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# Germplasm evaluation and transfer of Verticillium wilt resistance from Pima (*Gossypium barbadense*) to Upland cotton (*G. hirsutum*)

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**Abstract** Verticillium wilt (VW, *Verticillium dahliae*) is a worldwide destructive soil-borne fungal disease and employment of VW resistant cultivars is the most economic and efficient method in sustainable cotton production. However, information concerning VW resistance in current commercial cotton cultivars and transfer of VW resistance from Pima (*Gossypium barbadense*) to Upland (*Gossypium hirsutum*) cotton is lacking. The objective of the current study was to report findings in evaluating commercial cotton cultivars and germplasm lines for VW resistance in field and greenhouse (GH) experiments conducted in 2003, 2006, and 2007. In the study, 267 cultivars and germplasm lines were screened in the GH, while 357 genotypes were screened in the field. The results indicated that (1) VW significantly reduced cotton yield, lint percentage, 50% span length and micronaire, but not 2.5% span length and fiber strength, when healthy and diseased plants in 23 cultivars were compared; (2) some commercial cotton cultivars developed by major cotton seed companies in the US displayed good VW resistance; (3) many Acala cotton cultivars released in the past also had good VW resistance, but not all Acala cotton germplasm are resistant; (4) Pima cotton possessed higher levels of VW resistance than Upland cotton, but the performance was reversed when the root system was wounded after inoculation; (5) VW resistance in some conventional cultivars was transferred into their transgenic version through backcrossing; and (6) some advanced backcross inbred lines developed from a cross between Upland and Pima cotton showed good VW resistance. The successful development of VW resistant transgenic cultivars and transfer of VW resistance from Pima to Upland cotton implies that VW resistance is associated with a few genes if not a major one.

**Keywords** Upland cotton · Pima cotton · Verticillium wilt · Resistance · Interspecific breeding

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## Introduction

Verticillium wilt (VW, *Verticillium dahliae*) is a soil-borne fungal disease which causes annual cotton yield loss of 0.5–3.5% nationwide in the US and as high as 3.5–5.0% in the state of New Mexico (Blasingame and Patel 2005). The fungal hyphae penetrate root tips and enter the xylem vessels in the root system, where they proliferate and move throughout the xylem systems of the plants. Young cotton plants infected with *V. dahliae* show yellow or/and necrotic leaves, leaf shedding, stunted growth, wilting, vascular discoloration, and often die. Symptom development of VW is affected by many factors including pathogen population and virulence, cultivar, plant growth, temperature, soil microorganisms, irrigation, and fertilizers. The development and deployment of VW-resistant cultivars is the most efficient and economic method in management of the disease in cotton.

Cotton species and genotypes differ in resistance to VW, and *Gossypium barbadense* as a cultivated tetraploid species is highly resistant or tolerant (Wilhelm et al. 1974). In the past 50 years, cotton breeding has made substantial advance in progressively increasing the resistant level to VW. Some modern Acala cotton cultivars developed in California and New Mexico are highly resistant or tolerant such as Acala SJ-3, SJ-4, SJ-5, Germain's GC-510, and Acala Maxxa (Oakley 1998); and some other cultivars such as DP 51, DP Acala 90, ST 495, and HS 26 have moderate resistance (Smith et al. 1999). Resistance in Acala cotton is thought to be derived from *G. barbadense*. However, there has been no report to indicate the introgression of Pima germplasm to Acala cotton and the origin of the resistance (Zhang et al. 2005). Traditional genetic studies showed that resistance to VW in resistant *G. barbadense* and Upland cotton is polygenic with low to moderate heritabilities and with both additive and dominant genic effects (Verhalen et al. 1971; Cano-Rios and Davis 1981; Devey and Roose 1987; Aguado et al. 2008). Recent gene mapping studies using molecular markers have confirmed the results based on identification of multiple quantitative trait loci (QTL) for VW resistance (Bolek et al.2005; Wang et al. 2008; Yang et al. 2008; Jiang et al. 2009). Both genetic and QTL studies indicate difficulties in the transfer of VW resistance from *G. barbadense* to Upland cotton.

Since the mid-1990's, conventional cotton cultivars in the US, and some other major cotton-growing countries such as China and India, have been rapidly replaced by transgenic cotton, most of which were developed through backcrossing breeding. There have been numerous reports documenting the agronomic performance of these transgenic cotton cultivars in comparison with their non-transgenic counterparts (Jost and Cothren 2000; Verhalen et al. 2003; York et al. 2004; Bauer et al. 2006; Blanche et al. 2006). Due to the multi-genic nature of VW resistance in cotton, the resistance in some conventional cotton cultivars may be lost during the backcrossing process. However, whether the converted transgenic cultivars carry their parental resistance to VW remains unknown. To now, there are few reports on VW resistance in commercial cotton cultivars (Göre et al. 2009) and no report on VW resistance from breeding lines developed by interspecific crosses between Upland cotton and *G. barbadense*. The objectives of the present study were (1) to investigate the effects of cotton infection by *V. dahliae* on lint yield and fiber quality in the field; and (2) to screen cotton cultivars, germplasm, and breeding lines for VW resistance under both field and greenhouse (GH) conditions.

## Materials and methods

### Field evaluations

In Artesia Agricultural Science Center, Artesia, NM, USA, a field has been annually grown with Acala 1517 cotton for more than 50 years and has sustained a relatively uniform and heavy cotton infection by *V. dahliae*. This field naturally infested with *V. dahliae* served as a VW screening nursery for evaluating new cotton breeding lines and cultivars.

2003: The experiment was conducted to obtain data under field conditions on the severity of VW on 18 commercial cotton cultivars and five Acala 1517 cotton cultivars or strains from New Mexico and to compare the differences between healthy and infected plants in yield and fiber quality. To ensure a better VW infection, seeds were delinted and inoculated with *V. dahliae* (isolate BC, see below for more details) by soaking for 30 min in a conidia suspension containing $10^6$ conidia $ml^{-1}$ based on Bu and Li (1983). Similar seed-soaking techniques were also established for screening VW resistance in potato and strawberry (Jordan 1973; Platt and Sanderson 1987). The seeds

were air dried before sowing with a seeding rate of ten seed $m^{-1}$ in May 2003 with a row spacing of 1 m. The experiment was arranged in a randomized complete block design with three replications. The strip plot size was 1 row × 133 m long for convenience of mechanical planting. Cultural practices followed local recommendations. For simplicity, 50 consecutive plants in the middle of each plot were selected for destructive evaluation of VW based on vascular discoloration in the stem on Oct. 10, 2003. The 50 consecutive plants were cut above the soil line to assess stem infection by VW. The number of infected plants was divided by the total number of plants screened to calculate the percentage of infected plants (i.e. incidence). After the plants were mature, seedcotton from five healthy plants with no apparent leaf symptoms and five apparently heavily infected plants (see a VW scorinng system in the "GH screening" section for details) in each plot were bulk harvested, respectively. The seedcotton was weighed for yield determination and then ginned to determine lint percentage and lint yield (g $plant^{-1}$ was converted to kg $ha^{-1}$). A sub-sample of fibers from each plot was tested for fiber length, strength, and fineness at the Fiber Testing Lab of the Cotton Breeding and Genetics Program, New Mexico State University, Las Cruces, NM, USA.

2006: Entries consisting of 32 Acala cotton cultivars and germplasm lines were planted to the same field used in the 2003 experiment. The experiment was arranged in a randomized complete block design with two replicates. The plot size was 2 rows × 10 m long and the row spacing was 1 m. The cotton entries were planted on April 25, 2006. Cultural practices followed local recommendations. One row in each plot was used for destructive assessment of VW based on stem discoloration on Oct. 5, 2006, and for computing disease incidence following the same protocols used in the 2003 experiment. The other row was harvested for yield determination.

2007: The same 32 Acala genotypes evaluated in 2006 were re-tested in the same field using the same experimental design and plot size. Again, one row was used for destructive assessment of VW based on stem discoloration on Oct. 6, 2007. In the same field, 146 backcross inbred lines (BILs) and two parental lines-SG 747 (PVP 9800118) and Pima S-7, were tested in a randomized complete block design with two replicates. The plot size was 1 row × 10 m long. Evaluation of VW in the BILs and the parental lines was completed in the same manner as in other studies mentioned above. Cultural practices followed local recommendations.

In a separate field, in which severe and relatively uniform plant infections by *V. dahliae* had been observed, more than 100 breeding lines developed from our breeding program were divided into four tests, each of which was evaluated in a randomized complete block design with three replicates (2 rows × 6 m long). To assess stem infection, 30 plants per plot were cut at the soil line and discoloration in the stem was recorded. Disease incidence was computed as previously described.

## GH evaluations

2003: The investigation included nine Pima (*G. barbadense*) and 38 Upland cotton (*G. hirsutum*) genotypes. The seed was planted in a 10-cm plastic pot with four seed per pot (two hill per pot, two seed per hill) on January 18, 2003. The pots were filled with potting soil Scott 450 (Scott-Sierra Horticultural Products Co., Marysville, OH, USA) mixed with slow release Osmocote fertilizer (N : P : K = 14 : 14 : 14, Scott-Sierra Horticultural Products Co., Marysville, OH, USA). Each germplasm was grown in three pots.

*Verticillium dahliae* (isolate BC, a defoliation type), was isolated from field-infected cotton plants at the Leyendecker Plant Science Research Center, Las Cruces, NM. The isolate was grown on Czapek-Dox broth for 7 days at 22–25°C on a rotary shaker. The suspension was passed through a double-layer cheesecloth to separate spores from mycelia. The concentration of spores was determined using a hemacytometer, and appropriate dilutions were made to obtain a spore suspension with concentration of $10^5$ spores $ml^{-1}$. 35 days after planting, when seedlings were at 3–4 true leaf stage, root inoculation was made by drenching soil in each pot with 200 ml of spore suspension ($10^5$ spores $ml^{-1}$), resulting in $2.0 \times 10^7$ spores per pot.

After the disease symptom was first noticed 18 day after inoculation (DAI), evaluation was made three times (31, 40 and 78 DAI) for VW resistance based on total numbers of leaves, healthy, yellowish, necrotic or abscised leaves for individual plants. A rating system based on a scale from 0 to 5 (Wilhelm et al. 1974; Devey and Rosielle 1986; Devey and Roose 1987) was used to evaluate the plants for overall response to VW and a similar system was also established as a national

standard for screening cotton for VW resistance in China (Wu et al. 1999; Yang et al. 2008):

0. No symptom (healthy)
1. <25% chlorotic/necrotic leaves
2. 25–50% chlorotic/necrotic leaves
3. 50–75% chlorotic/necrotic leaves
4. >75% chlorotic/necrotic leaves
5. Complete defoliation or plant death

2007-Experiment A: The same 146 BILs tested in the field in 2007, together with the two parental lines were also evaluated in the GH in batches due to the high number of genotypes and limited GH space. In Batch 1, the lines tested were planted on July 3, and arranged in a randomized complete block design with three replicates with three pots per replicate per line. Seedlings were thinned to one plant per pot and inoculation with *V. dahliae* (isolate BC) was made on July 12 and 26 following the same method used in 2003. The inoculated plants were evaluated on Aug. 26 and 27 for VW resistance based on the aforementioned scoring system.

In Batch 2, the test was planted on July 2 and arranged in a randomized complete block design with two replicates with three pots per replicate per line. Seedlings were thinned to one plant/pot and inoculated with VW on Aug. 28. Evaluation for VW resistance was made on Sept. 30 in a same manner as for Batch 1 entries.

In Batch 3, the test was planted on Sept. 9 and arranged in a randomized complete block design with two replicates with three pots per replicate per line. Seedlings were thinned to four plants per pot and inoculated with VW on Sept. 24. The plants were screened for VW resistance on Oct. 27 in a same manner as for Batch 1 entries.

2007-Experiment B: Thirty-two Acala genotypes and 42 other commercial cotton cultivars were also tested in the GH. The 42 genotypes tested were mostly cultivars with the second generation of transgenic traits including Bollgard II (B2) and/or Roundup-ready Flex (RF) and these cultivars were submitted by seed companies for the official cotton variety test in New Mexico. The test was planted on Sept. 9 and arranged in a randomized complete block design with two replicates with three pots per replicate per line. Seedlings were thinned to four plants per pot and inoculated with *V. dahliae* (isolate BC) on Sept. 24.

To facilitate infection, the root system in each plant was wounded by pushing a 30-cm stainless steel or plastic ruler through the soil in the pots immediately after inoculation. In the 2007 experiments, yellowing and abscission of cotyledons were observed. For a better differentiation, these observations were taken into account by using a modified scoring system for VW resistance, as follows:

1. Healthy plants with no symptoms on leaves
2. One yellowish cotyledon
3. Two yellowish cotyledon
4. One cotyledon abscised
5. Two cotyledon abscised
6. Two cotyledon abscised and symptomatic true leaves
7. Complete defoliation
8. Dead plant

### Data analysis

The data was subjected to analyses of variance (ANOVA). For the two tests in the GH and field in 2003, separate ANOVA was performed first prior to the combined ANOVA using 23 common genotypes in both tests. For the comparative analysis between healthy and infected plants (as subplots) in the 23 genotypes (main plots), ANOVA based on a split-plot design was conducted. For the 42 commercial cotton cultivars tested in the GH in 2007, a two-way ANOVA was conducted. For the 32 Acala cotton genotypes tested in the fields in 2006 and 2007 and in the GH in 2007, a combined ANOVA was performed to partition genotype × test interactions (G × T). Due to the existence of G × T, separate ANOVA was then performed for the three individual tests. The same was done for the 146 BIL lines and their parents, but data from Batch 1 was excluded due to that plants in many entries did not incur any VW symptoms. The least significant difference (LSD) was used to compare differences between the cultivars or genotypes at $P = 0.05$.

## Results

### Effects of VW on lint yield and fiber quality

Across the 23 genotypes tested in the 2003 field experiment, the percentage of plants infected by

*V. dahliae* averaged at 27.81% with a range from 15.33% for NX 2429 to 49.33% for ST 457 (PVP 200200277; Table 1). Although no significant genotypic difference was detected for infection by *V. dahliae* since the *F* value for VW incidences was insignificant, FM 991 and NX 2429 had the lowest VW%; GC-546 RR (PVP 200200055), PHY 72 (PVP 200100115), PHY 78, STX 0003, DP 449 BR (PVP 200300135), DP 555 BR (PVP 200200047), NM 970513 and NM 970123 also had lower VW infection. However, ST 4892 BR (PVP 20000253), ST 457, and NM W1218 had the highest VW incidence.

Except for ST 457, lint yield in infected plants in all the genotypes was reduced by 17.2% in DP 458 B/RR (PVP 9800206) to 47.7% in FM 989 (PVP 200500107), as compared to their healthy plants (Table 1). On average, VW significantly decreased lint yield by 31.4% (1555 vs. 2266 kg $ha^{-1}$). For lint percentage, there was no significant difference between infected and healthy plants in six genotypes. The infected plants in 14 genotypes had lower lint percentage, but only three genotypes displayed an opposite trend. On average, the infected plants had significantly lower lint percentage (41 vs. 42%).

As far as fiber length was concerned, the difference in 2.5% span length between healthy and infected plants was with a range of −5 to 5% for the 23 genotypes and was within a 2% range in half of the genotypes. Overall, the difference (29.0 vs. 28.7 mm) was insignificant. However, infected plants in most of the genotypes tested, except for four genotypes, had reduced 50% span length (13.2 vs. 13.7 mm) and the difference (3.7%) was significant. In eight genotypes including DP 458 B/RR, DP 555 BR, BXN 49B (PVP 200200195), STX 0003, NX 2429, PHY 78, Acala 1517-99 (PVP 200000181), and All-Tex Atlas (PVP 9200188), the reduction was as high as 7–10%. The overall difference for fiber strength between healthy and infected plants was insignificant (216.7 vs. 218.5 kN m $kg^{-1}$). Surprisingly, most (15) of the genotypes tested had increased fiber elongation (by 1.57 to 18.56%) in their infected plants as compared to healthy plants, and the overall difference by 3.15% (9.2 vs. 9.5) was significant.

Interestingly, the most profound effect of VW was on micronaire, a measurement for fiber fineness and maturity. All the genotypes tested had reduced micronaire (3.71 vs. 4.59) by 6.04–27.77%. The average of reduction (19.17%) was significant (Table 1).

## VW resistance in commercial cotton cultivars evaluated in the GH without mechanical root wounding

Forty-seven cotton genotypes including mostly commercial transgenic Upland cotton cultivars were screened for VW resistance in the GH in 2003 (Table 2). The transgenic cultivars carried the first generation of transgenic traits including insect resistant Bollgard (B or BG) and/or herbicide resistant Roundup-Ready (R or RR). The percentage of infected plants with apparent symptom was low at 31 DAI, ranging from 0 to 83%. At 40 DAI, the percentage of infected plants increased to 66.7–100% except for three genotypes, indicating that the disease symptoms were progressing well. At 78 DAI, all the plants evaluated showed symptoms in most of the genotypes except for eight genotypes in which 77.8–91.7% plants showed VW symptoms. The results indicated that the inoculation was successful in that only a few plants escaped from VW infection at the third evaluation date (78 DAI), which ensured a lower experimenal error and a better differentiation for VW resistance between genotypes. Therefore, the disease rating results from the third evaluation date (78 DAI) were used for the final analysis. The disease rating from the second date (40 DAI) was significantly and positively correlated with the percentage of infected plants from the first date (31 DAI) and the disease rating from the third (78 DAI) date with coefficients of correlation ranging from 0.33 to 0.73. However, the rating results between 31 and 78 DAI were not significantly correlated.

In contrast to Upland cotton, more Pima cotton plants generally showed similar early symptoms (average infected plant percentage of 50.5 vs. 25.5% for Upland) such as yellowish or necrotic cotyledons or leaves, followed by vascular discoloration in later plant growth stages. However, there were much fewer completely defoliated or dead plants observed, indicating a good VW resistance in the Pima cotton germplasm (Table 2). In comparison with 57–4, a double haploid selection from obsolete Pima S-1 released in 1951, the modern Pima cultivars have improved VW resistance. The severity ratings for Pima S-6, PHY 57 (PVP 9900020), DP HTO (PVP 9800192) and DP 340 (PVP 200200111) were significantly lower than 57–4. Pima S-7 also had a lower disease rating, while Pima DP744 and PHY 76 were as susceptible as TM-1 and 57-4.

**Table 1** Effects of VW on lint yield and fiber quality in 23 Upland cotton genotypes, tested in VW heavily infected soil (Artesia, NM, USA, 2003)

| Genotype | VW (%) | Lint yield (kg ha$^{-1}$) | | Lint percent (%) | | 2.5% SL (mm) | | 50% SL (mm) | | Strength (kN m kg$^{-1}$) | | Elongation (%) | | Micronaire (unit) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H | I | H | I | H | I | H | I | H | I | H | I | H | I |
| DP 458 B/RR | 15.33 | 2522 | 1721 | 41 | 41 | 28.2 | 27.4 | 14.0 | 13.0 | 208.9 | 202.7 | 10.17 | 10.33 | 4.85 | 3.82 |
| DP 555 BR | 18.67 | 2360 | 1921 | 41 | 40 | 29.5 | 28.7 | 14.0 | 13.2 | 219.7 | 223.5 | 10.00 | 10.00 | 4.56 | 3.97 |
| DP 449 BR | 21.33 | 2159 | 1735 | 40 | 40 | 28.7 | 28.2 | 13.5 | 13.0 | 232.6 | 229.8 | 9.33 | 8.67 | 4.61 | 4.18 |
| SG 215 BR | 21.33 | 2581 | 1593 | 41 | 39 | 28.7 | 28.7 | 13.2 | 13.5 | 214.9 | 225.0 | 8.83 | 9.33 | 4.56 | 3.77 |
| ST 4892 BR | 22 | 2047 | 1481 | 40 | 39 | 29.0 | 27.4 | 14.0 | 12.7 | 215.0 | 212.5 | 10.33 | 11.17 | 4.47 | 3.83 |
| ST 457 | 22.67 | 2125 | 1564 | 41 | 40 | 27.4 | 27.4 | 13.5 | 12.4 | 215.3 | 224.8 | 9.33 | 9.00 | 4.70 | 3.53 |
| ST 580 | 22.67 | 2704 | 1869 | 42 | 40 | 29.2 | 27.9 | 14.0 | 12.7 | 208.3 | 219.2 | 9.00 | 9.33 | 4.67 | 3.83 |
| BXN 49B | 23.33 | 2184 | 1698 | 42 | 39 | 29.7 | 28.7 | 14.5 | 13.0 | 204.8 | 223.7 | 9.33 | 9.67 | 4.53 | 3.48 |
| STX 0003 | 24 | 2492 | 1640 | 40 | 40 | 29.0 | 29.2 | 13.2 | 14.0 | 208.7 | 202.7 | 9.00 | 9.67 | 4.15 | 3.27 |
| STX 5599 BR | 26 | 1799 | 1367 | 43 | 41 | 28.2 | 28.2 | 12.7 | 12.4 | 211.2 | 214.6 | 8.33 | 8.67 | 4.47 | 3.53 |
| NX 2429 | 26 | 2279 | 1192 | 38 | 41 | 29.2 | 30.2 | 15.0 | 14.0 | 245.4 | 227.6 | 8.00 | 9.00 | 4.73 | 3.85 |
| GC-546 RR | 26.67 | 2603 | 2155 | 40 | 41 | 30.2 | 28.7 | 14.0 | 12.4 | 218.2 | 220.6 | 9.67 | 9.33 | 4.53 | 3.65 |
| FM 989 | 26.67 | 2128 | 1402 | 40 | 38 | 29.7 | 29.7 | 13.7 | 13.5 | 238.5 | 246.7 | 9.00 | 9.67 | 4.73 | 3.65 |
| FM 989 BR | 27.33 | 2341 | 1266 | 42 | 39 | 29.7 | 30.0 | 14.2 | 14.5 | 227.9 | 241.3 | 9.00 | 9.33 | 4.53 | 3.36 |
| FM 991 | 29 | 2345 | 1610 | 41 | 41 | 30.0 | 28.4 | 13.7 | 13.2 | 214.7 | 205.4 | 8.50 | 9.00 | 4.43 | 4.03 |
| PHY 72 | 30 | 2194 | 1540 | 40 | 39 | 29.2 | 28.4 | 14.0 | 13.0 | 218.1 | 214.5 | 9.67 | 10.00 | 4.85 | 4.03 |
| PHY 78 | 30.67 | 2329 | 1357 | 39 | 40 | 30.7 | 29.5 | 14.5 | 13.5 | 213.2 | 220.6 | 9.33 | 8.67 | 4.26 | 3.59 |
| Acala 1517-95 | 31.33 | 2432 | 1369 | 41 | 39 | 27.9 | 28.2 | 13.5 | 13.2 | 202.8 | 206.0 | 9.33 | 10.00 | 4.47 | 4.20 |
| Acala 1517-99 | 32.67 | 1986 | 1170 | 40 | 40 | 27.9 | 27.9 | 13.5 | 13.0 | 210.6 | 210.7 | 9.67 | 9.00 | 4.85 | 3.91 |
| NM 970513 | 33.33 | 2626 | 1523 | 41 | 40 | 28.7 | 28.2 | 13.2 | 12.4 | 218.8 | 215.7 | 9.00 | 9.00 | 4.70 | 3.04 |
| NM 970123 | 38.67 | 2177 | 1385 | 40 | 39 | 29.0 | 30.5 | 13.5 | 13.2 | 224.0 | 223.8 | 8.67 | 8.33 | 4.61 | 3.33 |
| NM W1218 | 40.67 | 2039 | 1270 | 40 | 40 | 27.7 | 28.7 | 13.2 | 13.5 | 189.9 | 203.2 | 9.33 | 10.67 | 4.79 | 3.62 |
| All-Tex Atlas | 49.33 | 1659 | 1938 | 41 | 40 | 29.5 | 29.7 | 13.7 | 13.5 | 219.4 | 206.5 | 9.00 | 10.67 | 4.61 | 3.83 |
| Average | 27.81 | 2266 | 1555 | 41 | 40 | 29.0 | 28.7 | 13.7 | 13.2 | 216.6 | 218.3 | 9.21 | 9.50 | 4.59 | 3.71 |
| LSD (0.05) | ns | 554 | | 1 | | 0.5 | | 1 | | 25.7 | | 0.36 | | 0.58 | |

*VW%* percentage of plants with VW infection based on vascular discoloration of the stem from 50 consecutive plants in the middle of each plot, *ns* not significant, *LSD* least significant difference, *Lint percent* (%) percentage of lint weight in seedcotton weight harvested, *H* healthy plants, *I* infected plants, *DP* Delta and Pine Land Co. (now part of Monsanto), *ST* Stoneville Pedigreed Seed Co. (now part of Bayer CropScience), *FM* FiberMax (now part of Bayer CropScience), *PHY* phytogen (now part of Dow AgroScience)

**Table 2** GH evaluation of VW resistance in commercial cotton cultivars and lines (Las Cruces, NM, USA, 2003)

| Germplasm | Source | Type | VW%-DAI 31 | VW%-DAI 40 | VW%-DAI 78 | Rating-DAI 78 |
|---|---|---|---|---|---|---|
| FM 989 | FiberMax | Upland | 25.00 | 91.67 | 91.67 | 1.25 |
| FM 989 BR | FiberMax | Upland | 66.67 | 77.78 | 100.00 | 1.31 |
| DPLX 00513 BR | Delta and Pine Land | Upland | 8.33 | 75.00 | 100.00 | 1.33 |
| SG 125 | Delta and Pine Land | Upland | 16.67 | 33.33 | 91.67 | 1.50 |
| M315 (DP 60) | Delta and Pine Land | Upland | 0.00 | 66.67 | 100.00 | 1.67 |
| STX 5599 BR | Stoneville Pedigreed | Upland | 8.33 | 100.00 | 100.00 | 1.67 |
| ST 4892 BR | Stoneville Pedigreed | Upland | 16.67 | 81.82 | 90.91 | 1.92 |
| DP 449 BR | Delta and Pine Land | Upland | 16.67 | 91.67 | 100.00 | 2.00 |
| ST 4793 R | Stoneville Pedigreed | Upland | 41.67 | 91.67 | 100.00 | 2.00 |
| SG 215 BR | Delta and Pine Land | Upland | 15.38 | 75.00 | 75.00 | 2.03 |
| DP 458B/RR | Delta and Pine Land | Upland | 41.67 | 75.00 | 100.00 | 2.08 |
| HS 26 | Delta and Pine Land | Upland | 8.33 | 83.33 | 100.00 | 2.08 |
| STX 0003 | Stoneville Pedigreed | Upland | 16.67 | 91.67 | 100.00 | 2.28 |
| FM 989 R | FiberMax | Upland | 58.33 | 91.67 | 91.67 | 2.61 |
| DP 491 | Delta and Pine Land | Upland | 16.67 | 91.67 | 100.00 | 2.83 |
| DP 555 BR | Delta and Pine Land | Upland | 8.33 | 66.67 | 100.00 | 2.83 |
| PM 1560 BG | Delta and Pine Land | Upland | 0.00 | 72.72 | 100.00 | 2.83 |
| All-Tex Altas | All-Tex | Upland | 58.33 | 100.00 | 100.00 | 2.83 |
| SG 747 | Delta and Pine Land | Upland | 27.27 | 75.00 | 100.00 | 2.89 |
| BXN 49B | Stoneville Pedigreed | Upland | 16.67 | 90.91 | 100.00 | 2.89 |
| 33B | Delta and Pine Land | Upland | 30.43 | 79.41 | 100.00 | 2.99 |
| FM 991 R | FiberMax | Upland | 25.00 | 70.00 | 100.00 | 3.00 |
| NX 2429 | Syngenta | Upland | 41.67 | 100.00 | 100.00 | 3.33 |
| TM-1 | Delta and Pine Land | Upland | 71.43 | 100.00 | 100.00 | 3.89 |
| ST 580 | Stoneville Pedigreed | Upland | 36.36 | 81.82 | 100.00 | 3.89 |
| ST 457 | Stoneville Pedigreed | Upland | 16.67 | 90.91 | 90.91 | 4.11 |
| ST 474 | Stoneville Pedigreed | Upland | 0.00 | 20.00 | 100.00 | 4.56 |
| Acala PHY 72 | Phytogen | Acala | 50.00 | 83.33 | 100.00 | 1.25 |
| GC-546 RR | Stoneville Pedigreed | Acala | 58.33 | 100.00 | 100.00 | 2.00 |
| Nem-X | CPCSD | Acala | 0.00 | 11.11 | 77.78 | 2.06 |
| Maxxa | CPCSD | Acala | 0.00 | 22.22 | 100.00 | 2.22 |
| Acala 1517-99 | New Mexico University | Acala | 41.67 | 100.00 | 100.00 | 2.33 |
| NM 970123 | New Mexico University | Acala | 66.67 | 100.00 | 100.00 | 2.33 |
| NM W1218 | New Mexico University | Acala | 91.67 | 100.00 | 100.00 | 2.67 |
| NM 970513 | New Mexico University | Acala | 66.67 | 100.00 | 100.00 | 2.67 |
| Acala PHY 78 | Phytogen | Acala | 27.27 | 90.91 | 100.00 | 3.08 |
| PH 88 M-2983 | Phytogen | Acala | 63.64 | 90.91 | 100.00 | 3.33 |
| Acala 1517-95 | New Mexico University | Acala | 58.33 | 100.00 | 100.00 | 3.67 |
| Pima S-6 | USDA-ARS | Pima | 41.67 | 91.67 | 100.00 | 1.50 |
| PHY 57 | Phytogen | Pima | 72.73 | 81.80 | 100.00 | 1.64 |
| DP HTO | Delta and Pine Land | Pima | 33.33 | 75.00 | 100.00 | 1.71 |
| DP 340 | Delta and Pine Land | Pima | 41.67 | 91.67 | 100.00 | 2.04 |
| Pima S-7 | USDA-ARS | Pima | 50.00 | 100.00 | 100.00 | 2.08 |
| CH007 | Unknown | Pima | 83.33 | 100.00 | 100.00 | 2.42 |

**Table 2** continued

| Germplasm | Source | Type | VW%-DAI 31 | VW%-DAI 40 | VW%-DAI 78 | Rating-DAI 78 |
|---|---|---|---|---|---|---|
| DP 744 | Delta and Pine Land | Pima | 81.82 | 100.00 | 100.00 | 2.86 |
| PHY 76 | Phytogen | Pima | 25.00 | 87.50 | 87.50 | 3.03 |
| 57-4 | USDA-ARS | Pima | 25.00 | 43.75 | 100.00 | 3.83 |
| LSD(0.05) | | | | | | 1.84 |

*VW%* percentage of plants with VW symptoms in each replication, *DAI* days after inoculation, *Rating* degree of disease severity ranging from an 0 to 5 grading system: zero for no symptom in a plant to five for complete defoliation or death of a plant, *CPCSD* California Planting Cotton Seed Distributors (now part of Bayer CropScience), *LSD* least significant difference

There was also a significant genetic variation in VW resistance within the commercial Upland cotton cultivars, some of which exhibited a desirable VW resistance. In comparison with ST 474 (PVP 9400152) with the highest disease severity rating (4.56), 28 of 47 cultivars displayed significantly lower VW ratings, indicating better resistance. In comparison with the VW susceptible Upland cotton genetic standard, TM-1 (with a disease rating of 3.89), cultivars SG 125, SG 215 BR, DPLX 0513 BR, and DP 449 BR (PVP 200300135) from Delta and Pine Land Seed Co. (DP) showed significantly lower disease ratings (Table 2). M315, a root-knot nematode resistant germplasm with DP 60 background, was also resistant. ST 5599 BR (PVP 200300279), ST 4892 BR and ST 4793 R (PVP 200100213) from Stoneville Pedigreed Seed Co. (ST, now part of Bayer CropScience), and FM 989 and FM 989 BR from Bayer CropScience also had significant VW resistance. GC-546 BR and HS 26 (PVP 8600087) were confirmed to be moderately resistant to VW.

The Acala cotton cultivars or breeding lines tested had lower VW ratings than TM-1, with most ranging from 2 to 2.5, but only PHY 72 and GC-546 RR had significantly lower VW ratings (Table 2). New Acala cotton (1517-99, NM 970123, 970513, and W1218) developed in New Mexico after 1995 had better VW resistance than Acala 1517-95. However, Acala PHY 78 and Acala 1517-95 had similar VW severity ratings as TM-1, showing high VW susceptibility. Therefore, similar to Pima cotton, not all the Acala cultivars are resistant to VW.

Interestingly, ST 4892 BR and ST 4793 R, which are sister cultivars derived from the same genetic background ST 474, showed significantly higher VW resistance than their backcross parent, ST 474, which is highly susceptible to VW in the test (Table 2). BXN 49 B also had the ST 474 background, but it showed more tolerance (2.89) than the latter. The results indicate that the VW resistance in the transgenic version of ST 474 was likely derived from the donor parents of the transgenic traits. Similar results were obtained from the Bayer CropScience's FM (FiberMax) cultivars. Cultivars from FiberMax were initially developed from Australia and possibly contained Acala cotton background. FM 989 was resistant to VW, so was its transgenic stacked version FM 989 BR (average rating was 1.25 and 1.31, respectively); however, its herbicide resistant version FM 989 R was not as resistant with a severity rating of 2.61 (Table 2). Therefore, unintentional selection in the process of developing transgenic cultivars could result in random fixation of VW resistance gene(s) including the loss of VW resistance from the recurrent parent or gain of VW resistance from the donor parent.

## VW resistance in commercial cotton cultivars evaluated in the GH with mechanical root wounding

Mechanically wounding roots accelerated the development of VW infections in that, at 33 DAI, 24.4–84.0% (with an average of 55.8%) plants in Upland cotton showed symptoms, while almost all the plants (99.2%) in Pima cotton showed symptoms (Table 3). The difference in VW severity ratings between Upland (mean rating of 3.1; range of 1.54–5.17) and Pima cotton (mean rating of 5.1; range of 4.58–6.04) was significant based on an orthogonal contrast between the two groups. There were no significant differences in VW severity ratings among the ten Pima cotton genotypes tested. The results clearly indicate that the VW resistance in Pima was lost when roots were mechanically wounded at the seedling emergence.

**Table 3** GH evaluation of VW resistance in commercial cotton cultivars and lines by mechanical wounding (Las Cruces, NM, USA, 2007)

| Germplasm | Type/Source | Type | VW% | Rating |
|---|---|---|---|---|
| ST 4498 B2RF | Stoneville Pedigreed | Upland | 24.36 | 2.04 |
| ST 5458 B2RF | Stoneville Pedigreed | Upland | 37.50 | 2.08 |
| DP 161 B2RF | Delta and Pine Land | Upland | 41.67 | 2.29 |
| DP 164 B2RF | Delta and Pine Land | Upland | 37.50 | 2.42 |
| ST 4596 B2RF | Stoneville Pedigreed | Upland | 33.33 | 2.46 |
| PHY 375 | Phytogen | Upland | 54.17 | 2.50 |
| FM 1840 B2F | FiberMax | Upland | 27.24 | 2.56 |
| DP 143 B2RF | Delta and Pine Land | Upland | 54.17 | 2.58 |
| DP 174 RF | Delta and Pine Land | Upland | 50.00 | 2.75 |
| ST 4427 B2RF | Stoneville Pedigreed | Upland | 62.50 | 2.75 |
| PHY 315 | Phytogen | Upland | 41.67 | 2.75 |
| ST 6351 B2RF | Stoneville Pedigreed | Upland | 41.67 | 2.96 |
| ST 4554 B2RF | Stoneville Pedigreed | Upland | 66.67 | 3.00 |
| 65027 RF | All-Tex | Upland | 70.83 | 3.04 |
| PHY 485 WRF | Phytogen | Upland | 58.33 | 3.04 |
| FM 1880 B2F | FiberMax | Upland | 41.67 | 3.21 |
| SUMMIT B2/RF | All-Tex | Upland | 50.00 | 3.38 |
| DP 455 BR | Delta and Pine Land | Upland | 79.17 | 3.50 |
| APEX B2/RF | All-Tex | Upland | 79.17 | 3.50 |
| 65352 B2/RF | All-Tex | Upland | 71.79 | 3.84 |
| ST 5327 B2RF | Stoneville Pedigreed | Upland | 70.83 | 3.92 |
| 65016 RF | All-Tex | Upland | 83.97 | 4.04 |
| PM 3535 BR | Delta and Pine Land | Upland | 62.50 | 4.08 |
| ST 4357 B2RF | Stoneville Pedigreed | Upland | 66.67 | 4.08 |
| 65219 B2/RF | All-Tex | Upland | 79.17 | 4.46 |
| ST 4678 B2RF | Stoneville Pedigreed | Upland | 79.17 | 4.50 |
| DP 141 B2RF | Delta and Pine Land | Upland | 75.00 | 5.17 |
| PHY 725 RF | Phytogen | Acala | 25.00 | 1.54 |
| PHY 745 WRF | Phytogen | Acala | 41.67 | 1.92 |
| PHY 710 R | Phytogen | Acala | 62.50 | 2.50 |
| PHY 755 WRF | Phytogen | Acala | 54.17 | 2.96 |
| Acala 1517-99 W | New Mexico State University | Acala | 83.33 | 3.29 |
| Pima DP353 | Delta and Pine Land | Pima | 100.00 | 4.58 |
| Pima NM 06E1012 | New Mexico State University | Pima | 100.00 | 4.79 |
| Pima NM 06E1061 | New Mexico State University | Pima | 100.00 | 4.88 |
| Pima DP 744 | Delta and Pine Land | Pima | 100.00 | 4.92 |
| Pima Colbalt | Bayer CropScience | Pima | 91.67 | 5.04 |
| Pima S-7 | USDA-ARS | Pima | 100.00 | 5.07 |
| Pima DP 340 | Delta and Pine Land | Pima | 100.00 | 5.21 |
| Pima DP 353 | Delta and Pine Land | Pima | 100.00 | 5.29 |
| Pima PHY 800 | Phytogen | Pima | 100.00 | 5.33 |
| Pima NM 06E2032 | New Mexico State University | Pima | 100.00 | 6.04 |
| LSD (0.05) | | | 30.58 | 1.85 |

*VW%* percentage of plants with VW, *Rating* degree of disease severity ranging from one for no symptom to eight for dead plants, *LSD* least significant difference

Genotypic variation in VW resistance within Upland cotton was also noted (Table 3). Cultivars from FM and PHY continued to show better VW resistance with average ratings of 2.89 and 2.46, respectively, while cultivars from All-Tex had higher disease incidence (72.5%) and severity (3.71). Cultivars from DP and ST had similar disease incidence and severity (57.1 vs. 53.6%; 3.26 vs. 3.09). Most PHY cultivars displayed better VW resistance, especially PHY 725 RF and PHY 745 WRF with the highest VW resistance among the cultivars tested. DP 161 B2RF (PVP 200700413), DP 143 B2RF (PVP 200700011) and DP 164 B2RF (PVP 200700010) from DP, ST 4498 B2RF (PVP 200800230), ST 5458 B2RF (PVP 200800229) and ST 4596 B2RF from ST and FM 1840 B2F also showed better VW resistance.

## VW resistance in Acala cotton cultivars and germplasm

Representative Acala cotton cultivars released from New Mexico and California since the 1930s were screened for VW resistance in both the GH (2007) and the fields (2006 and 2007). Due to the fact that genotype × test interactions were detected, LSD was given for each test and the overall means across the three tests (Table 4). In comparison to one of the earliest Acala genotype Acala Original, Acala Young, Acala 1517-E2, Acala 1517-95, Acala 5, Acala SJ-3 and Acala SJ-4 displayed significantly higher levels of resistance in both 2006 and 2007 (Table 4). Acala 1517-SR3, Acala 1517-99, Acala Tex, Acala 1517-77BR in 2006, and Acala 1517-75, 1517-88, 1517-91, 1517-95, 1517-SR2 and Acala 29 in 2007 also showed some levels of VW resistance.

However, in the GH when roots were mechanically wounded immediately after inoculation at the seedling emergence, Acala 1517-88 and 1517-70 had the lowest ratings. Acala 1517-77BR, 1517C and Acala SJ-2 also showed signficantly lower VW incidences than Acala Original. Acala Young and 1517 C had signficantly lower disease severity ratings (Table 4). Again, Acala germplasm and cultivars from New Mexico and California contain VW resistance, but not all of them are resistant. The results in the field under natural infections were different from these in the GH when roots were wounded for infection.

## VW resistance in breeding lines derived from advanced backcrossing using Pima cotton (*G. barbadense*) as the donor parent

To broaden the Upland cotton genetic base and to introduce genes for VW resistance, fiber quality and other desirable traits into Acala cotton, hybridization between Upland SG 747 and Pima S-7 cotton and backcrosses were made for two generations using SG 747 as the recurrent parent. The resulting $BC_2F_1$ were selfed three times to develop $BC_2F_4$-derived BIL. One hundred and forty-six BIL lines, together with their parental lines were tested in both the GH (three tests) and field (one test), and a number of breeding lines from further pedigree selections were also tested in the field. Once again, ANOVA analyeses indicated significant effects from genotypes, tests and genotype × test interactions. Consistent with the earlier results in the present study, Pima S-7 showed better VW resistance than SG 747 in the field, but the trend was reversed in the GH when roots were wounded after VW inoculation. Compared with the resistant parent Pima S-7, several BIL lines including NMHT-15 and NMHT-65 showed consistently better VW resistance under the field and GH conditions (Table 5). Seven other BIL lines in three tests also showed better VW resistance (data not shown).

In the 2007 field experiment, 64 breeding lines derived from selections from the BIL populations were also tested for VW resistance. In comparison with the commercial Acala cotton cultivar 1517-99W, two lines had 45–50% reduction in VW incidence and nine other lines had >20% reduction in VW% (data not shown). This indicates that the resistance of VW from Pima cotton has been successfully transferred into Upland cotton.

Due to the genotype × test interactions, the correlations between different GH tests and the field test of the 146 lines and the two parents were positive, but not highly consistent. GH Test 1 and 3 were significantly correlated with the field test in VW incidences, i.e. percentage of diseased plants ($r = 0.175–0.252$, $r_{0.05} = 0.161$) and severity ratings ($r = 0.186–0.264$). However, results from Test 2 in the GH did not correlate with these from GH Test 1 and 3, but positively correlated with the results from the field ($r = 0.131–0.145$).

**Table 4** GH (Las Cruces, NM, USA, 2007) and field (Artesia, NM, USA, 2006-2007) evaluation of VW resistance in Acala cotton cultivars and lines

| Germplasm | Year of release | VW%-06 | VW%-07 | VW%-GH 07 | VW%-avg | Rating-GH 07 |
|---|---|---|---|---|---|---|
| Acala Young | 1929 | 38.00 | 8.00 | 60.12 | 35.37 | 2.37 |
| Acala 1064 | 1937 | 51.00 | 23.00 | 66.67 | 46.89 | 2.58 |
| Acala 1517 | 1939 | 50.00 | 29.00 | 62.50 | 47.17 | 2.63 |
| Acala 1517 C | 1958 | 55.00 | 18.00 | 50.00 | 41.00 | 2.13 |
| Acala 1517 D | 1960 | 43.00 | 22.00 | 70.83 | 45.28 | 3.88 |
| Acala 1517 BR2 | 1961 | 71.96 | 20.00 | 75.00 | 55.65 | 2.79 |
| Acala 1517-70 | 1970 | 57.85 | 19.00 | 33.33 | 36.73 | 1.67 |
| Acala 1517-75 | 1975 | 46.00 | 13.00 | 87.50 | 48.83 | 3.67 |
| Acala 1517-E2 | 1978 | 39.00 | 11.00 | 70.83 | 40.28 | 3.38 |
| Acala 1517-77 BR | 1982 | 30.00 | 22.00 | 37.50 | 29.83 | 2.04 |
| Acala 1517 SR2 | 1986 | 46.00 | 16.00 | 54.17 | 38.72 | 2.67 |
| Acala 1517-88 | 1988 | 49.50 | 16.00 | 29.17 | 31.56 | 1.42 |
| Acala 1517-SR3 | 1990 | 40.00 | 36.00 | 75.00 | 50.33 | 3.08 |
| Acala 1517-91 | 1991 | 62.38 | 16.00 | 75.00 | 51.13 | 2.79 |
| Acala 1517-95 | 1995 | 40.00 | 2.00 | 66.67 | 36.22 | 2.92 |
| Acala 1517-99 | 1999 | 31.00 | 38.00 | 83.33 | 50.78 | 2.96 |
| Acala Mesilla Valley | Unknown | 58.21 | 19.00 | 83.33 | 53.51 | 3.28 |
| Acala 4-42 | 1942 | 70.06 | 27.00 | 58.33 | 51.80 | 2.58 |
| Acala 44 WR | Unknown | 49.08 | 35.00 | 71.79 | 51.96 | 3.32 |
| Acala 5 | Unknown | 39.13 | 11.00 | 66.67 | 38.93 | 2.88 |
| Acala 2 | Unknown | 46.11 | 37.00 | 79.17 | 54.09 | 2.54 |
| Acala 29 | 1944 | 61.11 | 13.00 | 95.83 | 56.65 | 4.29 |
| Acala 51 | 1953 | 65.00 | 36.00 | 66.67 | 55.89 | 2.71 |
| Acala 40 | Unknown | 59.50 | 22.00 | 75.00 | 52.17 | 3.42 |
| Acala SJ-3 | 1974 | 36.00 | 10.00 | 66.67 | 37.56 | 2.92 |
| Acala SJ-4 | 1975 | 35.77 | 9.00 | 70.83 | 38.53 | 3.33 |
| Acala SJ-2 | 1973 | 65.00 | 27.00 | 44.32 | 45.44 | 2.46 |
| Acala Shafter Station | Unknown | 63.00 | 27.00 | 83.33 | 57.78 | 3.17 |
| Acala Wilt | Unknown | 64.00 | 22.00 | 45.83 | 43.94 | 2.67 |
| Acala Original | 1906 | 69.48 | 41.00 | 83.97 | 64.82 | 3.81 |
| Acala 8 | 1917 | 49.00 | 20.00 | 66.67 | 45.22 | 2.79 |
| Acala Tex | 1906 | 36.00 | 22.00 | 79.17 | 45.72 | 3.21 |
| LSD (0.05) | | 29.39 | 27.06 | 34.25 | 13.27 | 1.39 |

*VW%* percentage of plants with VW, *Rating* degree of disease severity ranging from one for no symptom to eight for dead plants, *GH* greenhouse, *avg* average, *LSD* least significant difference

## Discussion

### Effects of VW on lint yield and fiber quality

Cotton plant growth is affected by infection by *V. dahliae* to different extent with display of symptoms including yellow or/and necrotic leaves, leaf shedding, stunted growth, wilting, vascular discoloration, or death, leading to yield loss (Bassett 1974; Pullman and Devey 1982; Bejarano-Alcazar et al. 1997). Using data from two cotton cultivars in three successive years, Paplomatas et al. (1992) showed that the relationship between different inoculum density (propagules per gram of soil) of *V. dahliae* at planting time, and the disease incidence (percent foliar symptoms) followed a negative exponential curve, with correlation

**Table 5** Selected breeding lines of an advanced backcross population evaluated in the GH (Las Cruces, NM, USA, 2007) and field (Artesia, NM, USA, 2007) for VW resistance

| Genotype | GH-07 Rating-avg | GH-07 VW%-avg | Field-07 VW% |
|---|---|---|---|
| NMHT-15 | 2.00 | 54.17 | 7.00 |
| NMHT-37 | 1.50 | 35.67 | 17.00 |
| NMHT-65 | 1.04 | 24.67 | 13.00 |
| NMHT-118 | 1.17 | 29.00 | 24.00 |
| NMHT-124 | 1.04 | 16.67 | 26.00 |
| SG 747 (P1) | 2.96 | 79.33 | 32.00 |
| Pima S-7 (P2) | 3.75 | 91.50 | 14.00 |
| Min | 1.04 | 16.67 | 7.00 |
| Max | 5.09 | 100.00 | 54.00 |
| Mean | 2.76 | 62.28 | 27.25 |
| LSD (0.05) | 1.74 | 36.76 | 23.91 |

*NMHT* backcross inbred lines derived from two generations of backcrossing between SG 747 and Pima S-7 followed by three generations of selfing, *P1* recurrent parent, *P2* donor parent, *GH* greenhouse, *VW%* percentage of plants with VW symptoms, *Rating* degree of disease severity ranging from one for no symptom to eight for dead plants, *avg* average, *LSD* least significant difference

coefficients ranging from 0.71 to 0.96. The correlations between soilborne populations of the pathogen and ratios of lint yields of a VW-tolerant cultivar (Acala GC-510) over a susceptible cultivar (Acala SJ-2) were high (0.88–0.95). At higher inoculum density, the tolerant cultivar Acala GC-510 had higher lint yield than the susceptible cultivar Acala SJ-2. In the current study, apparently healthy (no symptoms) and infected (with symptoms) plants in 23 cotton cultivars with various VW resistances were compared for lint yield and fiber quality in the field with an average VW incident rate of 27.8%. Averaged across the 23 genotypes, in comparison to the healthy plants, plants with VW had a significantly reduced cotton yield, lint percentage, 50% span length and micronaire. But 2.5% span length and fiber strength were not affected, as expected, because fiber length is determined early in boll development (20–30 days post-anthesis). Surprisingly, fiber elongation in the infected plants was significantly higher than the healthy plants. VW deleteriously affects plant growth and development due to reduced phytosynthesis or premature defoliation, which in turn reduces the number of mature bolls and boll weight. Together with reduced lint percentage (by 2.38%), the present study indicated that lint yield can be reduced by more than 31%. The insufficient supply of phytosynthates would affect fiber maturity and cellulose deposition in developing fibers, resulting in shorter fibers, as demonstrated by reduced 50% span length, and more immature fibers (i.e. lower micronaire in the present study). The reduction in 50% fiber span length may in turn decrease fiber uniformity (a ratio between 50% span length and 2.5% span length). In fact, poor quality including low micronaire has long been associated with severe VW infection (Bugbee and Sappenfield 1970).

## Resistance in commercial cultivars

Even though there have been conference reports on evaluation of obsolete and commercial cotton cultivars for VW resistance (Wallace and Batson 1997; Wheeler and Schuster 2006; Wheeler 2007), no formal comprehensive paper has been published to address the issue. In the current study, 47 and 42 cotton genotypes, most of which were transgenic commercial cultivars representing the first and second biotech traits in cotton, repsectively, were screened for VW resistance in the GH in 2003 and 2007, respectively (Table 2, 3). The results showed that, except that all the six cultivars tested for All-Tex (a Texas High Plains cotton seed company) did not show any VW resistance (Table 3), all the major cotton seed companies have developed VW resistant cultivars such as, DPLX 00513 BR, DP 449 BR, SG 125, DP 161 B2RF, and DP 164 B2RF from Delta and Pine Land Seed Co. (now part of Monsanto); ST 5599 BR, ST 4892 BR, ST 4793 R, ST 4498 B2RF, ST 5458 B2RF, and ST 4596 B2RF from Stoneville Pedigreed Seed (part of Bayer CropScience); FM 989, FM 989 BR, and FM 1840 B2F from FiberMax (part of Bayer CropScience); and Acala PHY 72, PHY 725 RF, PHY 745 WRF, PHY 710 R, and PHY 375 from Phytogen Seed (part of Dow AgroScience). The VW resistance in the transgenic derivatives of FM 989 such as FM 989 BR, and DP 161 B2RF and DP 164 B2RF was confirmed by the field studies of Wheeler and Schuster (2006) and Wheeler and Woodward (2009) in Texas. The present study also indicates that Acala and Pima cottons are generally more resistant than Upland cotton, but not all of them are resistant. The results also demonstrate that breeding for VW resistance in the US continues to make progress. As a result, yield loss due to VW has

decreased in recent years (Blasingame 2002; Blasingame and Patel 2005, 2011).

However, when cotton genotypes were tested in field nursery soil infested with *V. dahliae*, results between the field and GH may not be congruent. When the same 23 and 32 genotypes were tested in both the field and GH in 2003 and 2007, respectively, the correlation between the disease incidences in the field and the severity ratings in the GH in 2003 and the correlation between disease incidences in the field and the GH were positively but not significantly correlated ($r = 0.156–0.169$, $r_{0.05} = 0.361–0.433$). The lack of correlation between the field and GH is likely due to several factors including differences in inoculum level and environmental conditions during infection and symptoms development. However, the results from the individual tests were highly significantly correlated with the average across the field and GH tests ($r = 0.630–0.711$, $r_{0.01} = 0.463–0.549$). Therefore, a combined screening from both the GH and field evaluations will be more reliable to assess cotton for VW resistance.

### Inoculation with root wounding

The hyphae of *V. dahliae* enter the xylem vessels in the root system by penetrating root tips in cotton plants. Defense in hindering the hyphae penetration represents the first line of resistance mechanism to *V. dahliae*. Artificial inoculation by mechanical wounding of the cotton root system would dismantle this line of defense in cotton. In this study, without mechanical root wounding, Pima cotton (*G. barbadense*) was found to be more resistant than Upland cotton. However, root wounding resulted in higher susceptibility of Pima than Upland cotton. The results clearly demonstrated that Pima cotton possesses some levels of anti-penetration mechanism against infection by *V. dahliae*. Mace et al. (1990) indicated that desoxyhemigossypol was the major phytoalexin in the Pima resistance response. Therefore, more detailed studies will be needed to understand the resistance mechanisms.

### Transfer of VW resistance from Pima to Upland cotton and inheritance nature of VW resistance

The present study provides the first line of evidence that VW resistance in Pima cotton (*G. barbadense*) has been successfully transferred into Upland cotton though backcrossing and repeated selfing followed by pedigree selections (Table 5). This was also evident in a released introgressed line, NM 24016 (Cantrell and Davis 2000), through pedigree selection from a cross between Pima and Upland (Zhang et al. unpublished). The success in the transfer of VW resistance from Pima to Upland through backcrossing or pedigree selection under non-VW conditions indicates that the genetic basis of VW resistance may not be complicated. Furthermore, examples in successful development of many VW resistant commercial Upland cotton cultivars is also an indication that VW resistance is associated with a few genes if not a major one. However, the challenge in the genetic study on VW resistance resides on the establishment of a reliable and consistent VW screening system with minimal experimental errors, as demonstrated here in the GH and field studies.

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