

**ASSOCIATION OF *AGROBACTERIUM*
TUMEFACIENS WITH COTTONSEED**

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Abstract

Agrobacterium tumefaciens has been associated with the occurrence of bronze wilt of cotton, a disease first recognized in 1995. The disease is characterized by the sudden wilting and bronzing or reddening of the foliage and occurs primarily on a number of short-season determinate cultivars. One of the complicating factors in demonstrating the bacterium's role in bronze wilt is the report that *Agrobacterium tumefaciens* is endophytic and ubiquitous in cotton. A collaborative research project was initiated to examine the frequency of occurrence of *Agrobacterium* spp. in seedlots of cotton. Cultivars used were DP 50, Sure-Grow 125, Sure-Grow 125RR, Sure-Grow 125BR, PM 1218 BR, PM 1220 RR, PM 1220 BR, and Stoneville 373. Two methods were used to assess the occurrence of *Agrobacterium* spp. with cottonseed. The first method, described by Bell (1999), involves planting cottonseed, harvesting seedlings 2 weeks after planting, removing a 1-cm proximal section of roots, grinding the root sections in sterile distilled water, and plating the homogenate on microbiological media. Media used to isolate bacteria included TSA, D-1, and EMB. Method 2 involves shaking seed in broths of media selective for the growth of *Agrobacterium* spp. (D-1 or D-5) for 48 hrs, homogenizing the seed and broth, and dilution plating the homogenate on TSA, D-1 and EMB. Bacteria were commonly detected on/in seed using either method. Biolog or FAME analysis indicated that none of the isolates from seed of any cultivar by the seed assay method (Method 2) were *Agrobacterium* spp. Bacteria from a number of genera commonly associated with plants were recovered, including *Enterobacter agglomerans*, *Pantoea agglomerans*, *Stenotrophomonas maltophilia*, *Pseudomonas* spp., *Paenibacillus* spp., and *Bacillus* spp. Artificially infesting seed with isolates of *Agrobacterium* and incubating in D-1 broth increased populations approximately two log units when seed were assayed 48 hrs after inoculation. By Method 1, *Agrobacterium tumefaciens/radiobacter* was isolated from PM 1218 BR and PM 1220 RR at very low frequencies in the Clemson and Arkansas laboratories, respectively. This preliminary information suggests that *Agrobacterium* spp. may be present at very low frequencies in cottonseed of some cultivars. The apparent latent infection of cotton seedlings by *Agrobacterium* spp. warrants further investigation. *Agrobacterium* spp. isolated from cotton are currently being characterized using PCR with primers specific for virulence genes of *Agrobacterium tumefaciens*.

References

Bell, A. A. 1999. *Agrobacterium* bronzing and wilt: Cultivar reactions and effects of temperature. p. 117-120 In Proc. Beltwide Cotton Conf., Orlando, FL. 3-7 Jan. 1999. National Cotton Council, Memphis, TN.