# BREEDING FOR BACTERIAL BLIGHT RESISTANCE IN COTTON

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## Abstract

Bacterial blight in cotton can cause significant injury to susceptible cotton lines. The pathogen is well-understood, screening methods are well-established, and resistance in the form of immunity to the disease has been developed. Although resistance is available, the U.S. cotton industry appears to periodically ignore bacterial blight. Interest in the disease again arose after several epiphytotics occurred in 2011-2012. The number of lines resistant to bacterial blight in the U.S. has increased greatly since 2015. This increase can partly be attributed to molecular marker assisted selection. If this selection continues, bacterial blight will likely again become a non-issue.

## Introduction

Bacterial blight in cotton, caused *Xanthomonas axonopodis* pv. *malvacearum*, has periodically been a problem in cotton for many years. Its primary symptom is a leaf spot with a distinctive shape (referred to as "angular leaf spot") with a distinctive appearance (water soaking). When the infection occurs on stems, it may cause a symptom referred to as "black arm", which may lead to plant lodging in severe cases. Late in the season, the bacteria may infect carpel wall of boll and contribute to boll rots. The most economic issues with bacterial blight is the occurrence of angular leaf spot causing severe loss of leaves early in the season, and increased boll rots late in the season.

Resistance to bacterial blight is perhaps the best understood host plant resistance relationship in cotton. Until recently, intentional incorporation of bacterial blight resistance into cultivars was abandoned by most U.S. cotton breeders, but remained an important breeding objective for cotton breeders in some other countries, particularly Australia and Brazil.

## Review of Breeding Work for Bacterial Blight Resistance

Inheritance of resistance to bacterial blight in cotton was first demonstrated by Knight and Clouston (1939) in the Sudan. They screened wild and cultivated cottons and identified the "B" series of genes that conferred resistance. This work was reported in a series of 10 papers with the last one published by Knight (1953). Curiously, the titles of all ten of the articles refer to bacterial blight by its "blackarm" symptom. This suggests that the "blackarm" symptom of the disease in this environment may have been relatively more important than the leaf spot or boll rot symptoms. Later, Innes et al. (1974) reported on the inheritance of resistance to bacterial blight in Uganda.

Dr. L.A. Brinkeroff at Oklahoma State University and Dr. L.S. Bird at Texas A&M University iled much of the bacterial blight resistance work in the U.S. in the 1950's through early 1980's. Much of this and other work concerning bacterial blight was reviewed Brinkerhoff (1970). Their work included development of methods (Bird and Blank, 1951), demonstrating the variation in the bacteria (Brinkeroff, 1963), and identification of races using host differentials (Hunter et al., 1968; Brinkeroff et al., 1984). They identified races by establishing a set of host differentials, but a new race (race 18) was found in Pakistan that was able to infect all of the previous race differentials (Hussain and Brinkeroff, 1978). Race 18 soon found its way to the U.S., and is now the dominant race found in natural infestations across the cotton belt (Thaxton et al., 2001).

Both Bird (1982) and Brinkerhoff et al. (1984) were able to develop effective immunity to bacterial blight by combining several single-gene resistance factors into a polygenic resistance background. Bird developed immunity in 101-102B, a line of G. hirsutum derived from a cross of Empire WR (G. hirsutum) and Bar 4/16, a Sudanese line of G. barbadense L. origin containing Knight's $B_2B_3$ blight-resistance genes. These resistant genes were then transferred to other backgrounds and have been made available to all U.S. cotton breeders.

My experience with breeding for resistance to bacterial blight began with my graduate school training in Bird's TAM-MAR program (Bird, 1982). In his program, he would inoculate with a mixture of four races, which usually

included races 1, 2, 12, and 18, with race 18 always included. Seedlings selected for resistance to seed deterioration and for germination at a cool time (13.3C) were scratch-inoculated with the mixture of races. Seedlings showing any susceptible reaction were discarded. The resistant seedlings were transplanted to greenhouse pots, and grown to maturity during the winter. Seed from the individual plants were planted in progeny rows the next summer, and superior progeny rows were selected and evaluated as strains in replicated tests. All progeny rows and subsequent seed increases were inoculated with the mixture of races (without surfactant at that time) using a tractor mounted, high-pressure sprayer. Spray tips were pointed up toward abaxial leaf surfaces, and plants were inoculated early in the mornings when stomates were open. Progeny displaying any susceptible plants were assumed to be segregating, and were discarded. The occasional susceptible plant in seed increases was rogued.

## Screening for Bacterial Blight Resistant Today

To my knowledge, U.S. cotton breeding programs that that have actively screened for resistance to bacterial blight in the past have included the TAM-MAR program (led by Bird, El-Zik, and Thaxton), some Texas seed companies, the Stoneville Pedigreed Seed Company (1970-1980's), and Thaxton at Mississippi State in the 2000's. Currently, Bourland (first at Mississippi State and subsequently at University of Arkansas), CSIRO (FiberMax germplasm in Australia), and Fundação MT in Mato Grosso, Brazil, actively screen for resistance. Recently, several major seed companies have begun using molecular markers to incorporate blight resistance into their lines (for example, Xiao, 2010). Most insist that the resistance of selections made by molecular markers should be confirmed with greenhouse or field inoculation.

Several factors have led to the relatively low breeding priority associated with bacterial blight resistance in the U.S. since about the 1960's. The first factor was the wide-spread use of acid-delinting of planting seed. As opposed to gin-run or mechanically delinted seed, acid-delinted seed are much less likely to carry the bacteria on the planting seed. A second factor has been the low yield loss usually associated with bacterial blight infections. Losses to bacterial blight are dictated by timing and environment associated with the infections. I was surprised that the wide employment of center pivot irrigation in the Mississippi River Delta in the 1980's and 1990's did not exasperate the disease. Finally, development of resistance to bacterial blight was generally not considered worth the extra work required to inoculate and screen materials.

Since 1978, Bourland has used the modifications of the procedures developed by Bird (1982) in his cotton breeding programs at Mississippi State University and the University of Arkansas (Bourland, 2004; 2013). Field spraying or scratch inoculation techniques are used to inoculate plants. At Arkansas, field spraying is done with a 4-row "Mud-Master" sprayer equipped with 110-gal tank and a sprayer with nozzles pointed upward to the abaxial side of leaves. Since the early 1990's, a silicon surfactant has been added to the inoculum (mixture of races, but always include race 18) to improve inoculation success. Without the aid of the surfactant, Bird and Blank (1951) employed 125-150 psi and Brinkerhoff (1963) employed about 400 psi when inoculating in the field. With the aid of the surfactant, we now use psi's of less than 50 psi. With improved inoculation efficiency, we are able to confidently spray-inoculate early generation populations in the field. Scratch inoculation of cotyledons in greenhouse plantings are used to evaluate cultivars and breeding lines. Ground powder of dried infected leaves is placed into distilled water (about 2 ml of ground tissue per 50 ml of water). Inoculation is done by dipping a modified drafting pen (or toothpick) into the solution, then scratching the abaxial surface of cotyledons. Depending on temperature of greenhouse, cotyledons can be evaluated for resistance after about 2 to 3 weeks.

Annually, Bourland inoculates all plants in his $F_2$ though $F_4$ populations (Bourland, 2004; 2013). Individual resistant plants are selected from the $F_4$ populations. Seed from the individual plants are evaluated as progeny in the $F_5$ and $F_6$ generations. The progeny are usually spray inoculated in the field, but are sometimes scratch inoculated to ascertain the exact proportion of susceptible plants. Selected progenies are promoted to strains and evaluated in replicated strain tests for up to four years. Annually, all seed increases of strains are inoculated and evaluated for bacterial blight resistance. The occasional susceptible plant are rogued from the seed increases.

## Bacterial Blight Resistant in Today's Cotton Lines

Since 1986, Bourland has released 88 germplasm lines (includes 15 lines released via Mississippi Agricultural and Forestry Experiment Station) and six cultivars. Seventy-six of the 88 germplasm lines and all six of the cultivars are resistant to bacterial blight. Although bacterial blight has not the primary focus of his breeding program, developing resistance to bacterial blight has remained a selection criteria because it is:

1. Relatively easy to establish and evaluate.
2. A distinctive characteristic that can help to describe releases.
3. Provides insurance against an epiphytotic (no concern with bacterial blight outbreak occurs).
4. Aids in maintaining pure lines by identifying segregating progenies and off-types in seed increases.
5. May enhance host plant resistance by adding to level of horizontal resistance.

Since 2013, entries into the Arkansas Cotton Variety Tests have been evaluated (using scratch inoculation) for resistance to bacterial blight (Table 1). The number of resistant lines greatly increased in 2016 and 2017. Prior to 2016, the University of Arkansas and International Seed Technologies were the primary sources of resistant lines with other sources providing occasional resistant lines. Since 2015, Bayer Crop Sciences, Monsanto and PhytoGen Seed Company have increased their offering of bacterial blight resistant lines. Many of these later increases were likely attributed to selection via molecular markers for resistance to bacterial blight.

Table 1. Number of lines resistant to bacterial blight in the 2013 – 2017 Arkansas Cotton Variety Tests, available at www.ArkansasVarietyTesting.com.

| | Number of lines by year | | | | |
|---|---|---|---|---|---|
| Bacterial blight response | **2013** | **2014** | **2015** | **2016** | **2017** |
| Susceptible | **24** | **24** | **13** | **13** | **16** |
| Intermediate | **4** | **0** | **4** | **10** | **7** |
| Resistant | **8** | **10** | **13** | **22** | **34** |
| Total number evaluated | **36** | **34** | **30** | **35** | **58** |
| **Source** | **Number resistant lines / Number lines evaluated** | | | | |
| Americot Inc. | **0/2** | **0/1** | **0/3** | **2/7** | **3/7** |
| Bayer Crop Science | **2/5** | **0/5** | **2/7** | **5/8** | **1/4** |
| International Seed Tech. | **-** | **2/4** | **3/4** | **2/3** | **2/3** |
| Crop Production Services | **1/4** | **1/4** | **1/4** | **1/5** | **0/4** |
| Monsanto | **0/9** | **1/4** | **3/8** | **6/9** | **4/10** |
| PhytoGen Seed Co. | **2/15** | **3/11** | **1/10** | **1/3** | **13/15** |
| University of Arkansas | **3/3** | **3/3** | **3/3** | **5/5** | **11/11** |

## Persistent Concerns

At present, some doubts are associated the availability of a good set of race differentials that can be used for identifying races. Without an established set of host differentials, it is difficult to ascertain what specific races are present. A second concern is the availability (purity and virulence) of races, which cannot be confirmed without race differentials. The question then arises of whether new races will evolve over time. The "immunity" described by Bird (1982) and Brinkerhoff et al. (1984) appears to be standing.

Access to cultures of multiple, virulent races of *X. axonopodis* is essential for establishing resistance to bacterial blight. If a plant is resistant to race 18, it will be resistant to all races. However, the inclusion of other races insures satisfactory inoculation even when race 18 cultures change and/or become less virulent. A breeding program that relies upon using naturally infected leaves for inoculum cannot be sure of the specific race(s) being used and cannot be assured of an ample supply of inoculum each year.

## Summary

Resurgence of bacterial blight has periodically occurred when screening for resistance and scouting of seed production fields is relaxed. Many of the issues involved with breeding for resistance have been solved. Field, greenhouse and molecular-marker methods can be used to identify resistant plants and lines, and the number of resistant lines have increased in recent years. Resistance has been incorporated into lines without any obvious deleterious effects. Although race 18 is dominant race in the U.S., available resistance to race 18 has not yet broken down, which seems to confirm that "immunity" is available. Bacterial blight will likely become a non-issue until screening for resistance is again relaxed.

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