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RELATIVE RECOVERY AND PATHOGENICITY OF *FUSARIUM* SPECIES ON COTTON

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Introduction

Upland cotton (*Gossypium hirsutum*) is considered to be the most important fiber crop grown in the world and the United States is ranked among the top five producing countries. The value of the cotton lint and seed produced in Alabama throughout the last decade has averaged $171,371,000 annually. Alabama produces cotton in the north, central and southern regions in 64 percent of the counties. Fusaria are reported to be among the most common fungi associated with the roots of cotton seedlings. *Fusarium* species are known to play a role in several diseases of cotton including the seedling disease complex, wilt, and boll rot. Therefore, a mycoflora study was conducted in 2000 and 2001 in order to identify *Fusarium* species found in association with specific parts of cotton and to observe the relationship between the age of the crop, its growth stage, and the incidence of selected fungi.

Materials and Methods

Plant samples were collected using a randomized systematic sampling pattern from fields representing the major cotton growing regions of the state. The fields were located at the Gulf Coast Substation in Baldwin county in south Alabama, the Wiregrass Substation in Henry county in southeast Alabama, the EVS Research Center in Macon county in central Alabama, and the Tennessee Valley Substation located in Limestone county in north Alabama. Cotton fields in each region were sequentially sampled at two weeks after planting (seedling stage), first bloom, full bloom, and maturity. Immediately after plants were collected, they were sealed in plastic bags and stored on ice prior to examination at Auburn University. After storage for less than 48 hr at 4 C, samples were washed in running tap water for 15 min then one 5-mm section was excised from the root hypocotyl transition zone of tap roots, petioles, leaves, and bolls of each plant. Plant tissues were surface sterilized in 95% ethanol followed by 0.10% sodium hypochlorite (NaOCl). Surface sterilized roots, petioles, leaves and bolls were then aseptically plated on acidified potato dextrose agar (APDA). The plates were incubated at 25 C for 3 to 10 days. Fungal colonies were immediately identified to species based on reproductive morphology and colony characteristics or subcultured on carnation leaf agar (CLA) for further identification. The number of colonies of each fungus per plate was recorded for each anatomical location, sample date, and test site. Relative recovery was calculated by dividing the number of colonies of individual fungi by the total number of all fungal colonies recovered from cotton tissue of each replicate and expressed as a percentage.

Pathogenicity tests were conducted in the greenhouse. Treatments consisted of *Fusarium equiseti, F. lateritium, F. longipes, F. moniliforme, F. oxysporum, F. proliferatum, F. semitectum, F. solani, F. subglutinans, Rhizoctonia solani, Thielaviopsis basicloa,* one control infested with sterile millet and one control without millet. To increase fungal inoculum, 75 cc of millet seed was placed in 250 ml flasks containing 150 ml of tap water. The millet seed was allowed to imbibe water for 24 hours after which the excess water was decanted and the flasks autoclaved twice at 121 °C for 20 minutes on subsequent days. Flasks were aseptically inoculated by adding a 5 mm diameter plug from the periphery of one week old PDA cultures. All flasks were incubated for 7 days at 25 °C under cool-light fluorescent illumination. Each flask was shaken daily to allow for thorough propagule dispersement.

Greenhouse tests were conducted using a Marvyn sandy loam (fine-loamy, siliceous, thermic typic kanhapludults) soil. A mechanical soil mixer was used to mix portions of the inocula with 500 g of sterile field soil to obtain an inoculum concentration of 1% (w/w). Infested and non-infested (control) soils were distributed to 10 cm diameter polystyrene plastic pots. Surface sterilized germinated cotton cv. Paymaster PM 1218BG/RR seeds (radicle 1 cm in length) were placed on the soil surface in each pot and covered with 2.5 cm of the appropriate soil. Each experimental unit was replicated six times with pots arranged in a randomized complete block design on a greenhouse bench. Parameters measured bi-weekly included cotton stand, height, and a disease rating. Taproots were assessed for disease Root and shoot dry weight were recorded at the end of 6 weeks concluding the termination of the experiment. This experiment was conducted three times.

Results and Discussion

A total of nine *Fusarium* species were isolated from cotton seedlings across all locations. *Fusarium* species isolated from cotton samples collected from the Tennessee Valley and southeast Alabama included *Fusarium equiseti*, *F. lateritium, F. oxysporum*,, *F. semitectum*, and *F. solani*. *Fusarium* species isolated from cotton samples collected from central Alabama included those previously mentioned plus *F. moniliforme*. Fusaria isolated from cotton samples collected from the Gulf Coast added the species of *F. longipes, F. proliferatum*, and *F. subglutinans* which have not previously been reported on cotton.

Fusarium oxysporum, F. solani, and *F. equiseti* were the most common species isolated across all locations. The relative recovery of *Fusarium oxysporum* and *F. solani* decreased as the season progressed and the majority of isolates were recovered from the roots. Relative recovery of *Fusarium equiseti* was highest at the end of the season and mostly isolated from the cotton bolls (Tables 1-4). Results from the greenhouse pathogenicity tests showed that all treatments had a significant impact on seedling stand except for *Fusarium semitectum* (Table 5). *Fusarium moniliforme*, *F. oxysporum, F. proliferatum, F. solani*, and *F. subglutinans* proved to be the most virulent species with the highest disease severity rating and resulting in significantly less biomass in comparison to the control (Tables 6 and 7).

Table 1. Relative recovery *Fusarium* species isolated at seedling stage.

***Fusarium* species**	**Root**	**Hypocotyl**
Fusarium equiseti	53% [a]	47%
Fusarium lateritium	0%	100%
Fusarium oxysporum	41%	59%
Fusarium proliferatum	50%	50%
Fusarium semitectum	80%	20%
Fusarium solani	57%	43%

[a] # colonies of individual fungi divided by total # of all colonies recovered.

Table 2. Relative recovery *Fusarium* species isolated at first bloom.

***Fusarium* species**	**Root**	**Petioles**	**Bolls**
Fusarium equiseti	0% [a]	20%	80%
Fusarium moniliforme	45%	22%	33%
Fusarium oxysporum	61%	20%	19%
Fusarium solani	63%	19%	18%

[a] # colonies of individual fungi divided by total # of all colonies recovered.

Table 3. Relative recovery *Fusarium* species isolated at full bloom.

***Fusarium* species**	**Root**	**Petioles**	**Bolls**
Fusarium equiseti	0%[a]	77%	23%
Fusarium oxysporum	54%	22%	24%
Fusarium semitectum	27%	60%	13%
Fusarium solani	17%	83%	0%

[a] # colonies of individual fungi divided by total # of all colonies recovered.

Table 4.Relative recovery *Fusarium* species isolated at maturity.

***Fusarium* species**	**Root**	**Petioles**	**Bolls**
Fusarium equiseti	11%[a]	24%	65%
Fusarium oxysporum	0%	77%	23%
Fusarium semitectum	0%	0%	100%
Fusarium solani	86%	0%	14%
Fusarium subglutinans	0%	0%	100%

[a] # colonies of individual fungi divided by total # of all colonies recovered.

Table 5. Impact of Fusaria on cotton stand.

Treatments	14 DAP	28 DAP	42 DAP
Fusarium equiseti	80%	60%	60%
Fusarium lateritium	80%	60%	60%
F. longipes	90%	70%	70%
Fusarium moniliforme	80%	30%	30%
Fusarium oxysporum	80%	70%	60%
Fusarium proliferatum	80%	60%	40%
Fusarium semitectum	100%	80%	80%
Fusarium solani	90%	70%	60%
Fusarium subglutinans	70%	60%	50%
Rhizoctonia solani	50%	30%	30%
Thielaviopsis basicola	50%	30%	30%
Control + millet	100%	100%	100%
Control	100%	100%	100%
LSD (P=0.05)	0.2	0.3	03

Table 6. Disease Severity Index.

Treatments	42 DAP
Fusarium equiseti	1[a]
Fusarium lateritium	1
F. longipes	1
Fusarium moniliforme	3
Fusarium oxysporum	3
Fusarium proliferatum	3
Fusarium semitectum	1
Fusarium solani	3
Fusarium subglutinans	3
Rhizoctonia solani	3
Thielaviopsis basicola	3
Control + millet	0
Control	0

[a] Disease severity index value, where 0 = no necrosis, 1 = < 33%, 2 = ≥33 to < 66%, 3 = ≥66 to ≤ 100% necrosis on roots or hypocotyl, 4 = dead taproot with lateral root growth above the dead area, and 5 = dead plant (Colyer, 1988).

Table 7. Impact of Fusaria on cotton biomass.

Treatments	Root Dry Wt (g)	Shoot Dry Wt (g)
Fusarium equiseti	0.4	0.13
Fusarium lateritium	0.4	0.11
F. longipes	0.4	0.1
Fusarium moniliforme	0.01	0.01
Fusarium oxysporum	0.1	0.04
Fusarium proliferatum	0.1	0.01
Fusarium semitectum	0.5	0.15
Fusarium solani	0.24	0.1
Fusarium subglutinans	0.1	0.04
Rhizoctonia solani	0.1	0.03
Thielaviopsis basicola	0.2	0.1
Control + millet	0.5	0.2
Control	0.6	0.2
LSD (P=0.05)	0.1	0.05