RESPONSE OF COTTON CULTIVARS TO 2015 BACTERIAL BLIGHT CAUSING ISOLATES IN FIELD AND GREENHOUSE TRIALS

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Abstract

Bacterial blight, which is caused by *Xanthomonas citri* subsp. *malvacearum*, was found on cultivars that are normally considered highly resistant to this disease in 2015. The disease was found on highly resistant cultivars in Amherst, Halfway, Lubbock, Plains, and Victoria, TX. A collection of over 100 isolates of the bacterium was made, which included entries from 16 locations in 2015. A small plot cultivar test in Plains, TX averaged 1.5% incidence of blight for resistant cultivars and 50.9% incidence of blight for susceptible cultivars. There was an average of 10% yield loss associated with the susceptible cultivars. So, clearly resistance did not diminish in any dramatic fashion, but this was the first time since resistant Fibermax cultivars were first planted in the region (since 2000) that any bacterial blight symptoms had been observed on multiple plants (i.e. in disease foci) on resistant cultivars. Greenhouse trials were conducted with these isolates. Water soaked symptoms were found on nearly 100% of inoculated Deltapine (DP) 1454NRB2RF, 58% of DP 1044B2RF, 15% Phytogen (PHY) 375WRF, 15% of Fibermax (FM) 1830GLT, and 8% of FM 2484B2F. Isolates from different locations behaved similarly on DP 1454NRB2RF, but incidence of water soaking could vary on the other cultivars by location of the isolate. Average water soaking symptoms ranged from 30 to 74% incidence on DP 1044B2RF (by location of isolate), from 1 to 41% on PHY 375WRF, from 2 to 48% on FM 1830GLT, and from 0 to 20% on FM 2484B2F. For a race shift to occur, it is expected that consistent water soaking symptoms would occur on a previously identified resistant cultivar (to race 18 of *X. citri* subsp. *malvacearum*). The greenhouse work was inconclusive as to whether minor gene(s) have been affected by some of these populations. It does not appear that a gene with a major effect on *X. citri* subsp. *malvacearum* has become ineffective. Resistant cultivars to race 18 are still the best method of controlling bacterial blight

Introduction

Bacterial blight in cotton is caused by *Xanthomonas citri* subsp. *malvacearum*. This pathogen is distributed worldwide (Hillocks, 1992). Symptoms of the disease include angular leaf spots (Fig. 1A), which are water soaked on the underside of the leaf (Fig. 1B), veinal water soaking (Fig. 1C), defoliation, and boll rot lesions (Fig. 1D). Race designations of a pathogen are a way of categorizing the ability of a population to reproduce on cultivars with different resistance genes. Brinkerhoff (1963) and Bird (1986) identified a set of ten cultivars that have been used to differentiate 19 races of *X. citri* subsp. *malvacearum* (Hunter et al., 1968). S295 was added to identify races 20 - 22 (Delannoy et al., 2005; Girardot et al, 1986; Wallace and El-Zik, 1989). The Texas A&M cotton breeding program previously maintained a set of bacterial isolates representing different races, and the set of differential cultivars that were used to identify different races. In 1991, 86% of the isolates in Texas were identified as race 18 and 14% were race 11 (Thaxton et al., 1992). They indicated that a shift had occurred in Texas, because earlier reports found that races 1 and 2 were the predominant races. In a survey conducted in 2000, samples were collected from Louisiana, Missouri, New Mexico, North Carolina, and Texas. All isolates tested positive for race 18, except one from Weslaco, TX that was found on Uzbekistan cotton (Thaxton et al., 2001). It was identified as race 2. Bacterial blight race surveys have been conducted in Australia, where all samples tested from 1983 to 1988 from South Wales, were race 18 (Allen and West, 1991). In previous surveys in Australia, race 1 was identified before 1974, except for one isolate of race 18. Races, 2-5, 7, 9, 10, and 18 were found between 1974 and 1983 (Allen and West, 1991). Race 18 was found in 76% of the strains in Pakistan by 1984 (Hussain, 1984). Ajene et al., (2014) identified races 1, 12, 13, and 16 in northern Nigeria. A survey in Iran during 2004 to 2005 (Madan et al., 2010) identified race 1 (16%), race 2 (8%), race 6 (57%), and race 18 (19%). Since the pathogen can infect seed, it is not surprising that race 18 has been found in many cotton growing regions, though not every region has seen the shift to race 18 that occurred in the U.S.

Field inoculations with local isolates of *X. citri* subsp. *malvacearum* have been conducted since 2000 in the Lubbock, TX area using replicated small plots with randomized order of cultivars, and incidence of blight from cultivars has been scored (Wheeler, unpublished data). A high level of resistance has been found in many of the Fibermax (FM) cultivars since they were first tested in 2000. Early testing did use an isolate confirmed by Dr. Peggy Thaxton to be race 18, and subsequent isolates used in the program performed identically on cultivars. Two of the early Fibermax cultivars in the Southern High Plains of Texas were FM 989 (PVP 009800259) and FM 958 (PVP200100208) and both listed resistance to race 18 of bacterial blight on the PVP application. This resistance was confirmed repeatedly in studies (Wheeler, unpublished data). Reports of bacterial blight occurring naturally in the Southern High Plains of Texas from 2000 – 2014 were found only on susceptible cultivars (author personal observations). In 2015, there were observations of bacterial blight appearing on resistant cultivars. A description of the events that were seen in the field and greenhouse tests on isolates that were obtained will be presented.

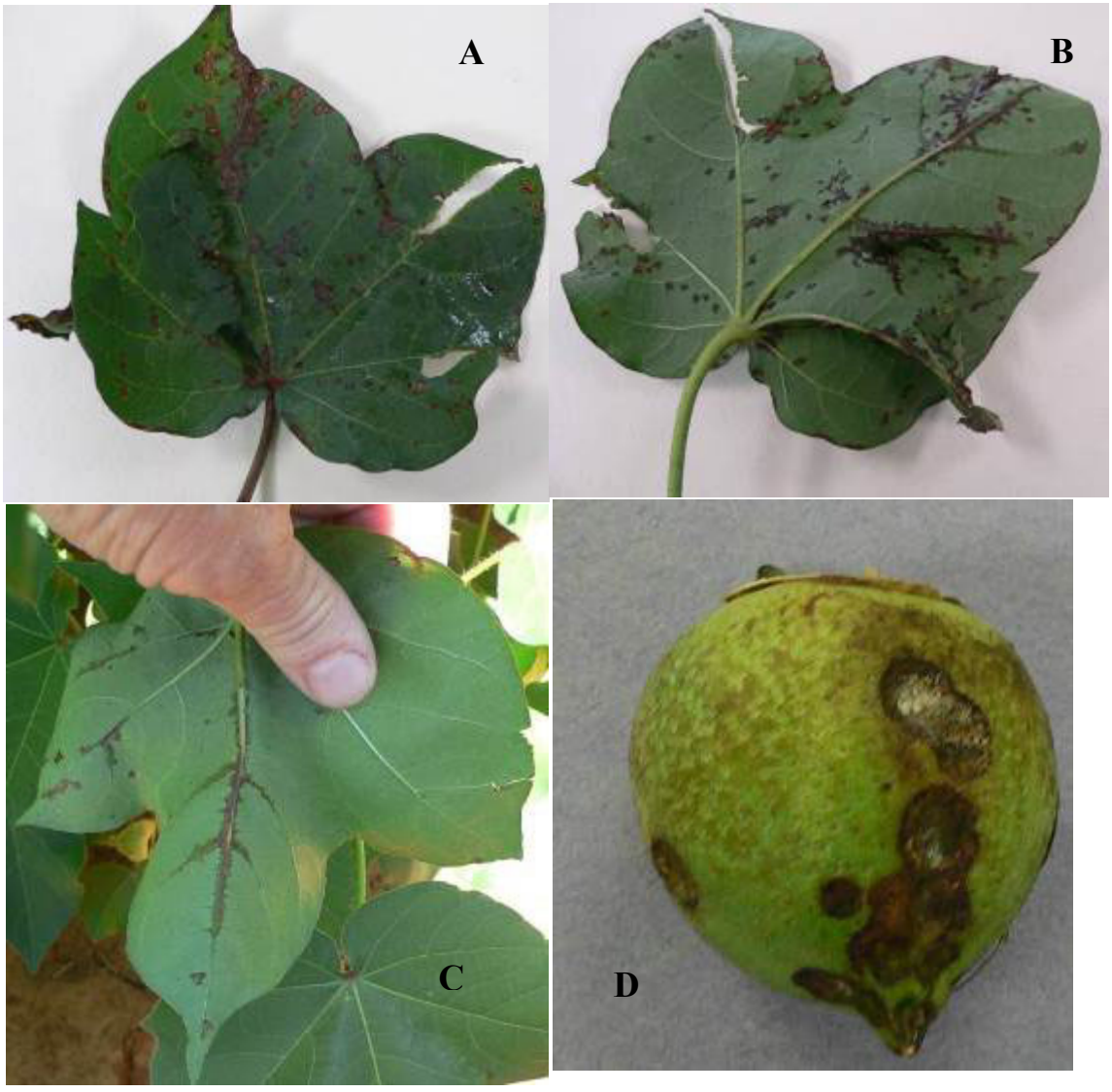

Figure 1. Typical bacterial blight symptoms A) leaf spots (topside); B) water soaked lesions (bottom side); C) Veinal water soaking; D) boll rot. Photo credit, T. Wheeler.

Materials and Methods

Field observations in Plains

A site where a small-plot cultivar trial was conducted developed bacterial blight. The producer of the field, which was located in Plains, TX contacted T. Wheeler initially about defoliation, which was occurring in his variety, FM 1830GLT. The leaves had either defoliated in the producer's fields, or the part of the leaf with blight lesions had fallen out, giving a ragged look to the leaves (Fig. 2). However, the disease continued in the test plots throughout the season, though not in the rest of the field planted with a resistant cultivar. The plots were two rows wide, 36 foot long, with 5 foot alleys, and 36 cultivars were planted in a randomized complete block design with four replications. The plots were rated on 5 and 26 August, and 16 September for blight incidence. A single plant was rated for blight symptoms at 20 locations within each plot. Locations were selected at regular intervals along the plot. Plots were harvested with a two-row cotton stripper, and the contents were weighed on load cells. A 1000 g subsample from the harvested plot was ginned to determine % turnout. Regression analysis (PROC REG, SAS version 9.3) was conducted on the average blight incidence for a plot and yield for the blight susceptible cultivars.

Figure 2. Symptoms of tattered leaves in a bacterial blight field, where lesions appeared to fall off the plant. Photo credit, T. Wheeler.

Bacterial blight sample from Victoria, TX

A sample was obtained from the cultivar FM 958, which is considered highly resistant to race 18. One isolate (C1) was obtained from the diseased leaf sample. It was increased in shake culture on Trypticase soy broth for 36 hours, then diluted by 100X and sprayed on a FM 2484B2F cotton plant in the field, with a surfactant Silwet L77 at 0.2% v/v (Wheeler et al., 2007). It was applied to runoff on the plant. Disease presence/absence was rated 14 days later, and symptomatic leaf samples were taken to re-isolate the pathogen.

Isolate collection and tests

Leaf samples were obtained from 16 locations in TX in 2015. When possible, the name of the cultivar or cultivars that had symptoms was included with the leaf samples. Bacterial isolations were made from the leaves by placing the area with a disease symptom in a sterile Petri dish (15 x 100 mm) and adding 5-10 ml of sterile deionized water. The lesion was cut into small pieces and after 20 min., three loops of water from the plates were streaked on potato carrot dextrose agar (Bird and Blank, 1951). Single *Xanthomonas* type colonies obtained from the streaked plates were streaked onto PCDA agar and maintained by restreaking every 3 wks.

Typical tests conducted in the greenhouse with these isolates included five cultivars: Deltapine (DP) 1454NRB2RF, DP 1044B2RF, Phytogen (PHY) 375WRF, FM 1830GLT, and FM 2484B2F. Tests were conducted in conetainers (SC10, Stuewe & Sons, Inc., Tangent, OR). Typical tests contained 5 cultivars x 9 isolates, plus a water check of each cultivar with four replications per treatment, arranged in a randomized complete block design. One isolate in each test was obtained in Seminole, TX prior to 2010. The bacterium would be grown for 3 to 7 days on PCDA and then scratched on the underside of the two cotyledons with a toothpick (sterilized) (Thaxton et al., 1992). After

inoculation, the plants were placed in a humidity chamber for one day at 100% humidity, and then moved into a growth chamber at 27 ºC for 13 days. Plants were rated for water soaked symptoms (plus/minus). The ten trials were combined and analyzed using a mixed model (PROC MIXED, SAS) with location of isolate and cultivar as the fixed factors. The Satterthwaite option was used to determine degrees of freedom, and T-Tests to determine (PDIFF option) differences between location effects on cultivar.

Results and Discussion

Field observations in Plains

The resistant cultivars did develop a small amount of disease at the first rating period, but lesions disappeared as the season progressed. In the susceptible cultivars, bacterial blight maintained throughout the growing season (Fig. 3). Yield loss associated with the susceptible cultivars averaged 10% (Fig. 4).

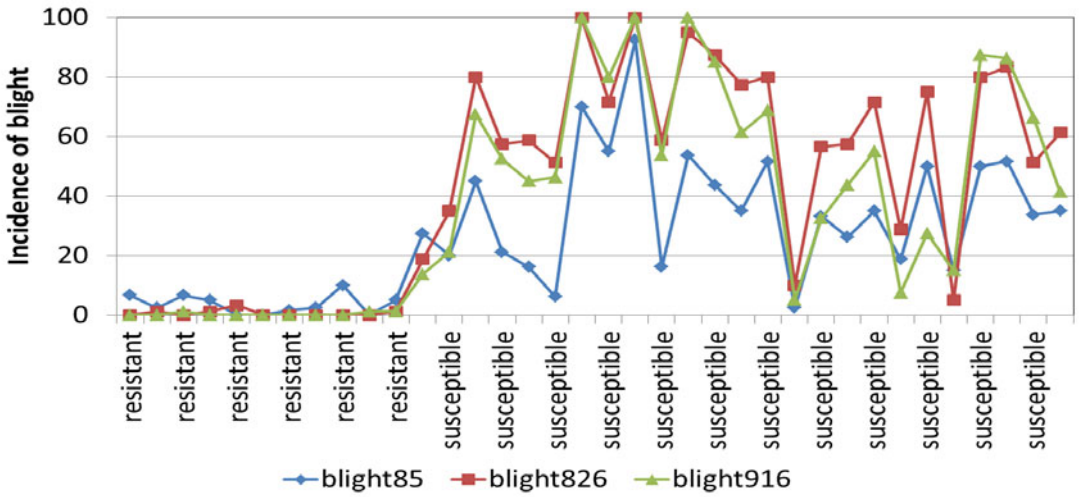

Figure 3. Dynamics of incidence of bacterial blight in a cultivar trial in Plains, TX in 2015. Blight was measured on 5 and 26 August and 16 September. Resistant or susceptible designations were determined by inoculated tests conducted elsewhere.

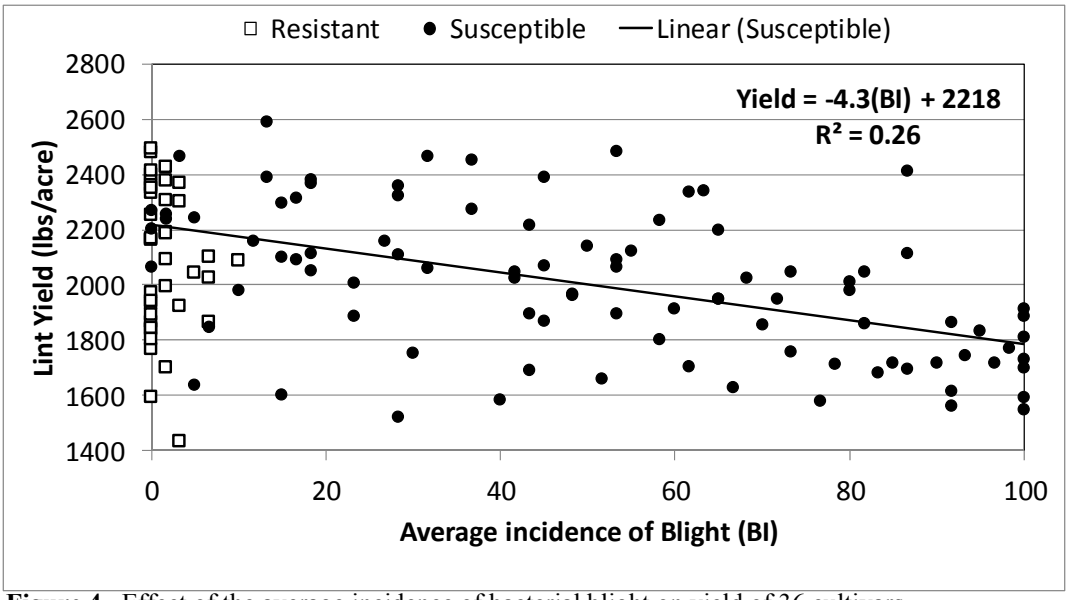

Figure 4. Effect of the average incidence of bacterial blight on yield of 36 cultivars.

Bacterial blight sample from Victoria, TX

FM 2484B2F which was sprayed with the C1 isolate, developed bacterial blight symptoms, and the bacterium was re-isolated from symptomatic leaves.

Isolate collection and tests

In 3 locations (Amherst, Lubbock, and Victoria), isolates were obtained from the resistant cultivars FM 1830GLT, FM 2484B2F, and FM 958, respectively. In all cases symptoms were present on multiple plants representing a foci of disease, and not on a single plant, which could have been a volunteer from a previous season or off-type of seed. In one location (Halfway), cultivars from which isolates were taken included a resistant cultivar, partially susceptible cultivar, and breeding line with no previous rating. At the Helms location (also in Halfway, TX, but several miles away from "Halfway" designation), some isolates were taken from a resistant cultivar (not currently marketed), and some from a susceptible cultivar (not currently marketed). In two locations (Reniform and Quaker, both in Lubbock, but at least 5 miles apart), isolates were taken from breeding lines without a rating for blight, and both were obtained from Pima cultivars in cotton breeders trials (obtained from TX and NM breeders). At all other locations, isolates were taken from susceptible cultivars. There was a significant cultivar ($P < 0.001$) and location x cultivar ($P = 0.09$) interaction. With the susceptible cultivar DP 1454NRB2RF, water soaked symptoms averaged 100%, and all locations behaved similarly with regards to water soaking. With DP 1044B2RF, water-soaking incidence averaged 58%, and some locations behaved differently than others (Fig. 5). With PHY 375WRF, FM 1830GLT and FM 2484B2F water-soaking incidence averaged 15, 15 and 8%, respectively; however, some differences between locations were observed.

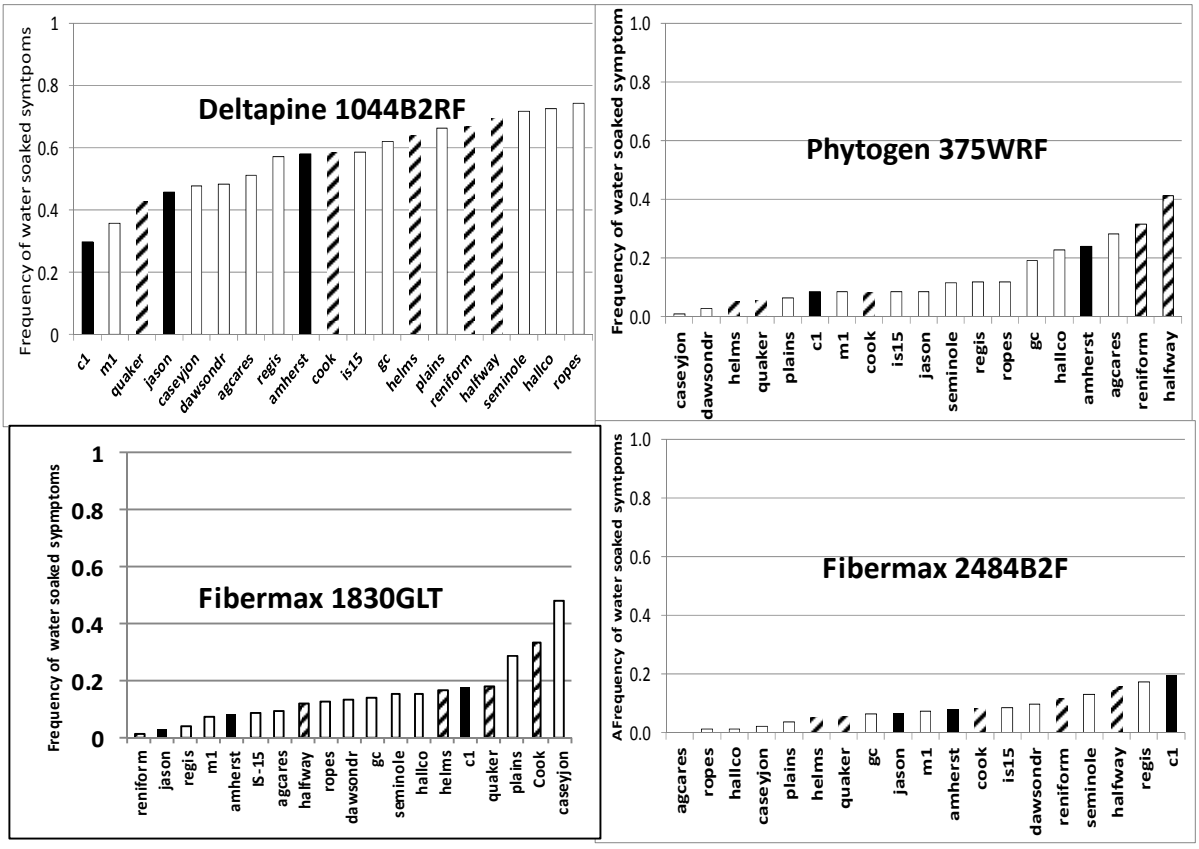

Figure 5. Proportion of tested plants with water soaked symptoms on the underside of the cotyledon, which had been scratched with an isolate of bacterial blight. Isolates from 19 locations were included in various tests. Black bars indicate isolates were taken from resistant plants, white bars indicate that all isolates were taken from susceptible plants, and patterned bars indicate that isolates were either from a mixture of resistant and susceptible plants, or that the rating was not known of the plants that isolates were taken from.

Our greenhouse assay has not historically been as consistent at eliciting disease symptoms as the field applications described for the C1 isolate from Victoria, TX. The methodology for the field application program is described in detail in Wheeler et al. (2007). This field application method has been used for 16 years successfully on many

different cultivars, and gives good symptoms and consistent results, which allow differentiation between resistant and susceptible cultivars. While we had previously used the greenhouse protocol, and it clearly can also differentiate between resistant (Fig. 6C) and susceptible plants (Fig. 6A and B), it also can be more variable in symptom development with cultivars that are not as susceptible. It appeared in some cases that water soaking symptoms were close to happening, because of chlorotic/necrotic areas that appeared, but that conditions may not have been quite good enough to produce water soaking (Fig 6D-F). A plant is either resistant or susceptible to water soaking, but some cultivars that were susceptible are more difficult to achieve susceptible symptoms.

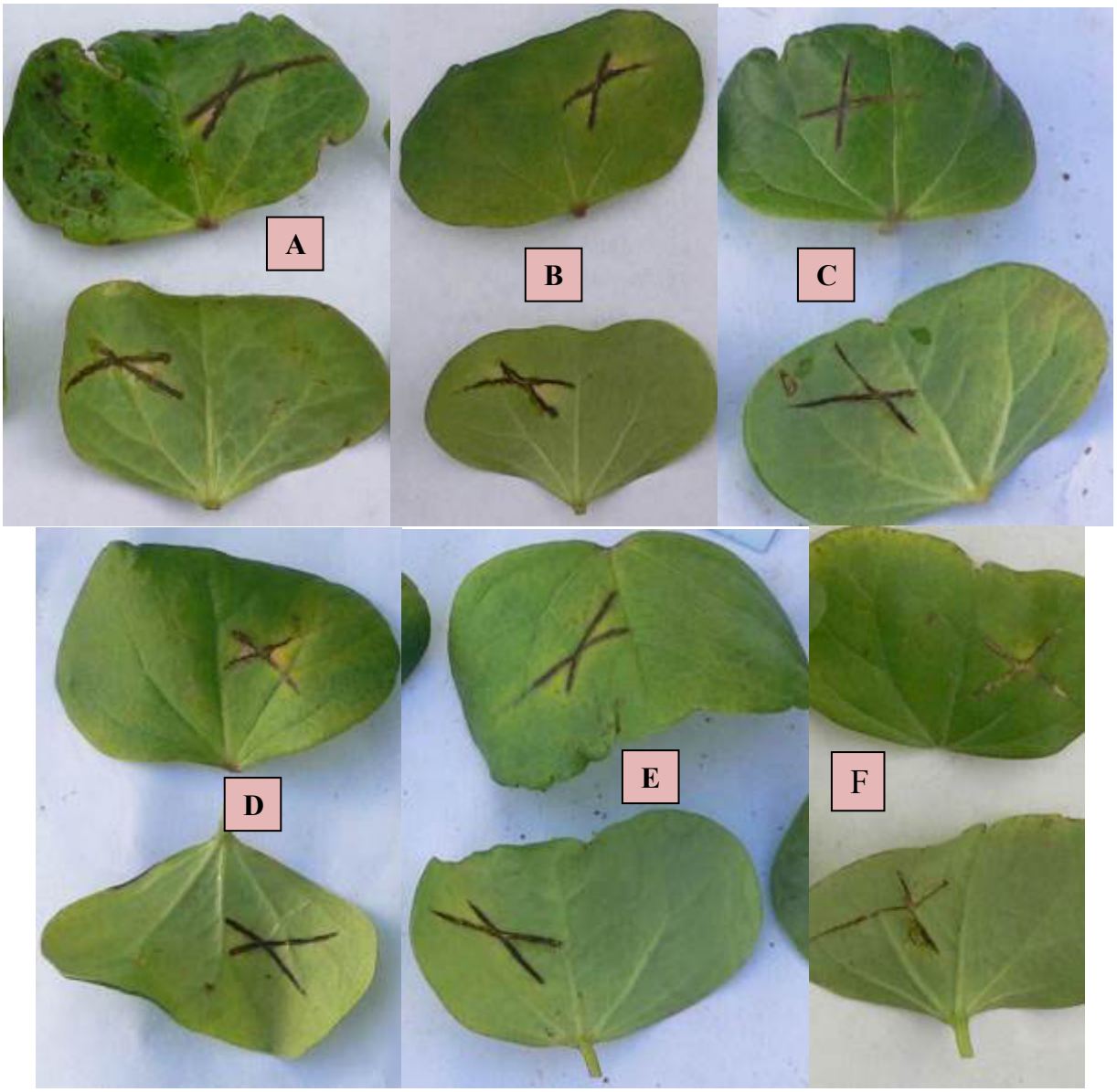

Figure 6. Water soaked symptoms on A) Deltapine (DP) 1454NRB2RF; B) DP 1044B2RF. C) Resistant response on Fibermax (FM) 2484B2F; D) Lack of water soaked symptoms on DP 1044B2RF, but note chlorosis on upper side of leaf. E) Lack of water soaked symptoms on Phytogen 375WRF, but chlorosis is apparent. F) Lack of water soaked symptom on FM 1830GLT, but slight chlorotic response to inoculation on topside of cotyledon. Photo credit T. Wheeler.

Summary

Bacterial blight was found in multiple field sites in Texas on cultivars that are resistant to race 18 of the pathogen. An isolate obtained from FM 958 was inoculated on FM 2484B2F in the field, developed symptoms, and then was re-isolated. Greenhouse studies with isolates obtained from field sites in 2015 did not consistently cause disease on cultivars that were considered to be resistant to the pathogen (race 18). At this time, there is insufficient evidence to conclude that a new race of *X. citri* subsp. *malvaceraum* exists in Texas. However, it is possible that some minor genes for resistance to the pathogen are no longer effective, but this needs to be proven with a differential set of genotypes. There is no evidence to suspect that genes with major effects on the pathogen are ineffective.

Acknowledgements

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