EVALUATION OF FUSARIUM WILT RESISTANCE IN COTTON CULTIVARS AND IDENTIFICATION OF PATHOGENIC RACES OF FUSARIUM OXYSPORUM F. SP. VASINFECTUM IN ALABAMA

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Abstract

Fusarium oxysporum f. sp. *vasinfectum* (FOV) is the causal agent of the fungal disease Fusarium wilt in cotton. Objectives of this study are to 1) Observe commercial variety responses to Fusarium wilt and root-knot nematodes (*Meloidogyne incognita*) and compare to yield and 2) Identify races of FOV present at the site of the Commercial Fusarium Wilt Trial. The projected outcome of these experiments is to be able to more effectively control the Fusarium wilt root-knot nematode disease complex in the southeastern United States with resistant varieties being the main control measures. Results showed four varieties having statistically similar yields to the resistant check M-315: Stoneville 4747 GLB2, Stoneville 4946 GLB2, Phytogen 427 WRF, and Phytogen 499 WRF. Eleven of sixteen cultivars showed statistically similar Fusarium wilt percentages to the resistant check M-315. Nine of sixteen cultivars tested had statistically similar root-knot egg reproduction factors compared to the resistant check M-315. There is a diversity of FOV races present at the Plant Breeding Unit, making this location ideal for a field trial. Races 1, 8, LA 108, and LA 127/140 were found to be present in 2014.

Introduction

The intensity of Fusarium wilt disease pressure is increased by the presence of the Southern root-knot nematode, *Meloidogyne incognita*. There are currently no curatives for this disease, and prevention methods available are minimal at best. This creates a dire need for research to discover cultivars that are resistant to both of these pathogens to help control disease severity and crop losses.

Fusarium oxysporum f. sp. *vasinfectum* (FOV) caused the loss of over $1.3 million and 3100 bales of cotton for 2013 in Alabama (Lawrence et al., 2014). Fusarium wilt and the root knot nematode (RKN) are two pathogens that put great pressure on cotton crops throughout the Southeast. There are currently no commercial cotton cultivars that are resistant to this disease complex. The only available option for control is to fumigate soils to reduce nematode populations; two downfalls to this control method are 1) the lack of economic feasibility for row crop farming to use these nematicides on a large scale and 2) the discontinuation of most effective fumigants due to environmental concerns. It is crucial to find other means of controlling and preventing this disease complex in order to decrease yield losses and economic losses for present day and future farmers.

FOV was first documented in an Alabama cotton field by Atkinson in 1892 (Atkinson, 1892). Symptoms and signs (Figures 1a.-1c.) that are present within affected cotton plants include chlorotic and necrotic leaves, abscission of affected leaves, wilting, reduced yield, and death. A relationship between the *Fusarium* pathogen and nematodes (Figure 1d.) was noticed early on (Atkinson, 1892). The fungal hyphae induce vascular discoloration and xylem blockage causing the plant to wilt.

The Commercial Wilt Trial has been utilized since 2003. Its purpose is to evaluate commercially available cotton cultivars for Fusarium wilt and root-knot resistance. Cultivars are provided by plant breeders and various companies for evaluation. Factors considered during evaluation are Fusarium incidence and severity, root-knot reproduction factors, and yield performance.

a. b. c. d.

Figure 1. a) Fusarium wilt foliar symptoms; b) Interveinal chlorosis and necrosis; c) Vascular discoloration of hypocotyl; d) Galling associated with root-knot nematode damage.

Methods

The Commercial Fusarium Wilt Trial was located at the Plant Breeding Unit of the E. V. Smith Research Center near Tallassee, Alabama. Sixteen commercial varieties that are commonly grown in Alabama and the Southeast were tested with this trial. Egg reproduction factors, Fusarium wilt incidence, and yield were compared to resistant (M-315) and susceptible (Rowden) checks. The trial was organized in a randomized complete block design with four replications. Plots were set up as 20 feet long one-row plots with 36-inch row spacing, with 6 feet alleys separating blocks. The trial was planted May 19, 2014 and maintained throughout the growing season using standard practices for pesticide and fertilizer applications as recommended by the Alabama Cooperative Extension System. Initial survival rates were taken 17 DAP and final survival rates were taken 101 DAP to determine plant survival rates by plot. Five wilt disease evaluations were taken throughout the season at 33, 43, 58, 73, and 93 DAP. Infected plants were counted and collected and the fungus was re-isolated onto half-strength APDA (acidified potato dextrose agar) 100mm plates using sterile techniques to confirm infection. FOV cultures were identified to race using four primers to sequence identification of pathogenic races of FOV according to Kim (2009) and Holmes et al. (2009): two EF-1α primers (EF1 and EF2) and two Beta-tubulin primers (BT3 and BT5). For root-knot nematode reproduction factor calculations, three root systems per plot were sampled at 63 DAP and root-knot eggs were extracted by shaking in 0.6% NaOCl for four minutes, and eggs were collected on a 25µm sieve and counted at 40X using an inverted Nikon TSX microscope. Data were statistically analyzed using Generalized Linear Mixed Models with SAS® PROC GLIMMIX using Tukey-Kramer's ($\alpha \leq 0.05$) with a negative binomial distribution function for count variables. Seed cotton yield was analyzed using a normal distribution function. Dunnett's *P*-values were calculated to compare entries to resistant and susceptible checks.

Results

The susceptible check Rowden averaged 30% wilt incidence and the resistant check M-315 averaged 2% wilt incidence; the commercial varieties with the lowest amount of disease present were Stoneville 4747 GLB2 and Phytogen 427 WRF with 1% wilt incidence. Eleven cultivars displayed statistically similar wilt percentages to the resistant check (Table 1): Phytogen 339 WRF, Phytogen 575 WRF, Phytogen 499 WRF, Phytogen 427 WRF, Deltapine 1321 B2RF, Deltapine 1252 B2RF, Deltapine 1050 B2RF, Stoneville 4747 GLB2, Stoneville 4946 GLB2, Stoneville 6448 GLB2, and FiberMax 1944 GLB2. Wilt percent incidences for each of these varieties was low with a range of 1-7%.

The susceptible check Rowden averaged 1871 root-knot nematode eggs per gram of root fresh weight (eggs/g RFW), and the resistant check M-315 averaged 270 eggs/g RFW. Nine varieties supported root-knot egg numbers that were statistically similar to the resistant check M-315 (Table 1): Croplan Genetics 3787 B2RF, Phytogen 499 WRF, Phytogen 427 WRF, Deltapine 1133 B2RF, Deltapine 1252 B2RF, Deltapine 1050 B2RF, Deltapine 1137 B2RF, Deltapine 1454NR B2RF, and Stoneville 4946 GLB2. Average root-knot eggs per gram of root for each of these varieties ranged from 703 to 1349.

The susceptible cultivar Rowden yielded an average of 684 lbs. of seed cotton per acre, and the resistant check M-315 yielded 1806 lbs. per acre. At .60¢ per pound (the average price of cotton lint in December 2014) the average profit per acre would be $292/acre for Rowden, and $569/acre for M-315. Four varieties were statistically higher yielding than the resistant check M-315: Phytogen 427 WRF, Phytogen 499 WRF, Stoneville 4747 GLB2, and Stoneville 4946 GLB2 with 2536, 2706, 2868, and 2521 lb. averages per acre of seed cotton yield. With the same .60¢ per pound average for 2014, the yields per acre for these varieties (at 40% lint production) would be $1094, $913, $1213, and $1134 per acre. This represents how imperative it is to be selective of the cultivar grown.

Fusarium isolates were taken from each trial and identified to race in order to determine the diversity of *Fusarium oxysporum* f. sp. *vasinfectum* at the field testing site. The predominant race identified was race 1, with 69 isolates being confirmed as race 1. Six isolates were confirmed to be LA 127/140. Five isolates of race 8 and five isolates of race LA 108 were identified. Races 3, 4, LA 110, and LA 112 were not identified at the field testing site in 2014. Although there were five total disease evaluations throughout the season, the first and the fifth were used for race identification. The first disease evaluation had races 1, 8, LA 108, and LA 127/140 present. The fifth disease evaluation had races 1, LA 108, and LA 127/140 present. This indicates a greater diversity at the beginning of the season as opposed to the end.

Table 1. Fusarium wilt incidence, root-knot eggs/g root, and yield for cultivars, 2014.

	Total wilt (%)	RK eggs/g root	Yield (lb/A)	Yield ($)
Croplan 3787 B2RF	13	1210	1664	399
PHY 375 WRF	9	2426	1443	346
PHY 499 WRF	5	1349	2536	609
PHY 339 WRF	2	1642	2312	555
PHY 427 WRF	1	906	2706	650
PHY 575 WRF	7	1940	1610	386
DP 1321 B2RF	3	1946	1997	479
DP 1133 B2RF	15	1094	1659	398
DP 1252 B2RF	7	909	1752	420
DP 1050 B2RF	6	1059	1812	435
DP 1137 B2RF	11	924	1799	432
DP 1454 NR B2RF	18	703	1399	336
ST 4747 GLB2	1	1573	2868	688
ST 4946 GLB2	2	1206	2521	605
ST 6448 GLB2	5	3331	2111	507
FM 1944 GLB2	6	2446	2044	491
M-315	2	270	1806	433
Rowden	30	1871	684	164

Summary

Races of *Fusarium oxysporum* f. sp. *vasinfectum* found at the Plant Breeding Unit were races 1, 8, LA 108, and LA 127/140. Race 1 was most predominantly found in 2014. Ranking the cultivars by yield indicated the highest yielding cultivars were Stoneville 4747 GLB2 followed by Phytogen 427 WRF, Phytogen 499 WRF, and Stoneville 4946 GLB2. Phytogen 427 WRF, Stoneville 4946 GLB2 and Phytogen 499 WRF supported lower root-knot populations and little FOV disease incidence. Stoneville 4747 GLB2 supported very low wilt incidence, but root-knot egg reproduction factors were not significant. Deltapine 1454NR B2RF supported lower numbers of root-knot nematode eggs per gram of root fresh weight than the susceptible check Rowden and other commercial cultivars tested in the trial. This variety performed the best when limiting root-knot nematode reproduction, but could be considered moderately susceptible to Fusarium wilt. Further testing will need to be done to confirm or deny resistance to FOV. Commercial cultivar selection is economically important to producers in fields with the Fusarium wilt Root-knot

nematode complex, and these results indicate cultivars are available for growers to help combat losses caused by this disease.

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