FUSARIUM WILT IDENTIFICATION AND ROOT-KNOT NEMATODE EFFECTS ON COMMERCIAL COTTON CULTIVARS IN 2010

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Abstract

Fusarium wilt of cotton is a serious fungal disease caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) and highly impacted by root knot nematode (*Meloidogyne incognita*). Both pathogens are common in most cotton-producing areas and often inhabit the same fields. Root knot nematode (RKN) trial numbers were tested to determine their effect on cotton lines. Along with this, the identification of different FOV races from various cotton lines from ten commercial companies was studied. Cotton plants exhibiting wilt symptoms were collected; the fungi isolated, and spores from the isolation cultivated on Acidified Potato Dextrose Agar (APDA). DNA was extracted using two sets of primers. ITS1 and ITS4 primers amplified the internal transcribed spacer (ITS) region, and sequences from the translation elongation factor (EF-1α) helped characterize the isolates. Results indicate that the population of *F. oxysporum* f. sp. *vasinfectum* in the southeastern United States is more diverse than previously recognized and additional research to categorize and monitor the identity of FOV present in Alabama is needed.

Introduction

Fusarium oxysporum f. sp. *vasinfectum* Snyder & Hansen, (FOV) is an adaptable fungus that causes the Fusarium wilting disease. FOV strains are rich soil inhabitants that have the ability to exist as saprophytes or persistent plant parasites that can be highly pathogenic, killing cotton plants. The fungus invades the root system directly through the root tips, at the formation point of lateral roots, or more frequently through wounds in the roots. (Agrios, 1988). Root-knot nematodes, *Meloidogyne incognita,* (RKN) increase the susceptibility of cotton plants to Fusarium wilt. The nematodes enter the cotton roots creating wounds allowing the fungus to penetrate into the root tissues. Losses to the cotton crop from the wilt-nematode disease complex have been greater specifically because of the root-knot nematodes (Powell 253-274). It is estimated that in Alabama alone, cotton growers loose an average of 2000 bales of cotton to the root knot nematode. The addition of Fusarium wilt increases losses to 6000 bales or approximately one million dollars of the production to the Fusarium wilt complex.

Eight pathogenic types or races of FOV have been described throughout the world, with the additional novel race 4 identified in California (Kim et al., 2005). Races of FOV were initially classified based on pathogenicity tests on okra, alfalfa, two cultivars of tobacco, and on different cotton species, *Gossypium hirsutum, Gossypium barbadense*, and *Gossypium arboretum* (Armstrong and Armstrong, 1958, 1978; Ibrahim, 1966) to distinguish six physiological races. However, lately a number of genetic markers have been used to further characterize these races. Worldwide strains of FOV can be classified into five major lineages currently based on sequence differences in the translational elongation factor, phosphate permase, and beta-tubulin genes and interginc spacer restriction enzyme (Kim et al., 2005). In 2001, the discovery of FOV race 4 in the San Joaquin Valley renewed interest in the various races of FOV around the United States.

The objectives of this project are to 1) examine the cotton cultivars response to root knot nematodes and Fusarium wilt effects; 2) determine cotton yield potential when challenged by the Fusarium wilt complex; and 3) and to classify FOV races in Alabama.

Materials and Methods

Field Trial:

Commercial cotton cultivars most frequently grown in Alabama were studied to determine their reaction to the soil borne pathogens, root-knot nematode (RKN) and Fusarium wilt. The trial was conducted at the E.V. Smith Research Center, Plant Breeding Unit, near Tallassee, AL. The soil is an Independence loams fine sand and is naturally infested with both pathogens. Cotton cultivars, Rowden and M-315 were included as the susceptible and resistant controls, respectively. All cotton entries were planted in single 20-foot rows on 36-inch centers, separated by 6-foot alleys. Four replications of the test entries and controls were evaluated in a randomized complete block design with a split plot restriction on randomization. Plots were planted May 14, 2010. All plots were maintained throughout the season using standard herbicide, insecticide, and fertility production practices as recommended by the Alabama Cooperative Extension System. Initial plant stand counts were made June 9 and wilted plants were counted and removed on June 23, July 7, July 21, August 4 and August 19. FOV isolations were done from the removed plants at each data. Root systems were surface sterilized in 0.6 % NaOCl for 1 minute and aseptically plated on acidified Potato Dextrose Agar (APDA). Three plants per plot were removed on July 22. The fresh root weights were recorded and nematodes were extracted from the root system by shaking in 1% NaOCl counted under the inverted microscope. Yields were collected utilizing a plot harvester on October 29. All data was analyzed using Generalized Linear Mixed Models procedures as implemented in SAS PROC GLIMMIX with a negative binomial distribution function for count variables. Percent wilted plants, RKN, root-knot egg, and seed cotton yield numbers were calculated.

Lab Trials:

Fungi were isolated from the hypotocol and upper tap roots of symptomatic cotton plants and grown on Acidified Potato Dextrose Agar (APDA) at 27°C for 7 days. To obtain genomic DNA, mycelia was taken from the APDA plate and ground into a powder in liquid nitrogen. DNA was extracted with a DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) using the manufacturer's protocol. The internal transcribed spacer (ITS) regions 1 and 2 were amplified in a Multigene Labnet thermocycler. The primers used were ITS1, ITS4, EF-1, and EF-2. The thermal cycles used for the PCR reactions were denatured using Y.Kim's protocol in "Characterization of California Isolates of *Fusarium oxysporum* f. sp. *vasinfectum*." PCR products were then cloned using the pGEM-T Vector Systems. Purification of the plasmid obtained was done using QIAprep Spin Miniprep Kit (Qiagen Inc.). The resulting amplified product was sent to Eurofins MWG Operon in a 25µl eppendorf tubes to be sequenced. Alignments of the sequences were done in Mega 4.1 software, and then analyzed by nucleotide blast in National Center for Biotechnology Information (NCBI).

Results and Discussions

The 2010 season, environmentally, was conducive for the root-knot nematode and Fusarium wilt pathogens. Fusarium wilt symptoms were initially visible in late June and were expressed with cotton leaf necrosis and wilting. Plant death occurred throughout July and August. The fungal pathogen was not found in the resistant M -315 cotton but was readily isolated from Rowden and the commercial cultivars. Over all the cotton submissions planted in 2010, Fusarium wilt incidence ranged from a high of 16.25% for the susceptible Rowden to a low of 0.4% for the resistant M-315 (Table 1). All commercial cotton cultivars except Delta Pine 0949 B2RF had fewer Fusarium wilt symptomatic plants than the susceptible Rowden. PhytoGen PHY 367 WRF and Stoneville ST 5458 B2RF displayed the fewest symptomatic plants and were followed by PhytoGen PHY 375 WRF, Delta Pine 1028 B2RF, PhytoGen PHY 485 WRF, Stoneville ST 4288 B2RF, and Delta Pine DP 1050 B2RF.

Several lineages corresponding to known races of *F. oxysporum* f. sp. *vasinfectum* were recognized by molecular markers and gene sequences used. The translation elongation factor-α gene was used to determine the relationships among the isolates from the diseased plants and data revealed the presence of races 1, 2, 4, and 8 within the Plant

Breeding Unit, Tallassee, AL. Further studies need to be to done to ascertain the discovery of the newly-described clades and potentially new clades in Alabama. Also, additional work conducted using the beta-tubulin and phosphate permease primer genes need to be undertaken to verify the lineages within Alabama.

Table 1. Average percent of Fusarium wilt incidence for cotton cultivars, confidence intervals, and *P*- values based on Dunnett's versus the susceptible check Rowden and the resistant check M-315.

		95 % Confidence Limit		Dunnett's P-value vs.	
Cultivar	**Mean**	**Lower**	**Upper**	**Rowden**	**M-315**
Deltapine DP 0949 B2RF	7.6	3.7	15.1	0.378	0.044
Deltapine DP 1028 B2RF	2.6	0.9	7.2	0.013	0.326
Deltapine DP 1050 B2RF	3.7	1.6	8.1	0.011	0.172
Fiber Max FM 1740 B2F	5.4	2.9	9.7	0.019	0.080
PhytoGen PHY 367 WRF	1.1	0.3	3.6	0.001	0.790
PHY 375 WRF	2.5	0.9	7.1	0.011	0.335
PHY 485 WRF	3.3	1.4	7.4	0.006	0.207
PhytoGen PHY 565 WRF	6.9	4.1	11.5	0.065	0.046
Stoneville ST 4288 B2F	3.4	1.6	7.2	0.004	0.188
Stoneville ST 5458 B2RF	1.6	0.6	4.6	0.001	0.560
Rowden	16.3	11.4	23.0		0.005
M-315	0.4	0.0	3.6	0.013	

The numbers of root-knot nematodes increase in all the cotton samples submitted. The standard susceptible cotton, Rowden, averaged 2149 root-knot eggs per gram of root while the M-315 resistant cotton supported only 88 eggs per gram of root (Table 2). All cotton cultivars supported higher populations of root-knot ($P \geq 0.001$) as compared to M-315 except PhytoGen PHY 367 WRF. This PhytoGen PHY 367 WRF was also the only cultivar less susceptible to the nematode as compared to the Rowden susceptible control.

Table 2. Root knot egg numbers (counts per g of root fresh weight), confidence intervals, and *P*-values based on Dunnett's versus the susceptible check Rowden and the resistant check M-315.

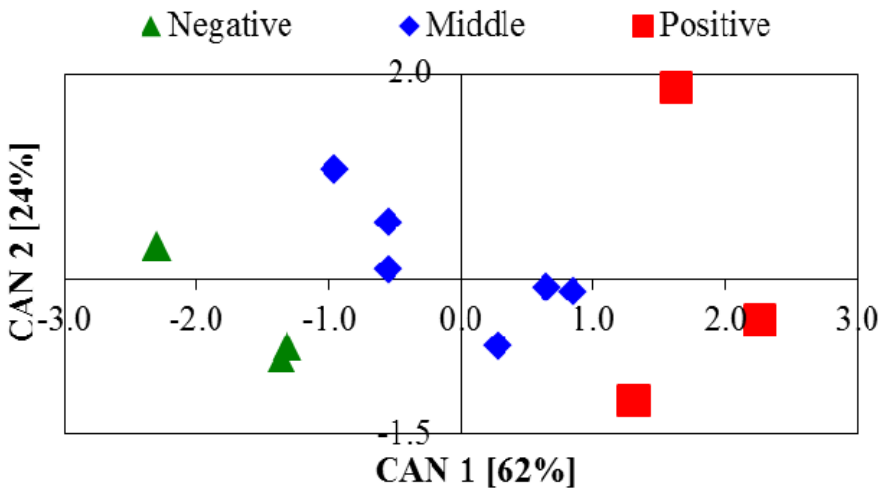
		95 % Confidence Limit		Dunnett's P-value vs.	
Cultivar	**Mean**	**Lower**	**Upper**	**Rowden**	**M-315**
Deltapine DP 0949 B2RF	775	347	1732	0.318	0.003
Deltapine DP 1028 B2RF	1092	488	2440	0.734	0.001
Deltapine DP 1050 B2RF	693	310	1550	0.225	0.005
Fiber Max FM 1740 B2F	1852	1176	2916	1.000	<.0001
PhytoGen PHY 367 WRF	382	171	853	0.023	0.067
PHY 375 WRF	1187	531	2652	0.835	0.000
PHY 485 WRF	1170	524	2616	0.819	0.001
PhytoGen PHY 565 WRF	1585	1006	2495	0.980	<.0001
Stoneville ST 4288 B2F	893	567	1406	0.217	<.0001
Stoneville ST 5458 B2RF	901	403	2013	0.484	0.002
Rowden	2149	962	4801		<.0001
M-315	88	39	197	<.0001	

Seed cotton yield varied from a low of 1151 lb/A in the susceptible Rowden to a high of 4467 lb/A in the PhytoGen PHY 367 WRF plots (Table 3). The PhytoGen PHY 367 WRF, PhytoGen PHY 565 WRF, Stoneville ST 4288 B2F, and Stoneville ST 5458 B2RF cultivars all produced more cotton ($P \geq 0.05$) as compared to Rowden. All of the cotton cultivars produced yields similar to the resistant cotton M-315 except Deltapine DP 0949 B2RF.

Table 3. Seed cotton yield (lbs per acre) for entries and checks, confidence intervals, and *P*-values based on Dunnett's versus the susceptible check Rowden and the resistant check M-315.

		95 % Confidence Limit		Dunnett's P-value vs.	
Cultivar	**Mean**	**Lower**	**Upper**	**Rowden**	**M-315**
Deltapine DP 0949 B2RF	1176	486	1866	1.000	0.020
Deltapine DP 1028 B2RF	1768	1078	2458	0.793	0.272
Deltapine DP 1050 B2RF	2316	1626	3006	0.150	0.951
Fiber Max FM 1740 B2F	1804	1114	2494	0.743	0.309
PhytoGen PHY 367 WRF	3467	2776	4157	0.000	0.691
PHY 375 WRF	1808	1118	2498	0.738	0.313
PHY 485 WRF	1535	845	2226	0.985	0.109
PhytoGen PHY 565 WRF	3133	2442	3823	0.003	0.991
Stoneville ST 4288 B2F	2603	1912	3293	0.042	1.000
Stoneville ST 5458 B2RF	2784	2094	3474	0.017	1.000
Rowden	1151	460	1841		0.017
M-315	2777	2087	3467	0.017	

Canonical discriminant analysis indicated the main force driving the differences along CAN 1 was seed cotton yield (r = 0.98). Cotton cultivars separated into three groups. PhytoGen PHY 367 WRF, PhytoGen PHY 565 WRF, and M-315 were the highest yielding. Phytogen 485 WRF, Deltapine DP 0949 B2RF and Rowden were the lowest yielding. The middle group cannot be distinguished from either extreme group. Differences in CAN 2 are driven by the root knot number, which is driven by egg number. Thus, PhytoGen PHY 367 WRF produced high yields while supporting few root-knot nematodes compared to PhytoGen PHY 565 WRF produced high yields and high numbers of nematodes.



Cultivar	Can1	Middle	Positive	Negative	Lbs./acre
Rowden	-2.29			0.32	1151
PHY 485 WRF	-1.35			-0.77	1535
Deltapine DP 0949 B2RF	-1.31			-0.65	1176
Fiber Max FM 1740 B2F	-0.95	1.06			1804
Deltapine DP 1028 B2RF	-0.54	0.09			1768
PHY 375 WRF	-0.54	0.55			1808
Deltapine DP 1050 B2RF	0.28	-0.65			2316
Stoneville ST 4288 B2RF	0.65	-0.08			2603
Stoneville ST 5458 B2RF	0.85	-0.13			2784
M-315	1.31		-1.19		2777
PhytoGen PHY 565 WRF	1.64		1.85		3133
PhytoGen PHY 367 WRF	2.26		-0.41		3467

Figure 1. Canonical discriminant analysis of cotton cultivar yield, nematode numbers and Fusarium wilt incidence. CAN 1 is seed cotton yield (r = 0.98) thus yield increases moving away from the Y axis. Differences in CAN 2 are driven by root knot egg numbers with higher numbers in the top than the lower red cultivars.

Conclusion

Phytogen 367 WRF, Phytogen 565 WRF, Stoneville 5458 B2RF, and Stoneville 4288 B2F cultivars all produced significantly more cotton than our Rowden control. Phytogen 367 WRF did respond as a resistant variety to the root knot nematode and did not allow the nematode population to increase over the season. The Fusarium wilt fungi were identified as races 1, 2, 4, and 8 with many more isolates that did not fit the known race or clade schemes. There is diversity of the Fusarium wilt fungal population.

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