GREENHOUSE SCREENING OF COTTON FOR FUSARIUM WILT RACE 4 RESISTANCE IN NEW MEXICO

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Abstract

In this study, 3,200 Upland cotton (*Gossypium hirsutum*) lines and 180 Pima lines (*G. barbadense*) were evaluated in the greenhouse or a lab for resistance to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) race 4 (FOV4). A total of 2,258 lines were screened in a commercial potting soil in the greenhouse under higher temperature (HT) conditions (24-32ºC), while 1,204 lines were screened in a FOV4-infected farm soil at a lower temperature (LT) setting (20ºC), both with artificial inoculations. The results showed that both temperature regimes produced similar disease incidence (81.2 for HT vs. 86.8% for LT), but LT caused much higher disease severity ratings (3.86 vs. 1.94) and plant mortality (81.7 vs. 7.5%) than HT. A growth chamber study confirmed that 20-23oC produced the highest disease incidence and severity and plant death. At 20ºC using a FOV4-infected farm soil, disease incidence, severity and plant mortality plateaued at 30 days post inoculation, and it is the best time to evaluate cotton for FOV4 resistance. Using the above evaluation methods to screen a set of 225 Upland germplasms, 60% of the lines showed 100% mortality; 37% of the lines had a mortality rate between 10 and 90%; and 3% of the lines did not display any apparent FOV4 symptoms. The results indicated that FOV4 resistance may be heterogeneous in existing germplasms and pedigree selection may increase frequencies of resistant plants.

Introduction

Fusarium wilt (FW), caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), is one of the most destructive diseases in the US and most cotton-growing regions in the world. In the US, FOV race 4 (FOV4) was reported in California in the early 2000s and recently in Texas and New Mexico (Kim et al., 2005; Holmes et al., 2009; Hutmacher et al., 2011; Halpern et al., 2018; Zhu et al., 2019b), and it has become an emerging threat to cotton production in the west Cotton Belt. Although FOV can infect cotton plants at any stage, leading to wilting, chlorosis and necrosis of leaves, stunting, defoliation, and plant death, FOV4 causes seedling death in highly susceptible Pima cotton genotypes during seedling emergence and early seedling growth stages. Because management of Fusarium wilt is extremely challenging due to the ability of FOV to persist in the soil for years, developing and using FW-resistant cultivars has proven to be the most and only cost-effective approach to control this disease (Zhang et al., 2015). Currently, no commercial Upland cotton cultivars grown in the US are known to be immune or highly resistant to FOV4.

The objectives of this study were, (1) to evaluate commercial cotton cultivars, advanced breeding lines, obsolete germplasm lines, and genetic populations for FOV4 resistance under the greenhouse or controlled conditions; (2) to identify factors that affect the development of FOV4-associated symptoms in cotton; and (3) to develop a fast and reliable artificial inoculation method to ensure successful screening of cotton for FOV4 resistance in the greenhouse or controlled conditions.

Material and Methods

FOV4 Isolate Used in the Screening

An isolate from Fabens, TX, identified to be the most virulent in our previous study (Zhu et al., 2019a), was used in

this study.

Cotton Germplasm Lines Screened for FOV4 Resistance

A total of 3,400 accessions, cultivars or lines were evaluated in this study, including:

- Commercial cultivars and elite breeding lines: 104 entries (Elite-104). These were from trials 17RB, 17HQ, and 16NV, each with 32 Upland cultivars and elite breeding lines, and trial 16B with 8 Pima cotton genotypes.
- A selected obsolete Upland cotton germplasm lines: 367 (AM-367).
- Upland lines from the National Cotton Germplasm Collection: 1,200 lines (US-610 and US-611).
- Exotic/Chinese Upland cotton germplasm lines: 225 (Exotic-225).
- A multi-parent advanced generation intercross (MAGIC) population: 550 recombinant inbred lines (RILs) and their 11 Upland parents (MAGIC-550).
- An introgressed MAGIC population: 330 lines and their 20 parents from random mating of crosses between 3 Upland cultivars and 17 chromosome substitution lines (CSLs) each with a pair of Pima 3-79 chromosomes/arms (RMBUP-330).
- A RIL population between resistant Pima S-6 and a Pima line: 170 (PimaRIL-170).
- Two RIL populations using resistant New Mexico Upland cultivars- NuMex COT 15 GLS and NuMex COT 17 GLS as parents: 190 (Upland-103 and Upland-95).
- Other advanced breeding lines and selections: 220 including Fabens-127.

Experimental Designs and Replications

For the 1,200 obsolete US Upland germplasm lines from the National Cotton Germplasm Collection, 10 plants per lines were evaluated in one replication first, due to its large size. Only the top most resistant lines were reevaluated in a second test with 10 plants per line.

For all the genetic populations and 367 obsolete germplasm lines, each test was arranged in a randomized complete block design (RCBD) with 2 replications (10 plants/line in each replication). Each test was repeated twice.

For commercial cultivars and elite lines, each of the trials (17RB, 17HQ, 16NV and 16B) was arranged in a (RCBD with 3 replications. Each test was repeated three times.

Inoculation Methods and Assessment of FOV4 Resistance

10 ml of 1×10^6 spores/ml conidial suspension of FOV4 were inoculated to emerged seedlings in each pot (10 plants/pot). The inoculum was pipetted onto the soil surface and lightly watered immediately after inoculation.

The foliar disease severity ratings were evaluated at 7, 14, 21, and 30 days post inoculation (dpi) using a 0-5 rating scale (Sanogo and Zhang, 2015), similar to these used by Zhang et al. (2012) for Verticillium wilt resistance, as the following:

0	no symptom
1	one wilted cotyledon
2	two wilted cotyledons or two cotyledons abscised
3	first true leaf wilted or three leaves abscised
4	whole plant wilted or more than three leaves abscised
5	dead plant

A Growth Chamber Study on Soil and Temperature

- Three types of soils: FOV4-infected farm soil, natural farm soil without FOV4, and commercial potting soil.
- Three temperature regimes: 20°C, 23°C, and 26°C.
- Six cotton lines with 3 replications in a RCBD.

Results and Discussion

Overview of Greenhouse Screening for FOV4 Resistance

A total of 2,258 lines were screened in a commercial potting soil in the greenhouse with a temperature range of 24 to 32ºC- high temperature (HT) regime (Table 1).

A total of 1,204 lines were screened in a FOV4-infected farm soil at a constant but lower temperature of 20ºC- low temperature (LT) regime (Table 2).

Table 1. Evaluation of cotton germplasm lines in a potting soil in the greenhouse at a high temperature (HT) regime ranging from 24-32ºC, 30 days post inoculation.

Study	No lines	Test	Incidence range	Incidence mean
AM-367	367	1	0.00-100.00	48.40
		2	100.00-100.00	100.00
Elite-104	104	1	53.24-100.00	96.06
		2	64.94-100.00	96.63
		3	26.75-100.00	63.38
MAGIC-550	550	1	0.00-100.00	89.43
		2	0.00-100.00	83.56
RMBUP-330	330	1	30.00-100.00	82.66
		2	56.25-100.00	96.78
US-610	610	1	0.00-100.00	77.85
		2	15.57-100.00	71.50
Fabens-127	127	1	0.00-100.00	66.67
PimaRIL-170	170	1	19.44-100.00	82.18
Mean/total	2258			81.16

Table 2. Evaluation of cotton germplasm lines in a FOV4-infected farm soil at a low temperature (LT) regime at 20ºC, 30 days post inoculation.

Study	No. lines	Test	Incidence range	Incidence mean
Upland-103	103	1	0.00-100.00	67.72
		2	16.50-100.00	90.98
Upland-95	95	1	57.82-100.00	95.60
		2	39.73-100.00	95.60
PimaRIL-170	170	1	59.82-100.00	93.81
Exotic-225	225	1	0.00-100.00	85.39
		2	0.00-100.00	72.68
US-611	611	1	0.00-100.00	92.30
Mean/total	1204			86.76

Temperature in Relation to Disease Severity Rating and Plant Mortality

Disease incidence did not differ between the two temperature regimes: 81.2% for HT vs. 86.8% for LT (Table 1 and 2). However, disease severity ratings significantly differed: 1.94 for HT vs. 3.86 for LT on a scale from 0 for a

healthy plant to 5 for plant death (Fig. 1). Plant mortality rates also significantly differed: 7.5% for HT vs. 81.7% for LT on a scale of 0-100% (Fig. 2).

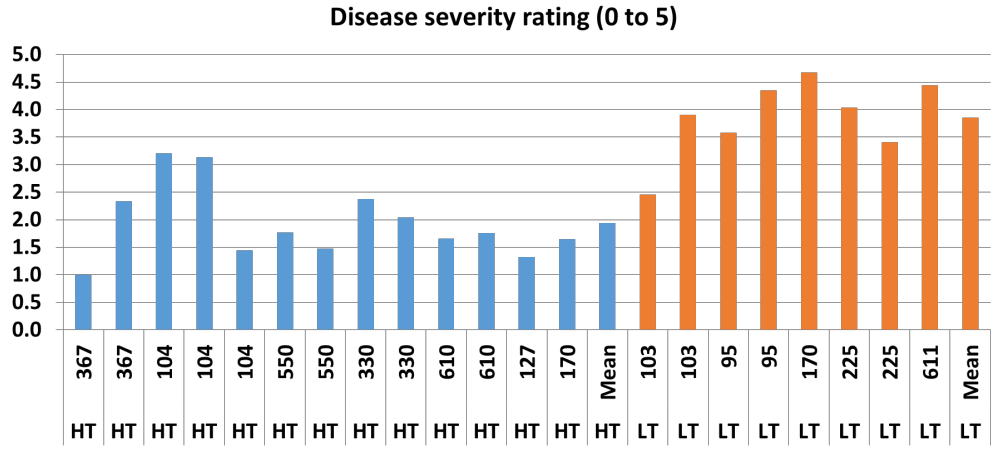

Fig. 1. Average foliar disease severity rating in different tests under a high temperature (HT) regime (24-32ºC) or a low temperature (LT) regime (20ºC).

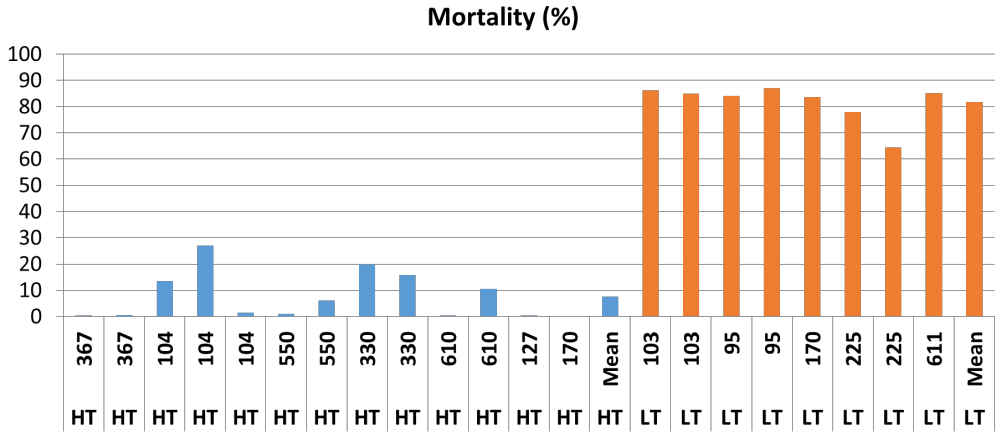

Fig. 2. Plant mortality in different tests under a high temperature (HT) regime (24-32ºC) or a low temperature (LT) regime (20ºC).

Time Course of FOV4 Infections at 20ºC

Within a week of inoculation, symptoms due to FOV4 appeared in a few of seedlings exhibiting one or two wilting cotyledons. Gradually, more plants displayed similar symptoms. Some plants showed necrotic or chlorotic leaves. Many seedlings dried up and died within two weeks of inoculation. At 30 dpi, most plants (~80%) died (Fig. 3).

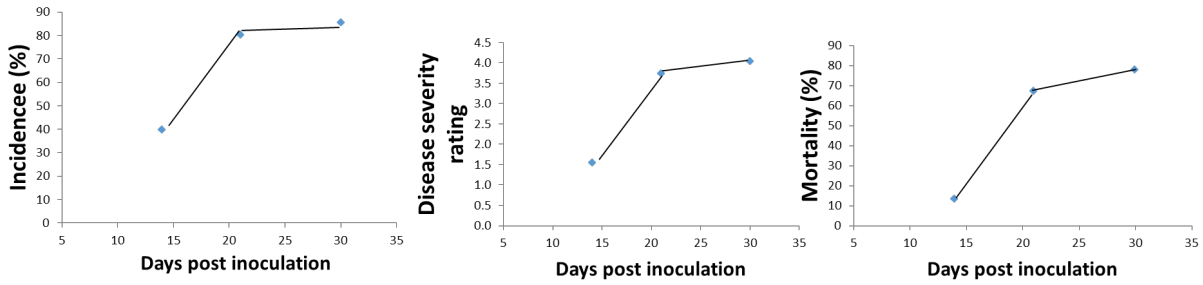

Fig. 3. Evaluation of cotton seedlings at 20°C.

A Growth Chamber Study on Temperature

At 23°C, FOV4 disease incidence, severity rating and plant mortality reached the highest. At 26°C, the plant damage due to FOV4 was lower than that at 20°C (Fig. 4).

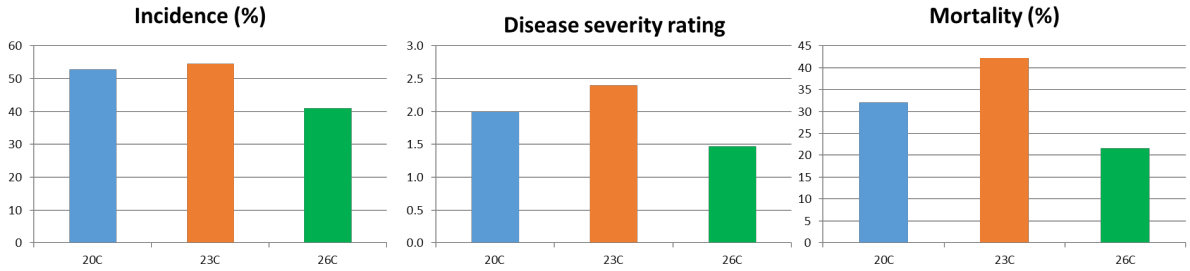

Fig. 4. FOV4 disease development at 20, 23 and 26°C, 21 days post inoculation.

A Growth Chamber Study on Soil Type

Using a FOV4-infected farm soil (at 10^4 spores/g), disease incidence, severity and plant mortality were not significantly higher than these using uninfected farm soil at 21 days post inoculation (Fig. 5). But both farm soils incurred a higher FOV4 damage than a commercial potting soil.

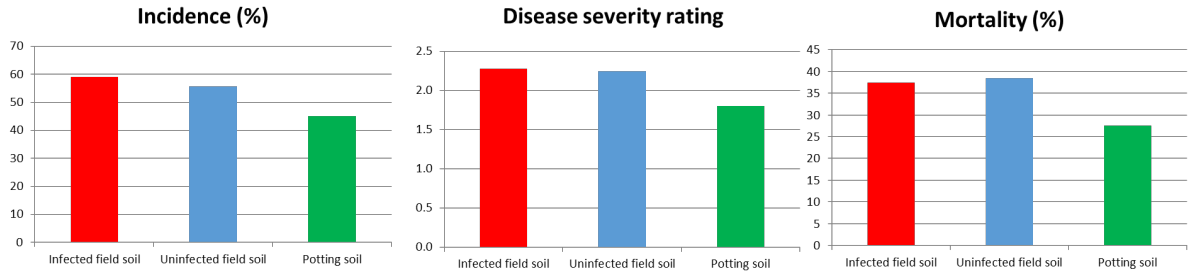

Fig. 5. FOV4 disease development in three different soil types, 21 days post inoculation.

Conclusions

1. Temperature is the key to successfully screen cotton for FOV4 resistance. 20-23°C causes the highest disease

incidence, severity and plant death in Upland cotton (80% or higher).

2. Using a FOV4 pre-infected farm soil is not necessary when artificial inoculation (at 1×10^6 spores per plant) is used. However, a farm soil is better than a commercial potting soil to induce higher FOV4 disease damage.

3. At 20-23ºC using a FOV4-infected farm soil, disease incidence, severity and plant mortality plateaued at 30 days post inoculation, and it is the best time to evaluate cotton for FOV4 resistance.

4. For example, using the above evaluation methods to screen a set of 225 exotic Upland germplasms, 60% of the lines showed 100% mortality; 37% of the lines had a mortality rate between 10 and 90%; and 3% of the lines did not display any apparent FOV4 symptoms.

5. Most Upland cotton lines are highly susceptible to FOV4 with 100% seedling mortality, but many existing germplasms may be heterogeneous in their resistance to FOV4. Pedigree selection may increase the frequencies of resistant plants.

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