ISOLATION OF *XANTHOMONAS AXONOPODIS* PV. *MALVACEARUM* FROM SYMPTOMATIC COTTON BOLLS

J. E. Woodward
Texas AgriLIFE Extension Service
Texas Tech University
Lubbock, TX
M.S. Kelley
Texas AgriLIFE Extension Service
Lubbock, TX
M.D. Dunlap
Texas AgriLIFE Extension Service
Wellington, TX

Abstract

A survey of seed collected from cotton bolls expressing a range of disease symptoms was conducted to determine the isolation frequency of *Xanthomonas axonopodis* pv. *malvacearum* (*Xam*). Samples of 750 bolls were collected from three adjacent plots of the susceptible varieties Deltapine (DP) 0912B2RF, Phytogen (PHY) 565WRF and Stoneville (ST) 5458B2F. Bolls were placed into classes (low, moderate or high) based on symptom expression. Lint and seed were hand harvested, ginned and subjected to acid-delinting. Seed were surface disinfested and placed in petri dishes containing potato carrot agar (PCA). Resulting muccoid isolates were streak-plated to PCA to obtain pure cultures. Pathogenicity tests were conducted to determine *Xam* isolates. Approximately 84% of bolls collected exhibited moderate to high levels of disease. Bacterial isoaltes were isolated from 26% of all seed assayed. Overall, the isolation of *Xam* increased from bolls exhibiting higher levels of disease. No *Xam* isolates were recovered from seed of PHY 565WRF or ST 5458B2F classified as low; whereas, 0.3% of DP 0912B2RF seed from that category were infected. The isolation frequency from DP 0912B2RF, PHY 565WRF and ST 5458B2F bolls classified as moderate were 0.5, 2.5 and 1.3%, respectively. Frequencies from the high category were 2.5, 2.8 and 1.6% for the respective varieties. Our results support previous studies indicating that *Xam* is seed borne and that levels of *Xam* can survive acid delinting.

Introduction

Bacterial blight of cotton, caused by the plant pathogenic bacterium *Xanthomonas campestris* pv. *malvacearum*, was first reported in Alabama (Atkinson, 1891). The bacterium has been reclassified as *X. axonopodis* pv. *malvacearum* (*Xam*) (Vauterin *et al.,* 2000). The disease has been reported from almost every country where cotton is grown (Hillocks, 1992). The existence of biological races of *Xam* has been identified following the breakdown of resistant genotypes grown in other regions of the world (Balasubramanyan and Raghaven, 1950). Characterization of *Xam* is complex and several race structures have been proposed. Ruano and Mohan (1982) have identified 19 races, with race 18 being the most prevalent (Thaxton *et al.*, 2001). Studies conducted by Gabriel *et al.* (1986) reported that the interactions fit the gene-for-gene model. Multiple gene complexes have lead to more stable resistance (El-Zik and Bird, 1970) and immunity is present in some upland varieties (Bird, 1962). Currently, a screening program is in place for the High Plains of Texas (Wheeler and Woodward, 2010). Yield losses following artificial inoculations approached 34% in highly susceptible varieties (Bird, 1959). Reports of losses under field conditions ranged from 35-59% (Leyendecker, 1950). Currently, losses due to *Xam* are considered negligible (Blasingame, 2010); however, severe outbreaks sporadically occur.

Cotton plants infected with *Xam* may exhibit a wide range of symptoms including angular leaf spot (Fig. 1a) and Black arm (Fig. 1b). Progression of the disease often results in premature defoliation (Fig. 1c). In addition to the aforementioned symptoms, *Xam* is capable of infecting cotton bolls (Fig. 1d). Invasion of bolls results in staining of cotton fibers and is thought to result in the surface contamination of seed (Hillocks, 1992). Tennyson (1963) reported bacterial blight developed in 20% of plants grown from infected seed, suggesting the potential for seed borne inoculum. Furthermore, the inability to eradicate *Xam* by acid delinting suggests internal contamination of seed (Hillocks, 1992). The objective of this research was to determine the isolation frequency of *Xam* from symptomatic cotton bolls.

Materials and Methods

Cotton bolls were collected from three adjacent plots of the varieties Deltapine (DP) 0912B2RF, Phytogen (PHY) 565WRF and Stoneville (ST) 5458B2F included in a field trial conducted in Collingsworth Co. Texas. Disease development originated from a natural field epidemic. The field had a history of cotton production and bacterial blight had been observed in previous years. A total of 750 bolls (250 per variety) were arbitrarily collected from the middle portion of the plant canopy. Bolls were categorized as having low, moderate or high levels of disease based on symptom expression, where: low = light water-soaked lesions with no penetration of the carpal wall; moderate = few, dark water-soaked lesions with slight penetration of the carpal wall; and 3 = numerous, dark water-soaked lesions and severe penetration of the carpal wall (Fig. 2&3). Lint and seed were removed from bolls by hand, ginned and subjected to acid delinting. Resulting seed were surface disinfested by soaking in a 0.5% sodium hypochlorite solution for 3 min. Seeds of each variety and category (n=960) were air dried and placed in petri dishes containing potato carrot agar (PCA) (Alexander, 2009). Characteristics of seed such as size, condition and maturity (based on color) were recorded. Petri dishes were incubated in the dark at 28°C for 48-hr. Resulting bacterial colonies were characterized and streak-plated on PCA to obtain pure cultures. Muccoid, *Xanthomonas*-like bacterial isolates were subjected to pathogenicity test as described by Alexander (2009). Sterile toothpicks containing each isolate were used to inoculate the cotyledons of DP 0912B2RF seedlings. Several non-muccoid isolates were included as negative controls. Plants were placed in a growth chamber incubated at 28±2°C with a humidifier was placed in the growth chamber to maintain humidity > 90% for 48-hr. Plants were maintained in the growth chamber for 10-d, at which point disease reactions, described as expanding water-soaked lesions, were rated.

Results and Discussion

Overall, hot and dry conditions were experienced across west Texas; however, extremely high irrigation capacity and abundant heat unit accumulation experienced within an irrigation span of the field surveyed lead to vegetative cotton growth which provided a conducive environment for bacterial blight. A wide range of symptoms attributed to *Xcm* were observed (Fig. 1) in all three varieties sampled. Boll samples were collected from the middle portion of the plant canopy in an attempt to standardize boll size and age. The majority (≈ 84%) of bolls collected were classified as having moderate or high levels of disease (Fig. 4). The arbitrary sampling of only symptomatic bolls may have limited the selection of bolls with low levels of disease. Differences in the proportion of immature versus mature seed were observed when comparing seed collected from bolls exhibiting low, moderate and high levels of symptoms (Fig. 5). Approximately 75% of seed obtained from bolls with low disease pressure were immature; whereas, 57 and 73% of seed collected from moderately and highly infected bolls were mature. Such differences in seed maturity may have resulted in an underestimate of infected seed for the low category. Little published information regarding the infection cycle of cotton by *Xam* is available. Overall, various microbes (both bacteria as well as fungi) were found to be associated with the seed assayed (data not shown). Results from the pathogenicity tests conducted show that *Xam* was isolated at higher frequencies from moderately and highly infected bolls for all varieties (Fig. 6). For DP 0912B2RF, *Xam* was isolated from 0.3, 0.5, and 2.5% of the seed obtained from bolls classified as low, moderate and high, respectively. None of the low disease seed of PHY 565WRF or ST 5458B2F were determined to be infected with the bacterium. Similar *Xam* isolation frequencies were observed in seed from moderately and highly infected ST 5458B2F (1.3 and 1.6%). Frequencies of 2.5 and 2.8% were determined to be associated with seed from moderately and highly infected PHY 565WRF bolls, respectively. Results from this study agree with previous reports that 1-6% of seed collected from symptomatic bolls are infected with *Xam* (Brinkerhoff and Hunter, 1963), and that acid delinting does not completely rid seed of *Xam* (Alexander, 2009; Hillocks, 1992). Other reports indicate isolation frequencies as high 30% (El-Nur, 1970). Information on the contamination of seed lots and bacterial blight epidemics is unknown. Low infection frequencies may serve as an initial inoculum source, thus areas should be carefully scouted prior to harvest especially in seed production fields.

Acknowledgements

The technical support of Ira Yates, Justin Spradley and Lindsey Thiessen is greatly appreciated. We also thank Texas Cotton State Support for partial funding of this project.

References

Alexander, A. 2009. Isolation frequency of *Xanthomonas axonodopis* pv. *malvacearum* from acid delinted and easiflo treated cotton seed. M.S. Thesis, Texas Tech University, 45p.

Atkinson, G. 1891. The black rust of cotton. Agricultural Experiment Station, of the Agricultural and Mechanical College, Alabama Agricultural Experiment Station Bulletin 27, Auburn University, Auburn, AL.

Balasubramanyan, R. and A. Raghaven. 1950. Bacterial blight of cotton in Madras. Indian Cotton Growing Review 4:118-120.

Blasingame, D., J. Banks, P. Colyer, R. Davis, W. Gazaway, N. Goldburg, R. Kemerait, T. Kirkpatrick, S. Koenning, J. Muller, M. Newman, M. Olsen, P. Phipps, G. Scuimbato, R. Sprenkel, J. Woodward, J. Wrather, and M. Patel. 2008. Cotton disease loss estimate committee report. Proc. Beltwide Cotton Conf. Pp. 294-297 (cd-rom).

Bird, L. 1959. Loss measurements caused by bacterial blight disease of cotton. Phytopathology 49:315 (abstr.).

Bird, L. 1962. Use of races of *Xanthomonas malvacearum* for establishing levels selection pressure in developing bacterial blight immune cottons. Proceedings Cotton Improvement Conferences 14, National Cotton Council, Memphis Tennessee, pp. 16-17.

Brinkerhoff, L. and R. Hunter. 1961. Frequency of cotton plants resistant to Fusarium wilt in some lines resistant and susceptible to bacterial blight. Plant Disease Reporter 45:126-127.

El-Nur, E. 1970, Bacterial blight of cotton in Sudan. Pest Articles and News Summaries 16:132-141.

El-Zik, K. and L. Bird. 1970. Effectiveness and specific genes and gene combinations in conferring resistance to *Xanthomonas malvacearum* in Upland cotton. Phytopathology 60:441-447.

Gabriel, D., A. Burges, and G. Lazo. 1986. Gene for gene interaction of five cloned avirulence genes from *Xanthomonas campestris* pv. *malvacearum*. Proceedings of the Natural Academy of Sciences of the USA 83:6415-6419.

Hillocks, R. 1992. Verticillium wilt. Pages 39-85 in: Cotton Diseases. R. J. Hillocks ed. CAB International, Wallingford, UK.

Leyendecker, P. 1950. Plant disease survey for New Mexico. Plant Disease Reporter 34:39-44.

Ruano, D. and S. Mohan. 1982. A new race of *Xanthomonas campestris* pv. *malvacearum* (smith) Dye in Panama State. Fitopatologia Brasiliera 7:439-441.

Tennyson, G. 1936. Invasion of cotton seed by *Bacterium malvacearum*. Phytopathology 26:1083-1085.

Thaxton, P. T. Brooks, and K. El-Zik. 2001. Race identification and severity of bacterial blight from natural infestations across the cotton belt. Proceedings of the Beltwide Cotton Conferences 1:137-138.

Vauterin, L., J. Rademaker, and J. Swings. 2000. Synopsis of the taxonomy of the genus *Xanthomonas*. Phytopathology 90:677-682.

Wheeler, T. and J. Woodward. 2011. Bacterial blight recommendations. Online http://lubbock.tamu.edu/files/2011/11/2010/Bacterial.pdf. Verified Jan 9, 2012. 3 p.

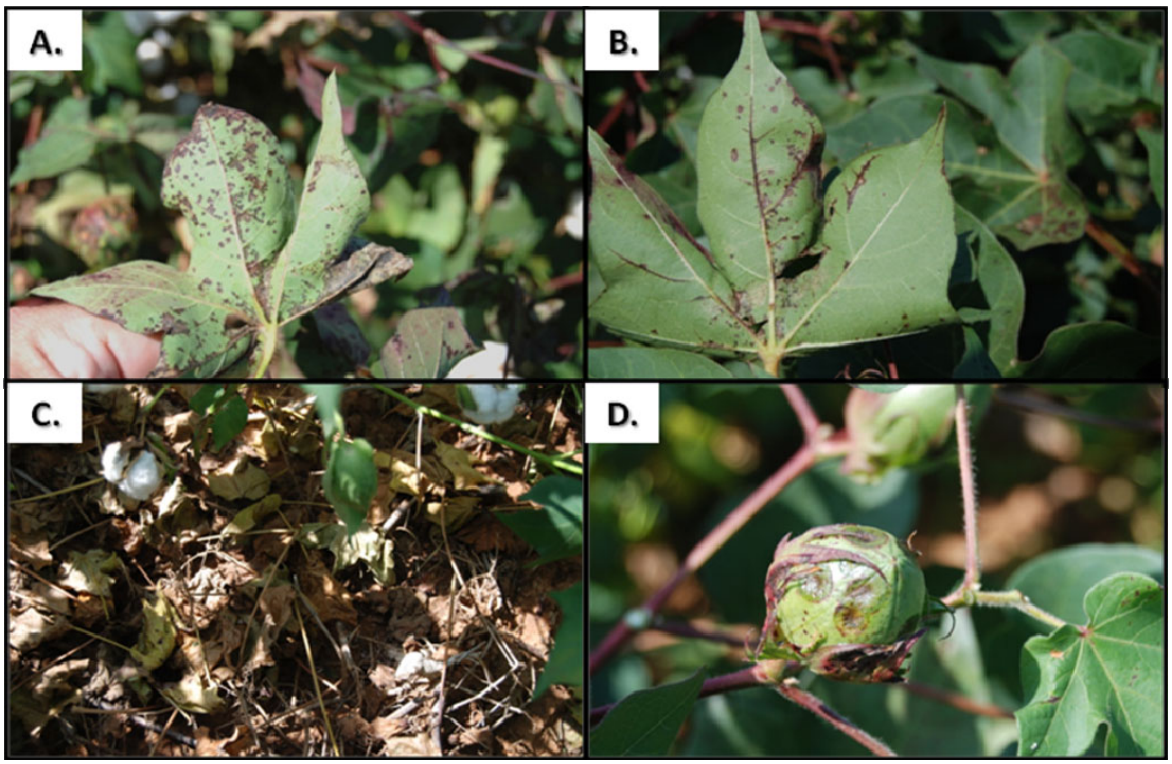

Figure 1. Appearance of symptoms associated with *Xanthomonas axonopodis* pv. *malvacearum* infecting cotton. (A) Angular leaf spot (note restriction of lesion within the veins), (B) Black arm (note: localized systemic infection), (C) Premature defoliation resulting from infection, and (D) Boll rot (note water-soaked lesions).

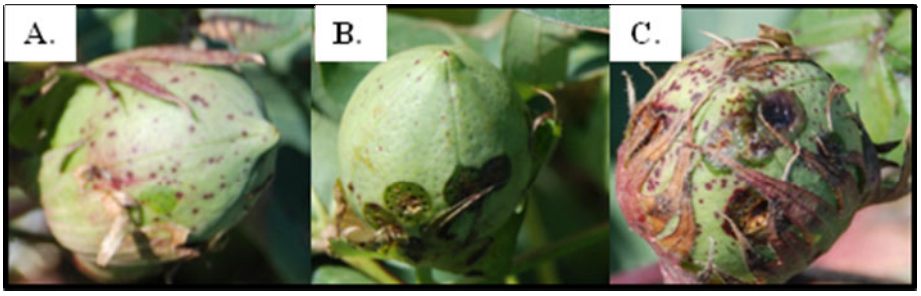

Figure 2. Classification of cotton bolls collected from the field exhibiting low (A), moderate (B), or high (C) levels of boll rot incited by *Xanthomonas axonopodis* pv. *malvacearum*.

Figure 3. Penetration of carpal wall and staining of lint following infection by *Xanthomonas axonopodis* pv. *malvacearum* for bolls characterized as having moderate (top) and high (bottom) levels of disease.

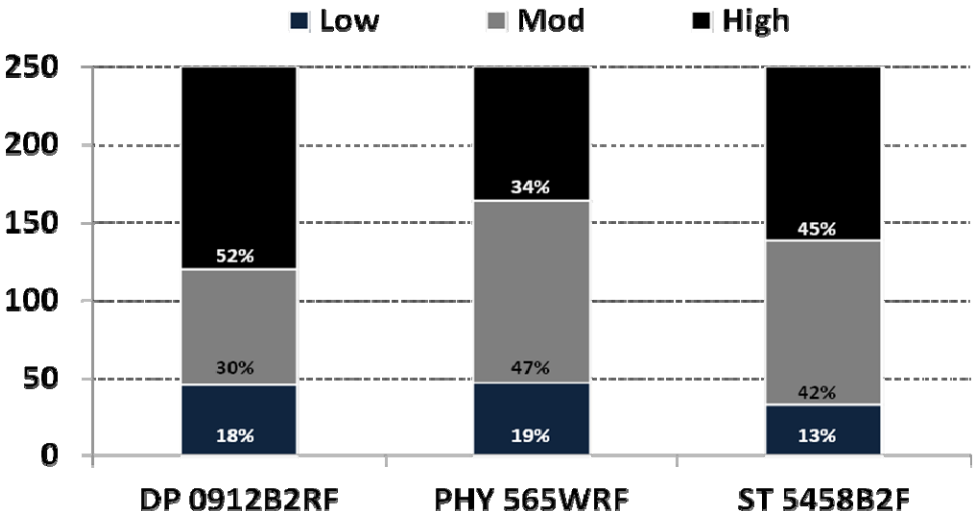

Figure 4. Classification of symptomatic bolls of three cotton varieties (Deltapine 0912B2RF, Phytogen 565WRF, and Stoneville 5458B2RF) exhibiting low, moderate or high levels of boll rot incited by *Xanthomonas axonopodis* pv. *malvacearum*.

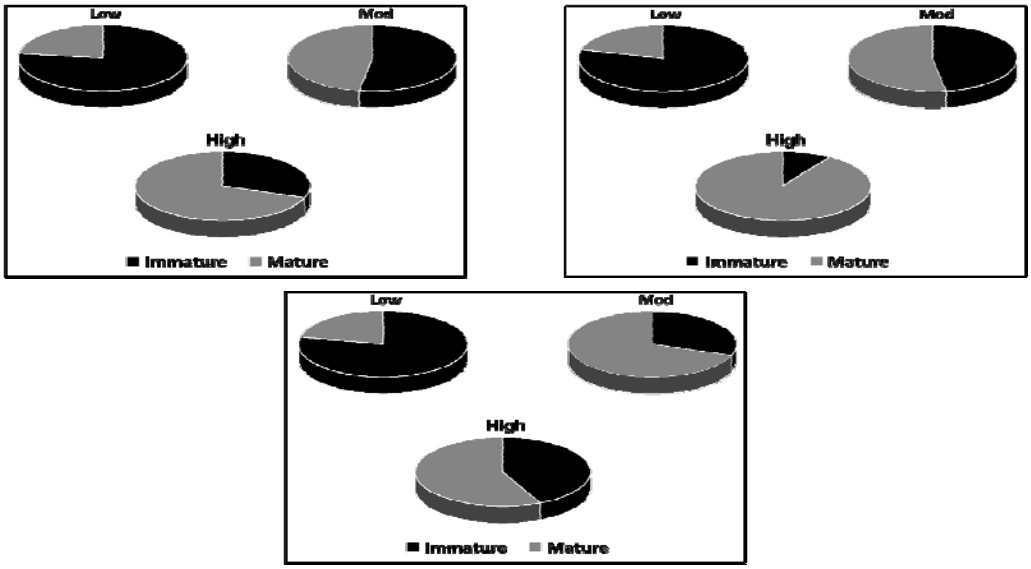

Figure 5. Distribution of immature and mature seed collected from Deltapine 0912B2RF (top left), Phytogen 565WRF (top right) and Stoneville 5458B2F (bottom) obtained from bolls exhibiting low, moderate or high levels of boll rot incited by *Xanthomonas axonopodis* pv. *malvacearum*.

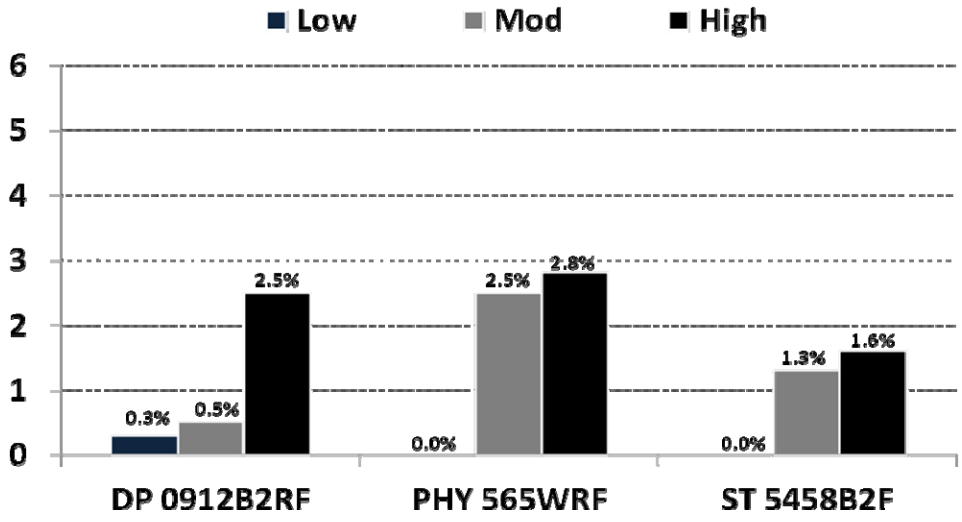

Figure 6. Isolation frequency of *Xanthomonas axonopodis* pv. *malvacearum* isolates following pathogenicity testing for three cultivars (Deltapine 0912B2RF, Phytogen 565WRF and Stoneville 5458B2F) obtained from bolls exhibiting low, moderate or high levels of boll rot.