco; (ii) the length of time after nematode invasion necessary for plants to be predisposed; and (iii) histologically the degree of colonization of root tissues by the fungus. No attempt was made to study the infection process nor to relate rate of fungal colonization to time following inoculation. A preliminary abstract has been published (10).

MATERIALS AND METHODS

The tobacco (*Nicotiana tabacum* L.) cultivars included in these greenhouse studies were 'Dixie Bright 101', 'Coker 316' (both susceptible to *M. incognita*) and 'N. C. 95', resistant to *M. incognita*. These cultivars are not known to differ in reaction to *R. solani*.

Uniform 4-week-old seedlings, approximately 5 cm high, were transplanted into sterilized plastic thumbpots containing a methyl bromide-fumigated, fertilized, 3:1 soil-sand mixture. After 1 week, seedlings

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[2] Department of Plant Pathology, North Carolina State University, Raleigh, N. C. 27607. The authors gratefully acknowledge the technical assistance of Mr. Larry Carter and the help of typists, Mrs. Josephine Crenshaw and Mrs. Joyce Johnson.

were transplanted into sterilized 15-cm clay pots containing a similar soil mixture. At transplanting, three open-end glass inoculation tubes (15 × 2 cm), previously sterilized in a 10% Clorox solution, were placed equidistantly around the periphery of each pot and arranged so that the end of each inoculation tube was in contact with the roots of the plants. Loose soil was added and compacted to secure the plant roots and tubes in place.

Nematode inoculum was produced and maintained on 'Homestead' tomato plants. Egg masses, removed individually from 6-week-old tomato cultures, were placed in water in small vials. Single vials, each containing four egg masses, were emptied into each of the three inoculation tubes. Thus, each nematode-inoculated plant was exposed to the larval hatch from 12 egg masses.

The culture of *R. solani* used in preliminary experiments (Isolate No. 289, 'Practicola type'), was isolated from cotton hypocotyls in North Carolina by Dr. R. T. Sherwood of the Department of Plant Pathology, North Carolina State University, Raleigh. *R. solani* cultures isolated during these preliminary tests were used as fungal inoculum in subsequent tests. The fungus was cultured on potato-dextrose agar (PDA) in 9-cm petri plates for 5 days at 25 C. To prepare inoculum, the entire contents of each plate were blended 30 sec with 200 ml of distilled water in a rotary blender. Each plant was inoculated by pouring 20 ml of the mycelial suspension down each of the three inoculation tubes. Four plants of each cultivar received treatments as follows: (i) *R. solani* 21 days after *M. incognita*; (ii) *R. solani* 7 days after *M. incognita*; (iii) *M. incognita* and *R. solani* simultaneously; (iv) *M. incognita* alone; (v) *R. solani* alone; and (vi) *M. incognita* and *R. solani* simultaneously after mechanical wounding. Noninoculated control plants were not included; however, *M. incognita*-resistant 'N. C. 95' which failed to develop significant necrosis in any treatment was used to measure treatment effects in *M. incognita*-susceptible cultivars.

The experiment was terminated 72 days after fungal inoculation. After isolations were made, roots were thoroughly washed and samples were fixed in formalin-alcohol-acetic acid (FAA), aspirated 30 min at 20 psi, dehydrated in a tertiary butyl alcohol series (2), and embedded in Fisher's Tissuemat (melting point 56 C). All samples were sectioned 10 mμ thick with a rotary microtome, mounted, and stained with safranin-fast green.

Preliminary experiments showed that *R. solani* colonized root tissues when *M. incognita* preceded the fungus by 3 weeks. To determine more precisely the time required for nematodes to predispose roots to *R. solani*, treatments (ii) and (vi) in subsequent tests were adjusted as follows: *R. solani* was added 10 days rather than 1 week after *M. incognita* and *R. solani* alone rather than with *M. incognita* was introduced following artificial wounding. The latter treatment was used to determine the importance of mechanical puncturing effects of nematode penetration on predisposition to the fungus. Four plants of each of the three cultivars were randomly arranged within each of the six treatments. Each treatment was replicated three times and inoculations were made as previously described. This experiment was terminated 60 days after fungal inoculation. Fresh top weights were determined by cutting the plants at the soil line and weighing.

Roots were thoroughly washed and rated according to six categories of root system necrosis: 0 = none; 1 = less than 10 percent; 2 = 11–25 percent; 3 = 26–50 percent; 4 = 51–75 percent; and 5 = 76–100 percent. Roots were again washed and isolations were made for fungal identification.

TABLE 1. Root necrosis in three flue-cured tobacco cultivars after exposure to various inoculation treatments with *Meloidogyne incognita* and *Rhizoctonia solani* (data were taken 60 days after fungal inoculation)[a].

Treatments	Cultivars[b]		
	'Dixie Bright 101'	'Coker 316'	'N. C. 95'
R. solani added 21 days after *M. incognita*	3.6	4.6	0.8
R. solani added 10 days after *M. incognita*	3.8	3.8	0.8
R. solani and *M. incognita* added simultaneously	2.1	2.2	1.3
M. incognita alone	2.5	1.9	0.3
R. solani alone	1.0	0.9	0.8
R. solani plus artificial wound[c]	0.9	1.0	1.1
LSD .05 for 2 cultivars within a treatment	0.8		
.01	1.1		
LSD .05 for 2 treatments within a cultivar	1.2		
.01	1.7		

[a] Data expressed as disease index (% visibly necrotic) 0 = none; 1 = less than 10%; 2 = 11–25%; 3 = 26–50%; 4 = 51–75%; 5 = 76–100%.
[b] 'Dixie Bright 101' and 'Coker 316'—susceptible to *M. incognita*; 'N. C. 95'—resistant to *M. incognita*.
[c] Roots were wounded by passing a sharpened spatula down the inoculation tubes.

Roots were air dried for 24 hr, then placed in brown paper bags and oven-dried 24 hr at 70 C, removed from the oven and exposed to the laboratory atmosphere for 2 hr to achieve moisture equilibrium and weighed.

RESULTS

R. solani was not isolated from 'N. C. 95' in any treatment group, but was isolated from 'Coker 316' and 'Dixie Bright 101' where *M. incognita* preceded *R. solani* by 10 or 21 days.

Root necrosis and fresh top weights were not significantly different among cultivars 'N. C. 95', 'Coker 316', and 'Dixie Bright 101' in plants inoculated with *R. solani* alone or *R. solani* followed by artificial wounding (Tables 1, 2). Differences in root dry weights among cultivars and treatments were not significant.

M. incognita-resistant 'N. C. 95' had less root necrosis than 'Coker 316' or 'Dixie Bright 101' when the nematode was applied alone (Table 1), although there were no statistical differences in fresh top weights (Table 2). Differences among cultivars when inoculated with nematodes and the fungus simultaneously were slight, although roots of 'Coker 316' and, to a lesser degree, 'Dixie Bright 101' were more necrotic than those of 'N. C. 95' (Table 1). There were no statistical differences in top weights (Table 2).

When *R. solani* followed *M. incognita*, the roots of nematode-susceptible 'Coker 316' and 'Dixie Bright 101' were consistently more necrotic than nematode-resistant 'N. C. 95' (Table 1; Fig. 1). These cultivars usually produced less fresh top weight than 'N. C. 95' (Table 2). Moreover a similar trend was noted when treatments were compared within root-knot susceptible cultivars (Table 2). For example, roots of 'Dixie Bright 101' were more necrotic in treatments

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FIG. 1. (Upper) Comparison of root necrosis in four replicates of the tobacco cultivars 'N. C. 95', 'Coker 316', and 'Dixie Bright 101' in which *Rhizoctonia solani* followed *Meloidogyne incognita* by 21 days (note the necrotic discoloration in *M. incognita*-susceptible 'Coker 316' and 'Dixie Bright 101').

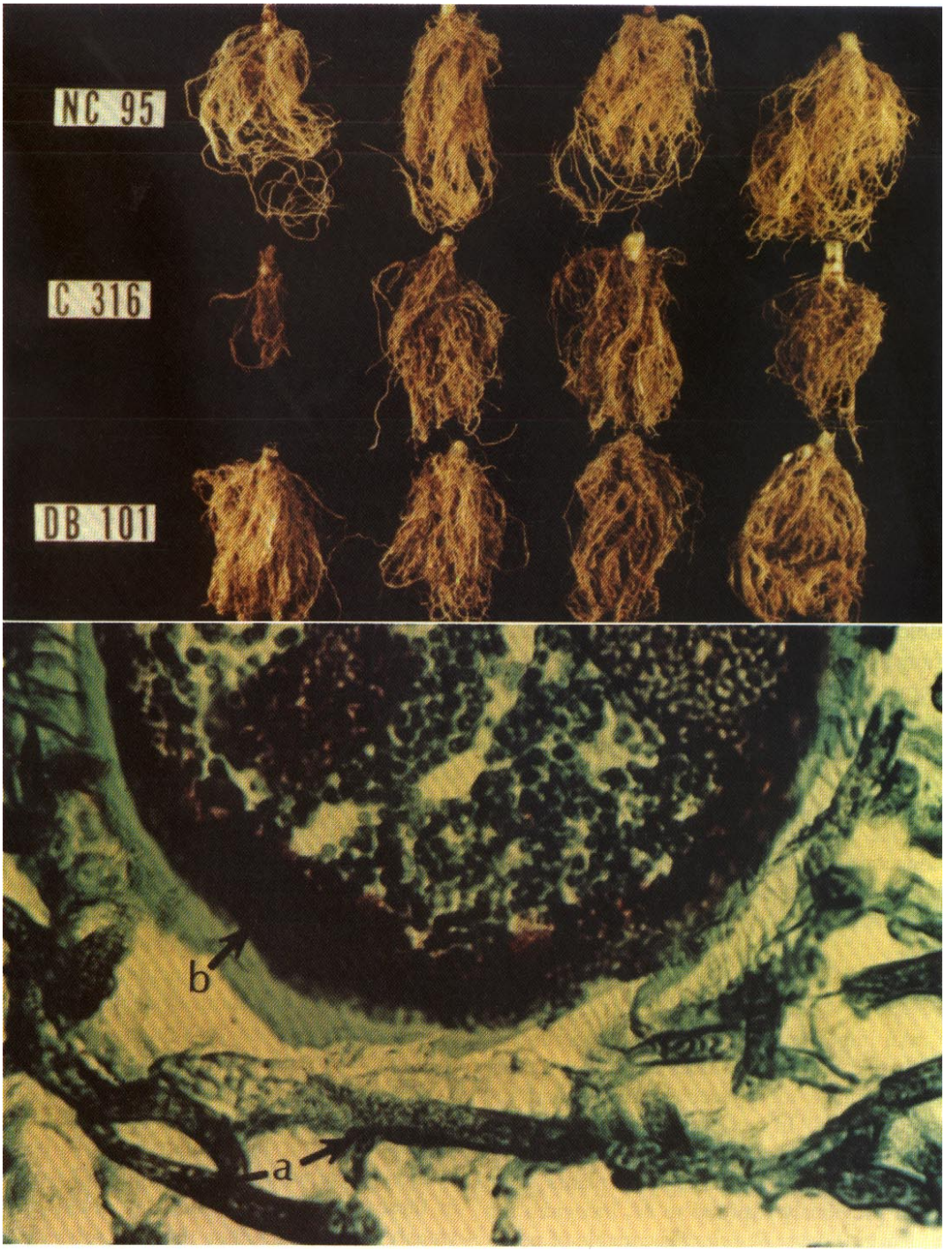

FIG. 2. (Lower) Cross section of a galled root of 'Dixie Bright 101' in which *Rhizoctonia solani* followed *Meloidogyne incognita* by 21 days. *R. solani* hyphae (**a**) and a portion of the female nematode (**b**) are shown (×400).

TABLE 2. Fresh top weights of plants of three flue-cured tobacco cultivars after exposure to various inoculation treatments with *Meloidogyne incognita* and *Rhizoctonia solani* (data taken 60 days after fungal inoculation and is expressed as average weight per plant in grams).

Treatments	Cultivars[a]		
	'Dixie Bright 101'	'Coker 316'	'N. C. 95'
R. solani added 21 days after *M. incognita*	96	74	115
R. solani added 10 days after *M. incognita*	89	97	120
R. solani and *M. incognita* added simultaneously	109	114	117
M. incognita alone	122	126	109
R. solani alone	113	111	99
R. solani plus artificial wound[b]	120	121	94
LSD .05 for 2 cultivars within a treatment	22		
.01	30		
LSD .05 for 2 treatments within a cultivar	25		
.01	34		

[a] 'Dixie Bright 101' and 'Coker 316'—susceptible to *M. incognita*; 'N. C. 95'—resistant to *M. incognita*.
[b] Roots were wounded by passing a sharpened spatula down the inoculation tubes.

where *R. solani* was added 10 days or 21 days after *M. incognita* than in any of the other treatments.

HISTOPATHOLOGY: *M. incognita*-susceptible 'Dixie Bright 101' exhibited extensive fungal colonization in treatments in which nematodes were allowed to become well-established for 21 days before fungal inoculation (Fig. 2). The hyphal strands did not appear to be restricted posteriorly or anteriorly in relation to the nematode. Vigorous hyphae encircled the nematode and were concentrated within the locus of nematode infection. Evidence of fungal invasion was not observed in *M. incognita*-resistant 'N. C. 95'.

DISCUSSION

An important aspect of these experiments is that *R. solani* alone does not inflict significant damage to tobacco plants beyond juvenile stages. In fact, this fungus is evidently unable to colonize healthy tobacco roots. If, however, root-knot susceptible plants have been previously exposed to *M. incognita* for at least 10 days, *R. solani* is capable of penetrating the roots and promoting necrosis. In this way, root-knot nematodes are capable of elevating normally minor pathogens to major status if present in significant numbers (9).

It is pragmatically impossible to speculate as to the number of such interactions operative in nature where countless microorganisms occur in the presence of root-knot nematodes. Powell (8) suggested that nematodes should not be considered only as detached entities in relation to plants, but as members of a complex biotic community. With respect to his suggestion, certain other fungi, ostensibly innocuous on tobacco, have been shown to penetrate and damage roots galled by nematodes (9). Mayol and Bergeson (4) have studied similar effects of soil-borne bacteria in conjunction with *M. incognita*. It is becoming evident that root-knot nematodes cause maximum destruction as components of a disease complex.

Predisposition of plants by *M. incognita* to attack by some microorganisms is most pronounced after the nematodes have been in roots for a period of time—up to 3 weeks in these experiments. This was noted previously with fungi other than *R. solani* (7, 9). This strengthens the belief that physiological changes in galled roots provide much

of the basis for root-knot nematode-fungus interactions. Such modifications must render roots highly favorable for fungal development—even to the extent that roots normally not colonized by a given fungus become susceptible. This does not imply that only galled regions are physiologically altered, but also includes areas removed from the site of nematode activity as indicated by Melendéz and Powell with *Fusarium* (5).

Regardless of the fundamental mechanisms involved, the key to control of interactions of the type dealt with here lies in nematode control. This fact is clearly illuminated in these studies by the virtual absence of root damage in *M. incognita*-resistant 'N. C. 95' in any treatment. It is further emphasized by the general lack of root necrosis in nematode-susceptible cultivars inoculated with *R. solani* only. This stresses the importance of nematode control in crop culture and imparts credence to Pitcher's suggestion that nematodes may be of more economic significance in inconspicuous and debilitating diseases than in the more spectacular and lethal plant diseases (6).

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