# Rhizoctonia Leaf Spot of Flue-Cured Tobacco in North Carolina

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**ABSTRACT**

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During the summer of 1984, a leaf spot disease not previously reported in the United States was observed on flue-cured tobacco throughout the area where the crop is grown in North Carolina. Symptoms began as small, circular, water-soaked spots that rapidly expanded into light green to tan lesions 2–6 cm in diameter with irregular margins. Tissue within the lesions was almost transparent, often displayed a pattern of concentric rings, and frequently dropped out leaving shot holes. Lesions, most common on lower leaves, were observed as high as 16 leaves (about 85 cm) up the stalk. Fungal mycelium was frequently present at the margins of lesions on the undersides of leaves, and occasionally, a hymenial layer and basidiospores of *Thanatephorus cucumeris* were observed. *Rhizoctonia solani* was isolated from these lesions and was shown to be the cause of them. The widespread occurrence and damage caused by the disease was attributed to the unusually cool, wet summer of 1984, which favored basidiospore formation and dissemination.

North Carolina is the leading producer of flue-cured tobacco in the United States. Numerous foliar and soilborne pathogens affect yield and quality of flue-cured tobacco, including the soilborne pathogen *Rhizoctonia solani* Kühn, which causes damping-off and sore shin (8). Sore shin is a root and crown disease that is usually of minor importance even though losses may reach 5–10% in individual fields. The disease is most severe when young plants are wounded at transplanting. The source of inoculum for sore shin is considered to be sclerotia and mycelium associated with plant debris. The only recommended control measure is avoidance of injury to transplants.

During the summer of 1984, numerous specimens of a leaf spot disease were received by the Plant Disease and Insect Clinic (Department of Plant Pathology, North Carolina State University) from counties throughout the region where flue-cured tobacco is grown in North Carolina. The disease was at first confused with brown spot caused by *Alternaria alternata* (Fr.) Keissler; however, *R. solani* was isolated consistently from the diseased tissue. The disease was most common on lower leaves but occurred as high as 16 leaves (about 85 cm) up the plant. Symptoms occurred on >80% of the plants in several fields surveyed, with most leaves having at least one lesion. Loss in yield exceeded 50% in one field. A similar, unreported leaf spot of flue-cured tobacco caused by *R. solani* had been observed previously on clinic specimens in North Carolina (R. K. Jones, *personal communication*). However, the severity and widespread distribution of this new disease plus the presence of the perfect stage of the pathogen in 1984 were unique.

*R. solani* (perfect stage *Thanatephorus cucumeris* (Frank) Donk) causes foliar blights of numerous crops (2,5,7,10) including tobacco (8,14). However, no previous report of this disease on flue-cured tobacco in the United States was found. This report describes the new foliar blight and the causal agent.

## MATERIALS AND METHODS

Tobacco leaves with characteristic lesions were collected in the field or obtained from specimens submitted to the Plant Disease and Insect Clinic, North Carolina State University, Raleigh. Leaf disks 3–9 mm in diameter were cut from the margins of lesions, surface-sterilized in 0.5% NaOCl for 30 sec, rinsed in sterile distilled water, and plated on 2% water agar (WA) and acidified potato-dextrose agar (APDA). After 24–48 hr of incubation at room temperature, hyphal tip transfers were made to fresh WA and APDA plates from colonies showing characteristics of *R. solani* (4). Cultures were maintained on PDA at room temperature (22–25 C). Ten isolates for study were obtained from five counties. All isolates were examined for presence of dolipore septa and multinucleate cells after staining hyphal tip and monilioid cells with a 0.05% solution of aniline blue in lactophenol (3). Anastomosis grouping of isolates was determined by the procedure of Parmeter et al (11). Tester isolates were obtained from S. B. Martin (Department of Plant Pathology, North Carolina State University). Mycelial growth rate was determined on Difco PDA at temperatures between 16 and 36 C. Tests consisted of three culture plates per isolate at each temperature, and the experiment was conducted twice. To induce the perfect stage of the foliar *R. solani* isolates, three methods were used: a nutrient step-down procedure wherein isolates were grown for 1 wk on PDA, then transferred to WA; a soil overlay procedure in which moist, pasteurized, sandy loam soil was placed over 1-wk-old nutrient agar cultures; and placement of rice grains colonized by *R. solani* into a mixture of equal parts of sand, soil, and peat in 10-cm-diameter clay pots, each containing a 6-wk-old seedling of the flue-cured tobacco cultivar Hicks.

Pathogenicity of the foliar isolates of *R. solani* to tobacco was tested with seedlings and detached leaves of Hicks tobacco. Partially expanded leaves were detached from greenhouse plants and placed in plastic moisture chambers containing moistened paper towels. Leaves were inoculated with either a hyphal fragment suspension or pasteurized soil infested with *R. solani*. The hyphal fragment suspension was obtained by blending 2-wk-old mycelial mats produced in 25 ml of potato-dextrose broth at room temperature. Soil inoculum was produced using the method of Ko and Hora (6). Undiluted, infested soil was placed in 1-g portions on leaves, which were checked daily for development of water-soaking and further lesion development. Tobacco seedlings and seed pots were inoculated in a greenhouse at 25–35 C with basidiospores of *T. cucumeris* that formed on the undersides of tobacco leaves (Fig. 1). Inoculated plants and seed pots were maintained in the greenhouse, watered regularly, and observed for leaf lesions daily. Reisolation of *R. solani* from inoculated leaves and seedlings was conducted on APDA and WA as described previously.

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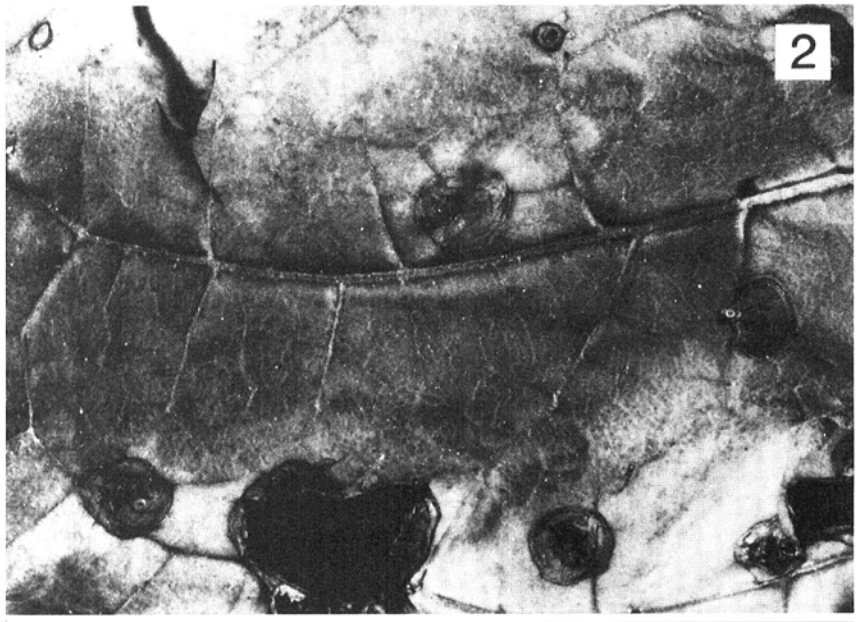



**Figs. 1–3.** Signs and symptoms of leaf spot of flue-cured tobacco caused by *Rhizoctonia solani*. **(1)** Hymenial layer on the underside of a Hicks tobacco leaf grown in the greenhouse. About 0.5 g of rice grains colonized by *R. solani* had been placed in the soil 4 cm from the base of the plant 5 days before photography. **(2)** Stages in symptom development ranging from small spots to shot-hole lesions. **(3)** Severe infection of flue-cured tobacco in the field.

## RESULTS

Symptoms in the field and greenhouse began as small, circular, water-soaked spots. Under very humid conditions, lesions enlarged rapidly, becoming transparent and light green with irregular margins. In less humid conditions, lesions expanded less rapidly and often developed a pattern of concentric rings and finally a shot-hole effect as the dead tissue dropped out (Figs. 2 and 3). A chlorotic halo was often present around lesions. Mycelium of *R. solani* was often present at the margins of lesions on the undersides of leaves, and occasionally, a hymenial layer also was observed. Inoculated plants developed symptoms similar to those of field-infected plants. Only 10–20% of inoculations with mycelial fragments or infested soil resulted in symptoms, whereas inoculations with basidiospores caused infection of all inoculated tobacco leaves. The isolates also caused postemergence damping-off and minor stem lesions on tobacco in addition to the leaf spot symptoms. The fungus was reisolated from all symptomatic tissue (34 of 34 attempts).

All isolates were similar in morphology, had light brown mycelium, and produced few or no sclerotia on PDA. Isolates produced sclerotia (2–8 mm in diameter) when incubated on WA and V-8 juice agar plates for 3 wk at laboratory temperature and when colonized rice grains were placed on WA, V-8, or PDA media. Growth rates were similar for all isolates, which averaged 11 mm of radial growth per day in the optimum temperature range of 24–28 C. Radial growth was 7, 9, 7, and 0.4 mm per day at 16, 20, 32, and 36 C, respectively. Only three of the 10 isolates were consistently positive in anastomosis tests with tester isolates, and all three isolates had an affinity with the AG 2 type 2 isolate. Attempts to induce the perfect stage of each of the 10 isolates were successful when colonized rice grains were placed in soil. All isolates were *T. cucumeris*. The soil overlay and nutrient step-down procedures were not successful.

## DISCUSSION

This is the first report of a leaf spot disease of flue-cured tobacco induced by *R. solani* in the United States. Symptoms are similar to the disease caused by *R. solani* on tobacco in Brazil (8) and Costa Rica (14). Symptoms of the disease can be confused with those of brown spot of tobacco caused by *A. alternata* in some specimens. Both diseases are characterized by a pattern of concentric rings and halos around the lesions.

The nature and source of primary inoculum are not known; secondary inoculum is thought to consist of basidiospores. Sclerotia and colonized debris are reported to be the source of initial inoculum for many *Rhizoctonia* diseases. The severity of the disease in

several fields and the widespread occurrence of the disease throughout the eastern half of the state is thought to be the result of a favorable environment (unusually wet and cool) that allowed production and dispersal of the basidiospores. The high incidence of disease, progression of symptoms from lower to upper leaves, and occurrence of a hymenial layer on leaf spots support this hypothesis. On the basis of the widespread occurrence of the disease in 1984 and clinic reports on the occurrence of the disease in previous years, the pathogen appears to be a common inhabitant of tobacco soils in North Carolina. We have not compared isolates of *R. solani* that cause tobacco sore shin or web blight of other crops with the tobacco leaf spot isolates.

All foliar tobacco isolates of *R. solani* that anastomosed belonged to AG 2 type 2. Members of this group are reported to cause root and crown rot of several crops (9,12,13). Most foliar blight isolates of *R. solani* belong to AG 1 (1,5,7,10) and usually do not affect other plant parts. The foliar isolates from tobacco caused damping-off and stem lesions on tobacco plants and also caused web blight of nightshade (*Solanum nigrum*) seedlings in greenhouse inoculation tests with basidiospores (H. D. Shew, *unpublished*). These isolates may therefore cause damping-off of tobacco seedlings or seedling and web blight of other crops and weed species.

At present, this disease does not represent a threat to production of flue-cured tobacco in North Carolina. The distribution and severity of the disease in 1984 were probably related to the unusual environmental conditions. Research will continue to better determine the relationship between the tobacco leaf spot isolates, other web blight isolates, and sore shin isolates of *R. solani*.

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