

# Frequency and Pathogenicity of *Fusarium* spp. Associated with Seedling Diseases of Cotton in Louisiana

P. D. COLYER, Assistant Professor, Red River Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Bossier City 71113

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## ABSTRACT

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Over a 2-yr period, *Fusarium* spp. were isolated from necrotic roots and hypocotyls of field-grown cotton seedlings at a frequency of 42%. Approximately 90% of the *Fusarium* spp. were identified as either *F. solani* or *F. oxysporum*. Other species isolated included *F. equiseti*, *F. moniliforme*, and *F. graminearum*. The different species and isolates within a species varied in virulence to cotton seedlings on the basis of pathogenicity tests conducted in the greenhouse. Few isolates caused postemergent damping-off, but many caused root and hypocotyl necrosis and/or death to the taproot. The *F. solani* isolates were more virulent than the other species.

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Seedling diseases of cotton are a major problem in cotton production. In 1985,

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37,895 bales, or 4%, of Louisiana cotton were lost to seedling diseases (7). *Rhizoctonia solani* Kühn, *Thielaviopsis basicola* (Berk. & Br.) Ferr., and *Pythium ultimum* Trow are the major fungal components associated with seedling diseases (3,4,10,14). *Fusarium* spp. are often associated with diseased plants, but their pathogenicity is controversial. Several researchers have

reported only mild virulence and consider the fungus a secondary invader (3,4,6,8,9), while others have reported a high degree of virulence (2,13,15). Nevertheless, researchers agree on the frequent association of *Fusarium* spp. with diseased seedlings (1,6,8,9,12,13). The object of this research was to identify the species of *Fusarium* involved in cotton seedling diseases in Louisiana and to determine their pathogenicity and relative virulence.

## MATERIALS AND METHODS

**Isolation and identification of fungi.** Isolations were made from 4- to 6-wk-old field-grown cotton seedlings. Seedlings were removed from the field and washed under tap water to remove any adhering soil. Necrotic root or hypocotyl tissue was excised from the seedling, submerged in 70% ethanol for 30 sec, and surface-

disinfested in 0.5% (w/v) NaOCl for 3 min. Some sections were plated on fresh potato-carrot agar acidified to pH 4.0 with lactic acid (APCA) (5) and incubated at 24 C for 7 days. Other sections were plated on water agar (WA) and incubated at 24 C for 24 hr, at which time hyphal tips were transferred to APCA. Single spores of isolates tentatively identified as *Fusarium* spp. were transferred to carnation leaf agar (CLA) and identified according to the classification scheme of Nelson et al (11).

**Pathogenicity.** An inoculation test was used to determine the pathogenicity of *Fusarium* isolates. Cotton seedlings were

prepared by surface-disinfesting cultivar Gumbo 500 seeds for 5 min in 0.5% aqueous NaOCl and germinating the seeds in petri plates containing WA. The seeds were incubated at 36 C for 4 days before use. Only healthy seedlings in the two-cotyledonary stage with well-developed root systems were used.

Fungal inocula were prepared by culturing *Fusarium* isolates from CLA plates in flasks containing 100 ml of fresh potato-carrot broth in a shaking water bath at 24 C for 4 days. In 1985, the resulting fungal suspensions were filtered through cheesecloth and utilized in pathogenicity tests. In 1986, the filtrates

were centrifuged to concentrate the conidia, and the resulting pellet was resuspended in sterile distilled water and adjusted to a final concentration of  $1 \times 10^6$  conidia per milliliter.

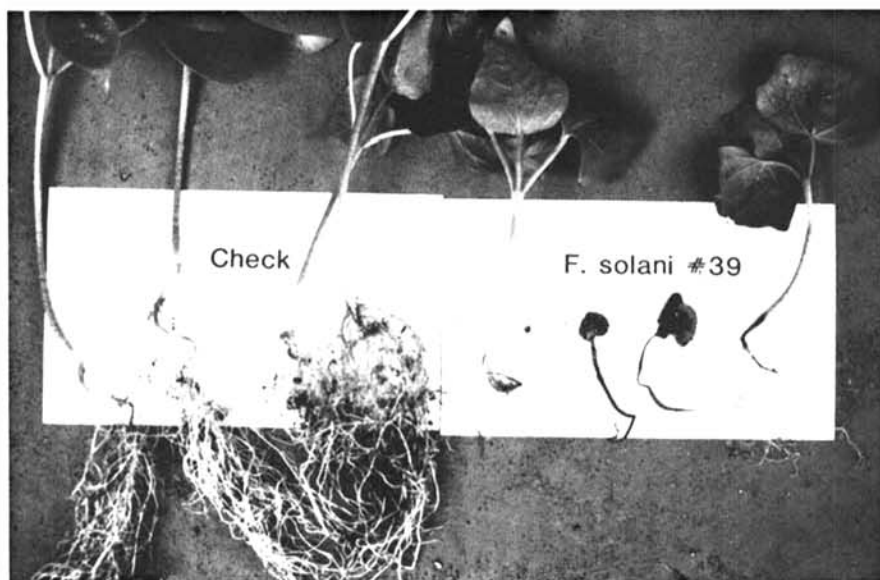
Aseptic cotton seedlings were dipped in the conidial suspensions and planted in sterilized Peter's potting mix. Nine seedlings per isolate were evaluated. The plants were maintained in a greenhouse at  $21 \pm 5$  C for 30 days, at which time they were removed from the pots, washed under tap water, and indexed for root or hypocotyl discoloration. The root-hypocotyl disease index (RHDI) was: 0 = no necrosis, 1 = <33%, 2 =  $\geq 33$  to <66%, 3 =  $\geq 66$  to  $\leq 100\%$  necrosis on the roots or hypocotyl, 4 = dead taproot with proliferation of adventitious lateral roots above the dead area, and 5 = dead plant. Isolations from inoculated seedlings on APCA were made to verify the presence of *Fusarium* spp. The mean RHDI for each isolate and species was then calculated. Mean indices for each species were compared, using an analysis of variance and Duncan's multiple range test at  $P = 0.01$ .

**Table 1.** Frequency of *Fusarium* spp. isolated from diseased field-grown cotton seedlings

Species	1985		1986	
	Number <sup>a</sup>	Percent <sup>b</sup>	Number	Percent
<i>F. solani</i>	34	34	40	49
<i>F. oxysporum</i>	49	49	30	37
<i>F. equiseti</i>	8	8	12	15
<i>F. moniliforme</i>	6	6	...	...
<i>F. graminearum</i>	3	3	...	...

<sup>a</sup>Number of seedlings from which each species was isolated.

<sup>b</sup>Percentage of total isolations from which each species was isolated.



**Fig. 1.** (Left) Uninoculated seedlings and (right) seedlings inoculated with *Fusarium solani*.

**Table 2.** Disease indices from cotton seedlings inoculated with *Fusarium* spp.

Species	1985		1986	
	Number of isolates	RHDI <sup>a</sup>	Number of isolates	RHDI
<i>F. solani</i>	26	3.29 a <sup>c</sup>	19	3.14 a
<i>F. oxysporum</i>	33	2.75 b	24	2.62 b
<i>F. equiseti</i>	6	2.62 b	4	2.50 b

<sup>a</sup>Mean root and hypocotyl disease index of nine seedlings per isolate on a 0-5 scale, where 0 = no necrosis, 1 = <33%, 2 =  $\geq 33$  to <66%, 3 =  $\geq 66$  to  $\leq 100\%$  necrosis on roots or hypocotyl, 4 = dead taproot with proliferation of adventitious lateral roots above the dead area, and 5 = dead plant.

<sup>c</sup>Mean values followed by the same letter do not differ significantly ( $P = 0.01$ ) according to Duncan's multiple range test.

## RESULTS

**Isolation and identification of fungi.** A majority of the isolations from diseased cotton seedlings in 1985 and 1986 were identified as *Fusarium* spp. In 1985, 42% of the isolations were *Fusarium* spp. and 43% were *Rhizoctonia* spp. In 1986, 43% of the isolations were *Fusarium* spp. and 37% were *Rhizoctonia* spp. The majority of the *Fusarium* isolates were either *F. oxysporum* Schlecht. or *F. solani* (Mart.) Appel & Wr. (Table 1). Other species included *F. equiseti* (Corda) Sacc., *F. moniliforme* Sheld., and *F. graminearum* Schwabe.

**Pathogenicity of *Fusarium* spp.** Nearly all the isolates produced disease symptoms on the seedlings similar to those shown in Figure 1. Differences in virulence among species were observed in the pathogenicity tests (Table 2). The isolates of *F. solani* had significantly higher RHDI ( $P = 0.01$ ) than did *F. oxysporum* and *F. equiseti*. The results from 1985 and 1986 were similar.

Figure 2 shows the distribution frequencies of RHDI for the isolates as a percentage of the total for each species. Since results from both years were similar, the data were combined. All three species of *Fusarium* were pathogenic to cotton seedlings. The most frequently occurring RHDI's were 2 and 3, indicating moderate virulence by a majority of the isolates. Whereas 8% of the seedlings inoculated with *F. solani* were killed (RHDI 5), only 1.6 and 1.1% of the seedlings inoculated with *F. oxysporum* and *F. equiseti*, respectively, were killed. Less than 1% of the isolates were nonpathogenic (RHDI 0). *F. solani* was the most frequent highly virulent species (RHDI 3, 4, and 5), followed by

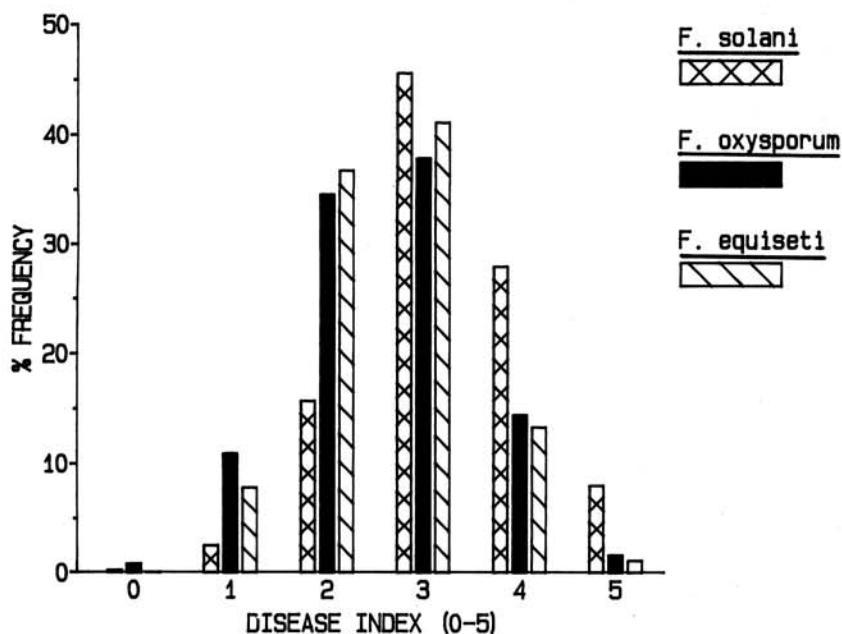


Fig. 2. Frequencies of *Fusarium* spp. isolated from cotton seedlings for each disease index category, where 0 = no necrosis, 1 = <33%, 2 = ≥33 to <66%, 3 = ≥66 to ≤100% necrosis on roots or hypocotyl, 4 = dead taproot with proliferation of adventitious lateral roots above the dead area, and 5 = dead plant.

*F. oxysporum* and *F. equiseti*.

## DISCUSSION

The role of *Rhizoctonia* as a pathogen of cotton seedlings, as well as of other crops, is widely known. The involvement of *Fusarium* spp. in cotton seedling disease, however, is uncertain. Although the high incidence of *Fusarium* spp. associated with diseased seedlings has been generally acknowledged (1,6,8,12,13), their pathogenicity and relative virulence have been questioned (3,4,9).

A negative correlation between the number of isolations of *Fusarium* and disease severity was reported from loessial soils in Tennessee (9). These authors concluded that *Fusarium* spp. are not important pathogens in the cotton seedling disease complex, although *Fusarium* spp. were the most frequently isolated fungi. The species of *Fusarium* involved were not identified. In a 4-yr study in Arkansas, more than 45% of all fungi isolated from diseased seedlings in each year were *Fusarium* spp., primarily *F. oxysporum*, *F. solani*, and *F. moniliforme* (6). Some isolates of *F. moniliforme* and *F. oxysporum* were reported as being mildly pathogenic in pathogenicity tests, although distinct lesions and reduced root systems were observed. *F. moniliforme* and *F. solani* were isolated from diseased seedlings in Oklahoma at frequencies of 61 and 19%, respectively (12). In pathogenicity tests, several isolates of *F. moniliforme* were reported to be only mildly pathogenic, but the high frequency of isolation was a

concern for the investigators.

Several researchers have, however, reported highly virulent *Fusarium* spp. on cotton. Woodroof (15) reported extreme pathogenicity of cotton seedlings by *F. moniliforme* that caused a dry root rot and dwarfing of plants. Nine species of *Fusarium* were recovered from diseased seedlings in Mississippi, and the incidence of *F. oxysporum* was positively correlated with root and hypocotyl disease indices (13). Other species isolated, but not evaluated in pathogenicity tests, included *F. solani*, *F. equiseti*, and *F. moniliforme*. In one study, the percentage of emergence of seedlings from fumigated soil artificially infested with *F. solani* was significantly lower than that from noninfested soil (2).

In this study, *Fusarium* spp. represented approximately 42% of all fungi isolated from diseased seedlings. The primary species were *F. oxysporum* and *F. solani* with low levels of *F. equiseti*, *F. moniliforme*, and *F. graminearum*. These species are similar to those reported by others. *F. graminearum* was isolated at a frequency of less than 1% from cotton seedlings in Mississippi (13). Different classification schemes utilized to identify the *Fusarium* spp. may explain some differences in species composition.

Under the conditions in this study, *Fusarium* spp. were pathogenic and often highly virulent. Although few isolates actually caused damping-off, they did cause extensive root and hypocotyl necrosis, and some decay of the taproot

with lateral roots proliferating above the dead area. Many of the disease symptoms observed in the pathogenicity tests were observed on seedlings in the field in Louisiana. The *F. solani* isolates were more virulent than the other species.

The differences in pathogenicity and virulence observed between this study and others may be related to the conditions under which pathogenicity testing was conducted. The high level of virulence and the frequency of isolation indicate *Fusarium* spp. are important in the etiology of cotton seedling diseases in Louisiana. Current control efforts are often directed at *Rhizoctonia* spp. and *Pythium* spp. This strategy may explain the high incidence of *Fusarium* on diseased seedlings. Future management practices should include control of *Fusarium* spp. in seedling diseases of cotton.

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