Agrobacterium Bronzing and Wilt of Cotton: Epidemiology and Control

A.A. Bell
USDA, ARS, Southern Crops Research Laboratory,
College Station, Texas, USA 77845

ABSTRACT

Bronzing and sudden wilt syndrome that develops in USA cotton plants during boll development in hot weather have been associated with infections of roots by Agrobacterium biovar 1 strains. Conditions favouring the multiplication of the bacteria in roots and development of symptoms include: high clay content in soil, high soil temperatures (above 30ºC), excess nitrogen and/or irrigation and deficiencies of phosphorus, sulphur and/or potassium. Certain isolates of Fusarium species, Talaromyces flavus, Pseudomonas aeruginosa, Burkholderia cepacia and Bacillus subtilus were antagonistic to Agrobacterium and alleviated symptom development when added to pasteurized soils before planting Agrobacterium infested seed. Increasing the amounts of phosphorous and sulphur in the fertilizers decreased bacterial multiplication in roots as well as bronzing and sudden wilt symptoms. Cultivar resistance was evaluated by incubating seed in germinating towels wet with bacterial suspension for 24 hours at 30ºC, planting in pasteurized soil containing 15.0-2.5-25.0 fertilizer containing chelated minor elements. Generally short-season cultivars that fruited early were more susceptible than cultivars that fruited at higher nodes and were heat tolerant. Some short season cultivars were more resistant than others.

Introduction

Short season production systems that require fewer pesticide applications have been developed for cotton production in marginal areas of the USA Cotton Belt. They are based on short-season cultivars that set and mature fruit more rapidly. Practices such as early planting, preplant fertilization and application of granular systemic insecticides for control of early insects, have been used to facilitate early boll set and crop development. In 1995 and 1996, many short-season cultivars were severely affected by a disease referred to "sudden wilt", "copper top", "red top", "bronzing", "early fade out", or "*Pseudomonas* wilt," depending on locality. Fields in Texas, Louisiana, Arkansas, Mississippi, Tennessee and North Carolina suffered losses of as much as 50% of the potential crop. A few fields in Texas were affected so severely that they were not harvested. In 1997, the disease also appeared in Georgia and California, indicating that it is present throughout the USA Cotton Belt. It is especially severe on heavy clay soils or silt loams, such as those in river bottoms. It generally affects plants most severely in July or August, after heavy fruit loads have set and hot, moist conditions prevail. A prolonged dry period in late June or July followed by rain or irrigation, often precedes the appearance of severe disease symptoms.

Diseased plants show extensive rotting of the fine tertiary roots and most of the original secondary roots have been lost, so that few, if any, sizeable secondary roots remain. Necrotic scars remain on the healthy roots at sites where the fine roots were rotted away. A bacterium that was consistently found in the necrotic root scars from several hundred diseased plants collected across the USA Cotton Belt, was identified as a strain of *Agrobacterium* biovar 1 (Bell *et al.*, 1997). Only *Agrobacteriumtumefaciens* biovar 2 had previously been reported from cotton under field conditions, and it caused typical crown galls as well as root galls (Zutra and Orion, 1982). *Agrobacterium* biovar 1 species are named according to the presence or absence of infectious tumor-inducing (Ti) or root-inducing (Ri) plasmids (Krieg and Holt, 1984): *A. radiobacter* has no Ti or Ri plasmid, *A. tumefaciens* contains a Ti plasmid, and *A. rhizogenes* contains an Ri plasmid. However, not all of 86 isolates from cotton contained infectious plasmids, and those isolates that contained plasmids induced both abnormal roots and galls on cotton roots with the predominant response depending on environmental conditions and the specific isolate (Cui *et al.*, 1997). None of 86 isolates from cotton caused tumours on the crown or aerial parts of the plant. The cotton isolates behave like the rhizogenic *Agrobacterium* biovar 1 isolates found in cucurbits in England (Davioud *et al.*, 1988) and melons in Japan (Sawada *et al.*, 1995). They have been called both *A. rhizogenes* and *A. tumefaciens* by different investigators, consequently, no species identification has been given to the cotton isolates, even though all isolates are very similar in characters controlled by the chromosome that is consistent with a single species. The host range of the cotton *Agrobacterium* has not been determined but *Agrobacterium* biovar 1 isolates similar in appearance and behaviour to those from cotton have been isolated

in the USA from seeds and roots of soybeans, peanuts, dry beans and sorghum. In 1998, a similar bacterium was isolated from galls on cantaloupe roots from Puerto Rico.

Causal Organism

Agrobacterium biovar 1 from cotton can be readily isolated and characterized (Schaad, 1988). The bacterium can be obtained readily from several tissues: necrotic scars or pustules left on tap roots due to the rotting of the secondary root; callus cells formed from root scars on old tap roots; undifferentiated tumors which are 2-30 mm wide on secondary roots or lower tap root; or even apparently healthy roots from plants more than 1 month old. *Agrobacterium* concentrations in these tissues generally vary between 1-100 million colony forming units (cfu) per gram of fresh root with the highest concentrations in newly formed callus or gall tissues.

Isolation of the bacterial pathogen from cotton tissue is generally best accomplished by grinding tissues in water and spreading dilutions on D-l medium (Schaad, 1988). The *Agrobacterium* biovar 1 from cotton grows readily on either biovar 1 or biovar 3 medium (Ibid.) and unlike *Agrobacterium* biovar 2, does not produce acid on PDA plus 0.08% calcium carbonate. Most isolates show all the characteristics considered typical for biovar 1 (Ibid.), except some isolates do not form 3-ketolactose. This variation was also noted for *A. rhizogenes* biovar 1 isolated from cucumber in England. The cotton pathogen shows unusually high temperature adaptation for an *Agrobacterium*; that is, it grows at 43$_{o}$C. It also produces more constitutive endopolygalacturonase (pH 4.5 optimum) than biovar 1 isolates belonging to *A. radiobacter* or *A. tumefaciens*. In this respect, it is similar to *Agrobacterium vitis* that causes root rot of grape (Rodriguez *et al.*, 1991).

Fatty acid profiles of *Agrobacterium* biovar 1 isolates from cotton are similar to those of type strains of *A. radiobacter* and *A. tumefaciens* (Bouzar *et al.*, 1993). The cotton isolates usually have lower concentrations of the 17:0 CYCLO and 19:0 CYCLO w8c fatty acids than the type strains of the two species. A few of the cotton isolates also contain the 16:0 3OH fatty acid not found in the type strain of *A. tumefaciens* biovar 1. Four different profiles of fatty acids were found among 10 isolates from cotton, indicating considerable variation in the pathogen.

The *Agrobacterium* biovar 1 isolates from cotton did not induce stem tumours on cotton, tobacco, tomato, Kalanchoe, Fava bean, snap bean, carrot or sunflower and induced roots only occasionally on carrot root discs and tomato stems. Some isolates induced tumours on secondary cotton roots in neutral soils, but only root deterioration followed by appearance of replacement roots were observed in alkaline soils. White replacement roots that emerge from sites where the original secondary roots are killed have the appearance of transformed roots. However, it has not been determined if transfer DNA is incorporated in these roots or the root tumours.

Many cotton isolates contain a plasmid similar in size to the Ti plasmid in *A. tumefaciens* type strain B6 (Cui *et al.*, 1988). Twenty-five of 86 isolates also gave positive PCR amplification of a 730 base pair section overlapping virC1 and virC2 genes. Sequence analysis of the 730 bp amplification product cloned from isolate 34B from seed of the cotton cultivar Stoneville 132 showed greater than 70% nucleotide identity to virC gene sequences from five other known Ri- and Ti-plasmids from *A. rhizogenes* and *A. tumefaciens* (Cui *et al.*, 1997). This plasmid section is highly conserved in structure when present in infectious Ri and Ti plasmids, but it is not always present (Sawada *et al.*, 1995).

Symptoms

The most dramatic symptoms of the disease develop during fruit development and become progressively more severe as bolls approach maturity. Plants may totally collapse and develop sudden wilt overnight or within a few days of the first symptoms. In clay soils, the plants at the end of a row are often most severely affected and may totally collapse and die with leaves attached, while internal plants in the row, even those next to the end ones, have few symptoms. In other soils, plants may die randomly throughout the field with premature opening of bolls. Usually, the loss of turgor in leaves is not apparent. However, the tops of diseased plants become slightly chlorotic, leaves become bronzed or reddened and leaves, bolls and flower buds abscise. Leaves also may show epinasty and lack the gloss of healthy leaves. Sulfur fertilizers (gypsum or Epsom salts) partially alleviate these symptoms in the greenhouse.

Collapse and blackening of phloem and bark tissues sometimes occurs on petioles of leaves and bolls and on the main stem near the boll petioles. These darkened lesions vary from a few millimetres to many centimetres in length, and, in some cases, the entire terminal end of a branch or stem becomes blackened and all leaves die or abscise. Young bolls often abscise or fail to develop, showing blackening or splitting of the boll carpels before maturity. In less severe cases, bracts may become necrotic prematurely and leaves subtending the boll and the fruiting branch may develop nectrotic margins and defoliate. All of these symptoms can be greatly reduced by applying triple superphosphate granules to the soil to supply continuous excess phosphorus, indicating that the bacterium may interfere with phosphorus uptake or transport in the plant. This conclusion is supported by the finding that roots of wilting plants are unable to pump water by active root pressure unless they are given phosphate salts.

Extensive rotting of the secondary and tertiary roots often leaves only the large tap root and a few secondary roots. These remaining roots appear healthy, except for the necrotic scars or pustules caused by the rotting of roots once attached to them. Proliferations of callus cells sometimes grow from scars, especially in the presence of high moisture levels. Galls generally break off and remain in the soil when plants are pulled in the field, but galls can be observed if plants are gently lifted with a spade. Galls on some cultivars are very small and may be confused with those caused by root knot nematodes. The largest galls were on roots of Acala cultivars.

Although the pathogen enters and moves in the xylem vessels, it rarely causes any vascular browning in xylem tissue of either the stem or taproot. Similar behaviour has been reported for *A. vitis* in grape (Tarbah and Goodman, 1987). The absence of vascular browning in the lower stem most readily distinguishes *Agrobacterium* wilt from *Fusarium* or *Verticillium* wilt. In *Agrobacterium* wilt and the fungal wilts, the plants may recover and make new growth, especially from the lower stem nodes. However, these plants produce little or no fiber and any that is produced is of poor quality. Additionally, embryos in seed may be aborted or underdeveloped.

Disease Cycle and Epidemiology

Agrobacterium biovar 1 from cotton survives in seed, soil or water in the absence of its host. As with the bacterial blight pathogen, the bacteria in seed are most readily detected by planting surface-sterilized seed in sterile sand and then isolating the bacteria from the young seedling root. The bacteria apparently infect the roots through the natural wounds created by the emergence of secondary roots, since these are the tissues where it first appears and is most concentrated. Numbers of the bacteria are higher on nematode infested roots than on roots without nematodes, indicating that wounds caused by nematodes may also be infection sites. Likewise, lesions caused by *Rhizoctonia* have much higher *Agrobacterium* populations than adjoining healthy tissues, indicating that penetration by fungi may allow infection of hypocotyls by the bacteria. *Agrobacterium* biovar 1 can be isolated consistently from cotyledons, but it is uncertain whether this infection occurs in the soil before emergence or is due to systemic introduction of the pathogen. Bacteria first appear in the lower 2-4 leaves at about the same time that the first secondary roots are rotted completely at their point of attachment to the primary root (i.e., 3-5 weeks after planting). This indicates that the bacteria are transported systemically through the xylem.

providing wounds and inhibiting natural antagonists. These complex microbial interactions may explain why plants growing side by side can exhibit very different symptom severity even though both are infected by *Agrobacterium*.

Agrobacterium biovar 1 appears to reach the developing seed through the xylem. In plants with developing bolls, it can be isolated from xylem eluates and accumulates in xylem fluids forced from severed stems by natural root pressure. Small necrotic lesions on leaves near bolls usually yield the bacteria, if the leaves are thoroughly washed and incubated at 100% relative humidity for 24 hours before samples are taken. The bacteria in foliar parts are apparently often latent and bound to host cell walls since they do not disperse into water. It is necessary, therefore, to stimulate new bacterial growth before the bacteria can be readily isolated.

The damage to cotton tissue probably involves an endopolygalacturonase (pH 4.5 optimum) enzyme. There is considerable evidence that an endopolygalacturonase (pH 4.5 optimum) is involved in grape root rot caused by *A. vitis* (Rodriguez *et al.*, 1991). In several assays, *Agrobacterium* biovar 1 isolates show endopolygalacturonase activity equivalent to that of *A. vitis* isolates, whereas *A. rhizogenes* biovar 2 isolates that cause hairy root in other plants showed little or no enzyme activity.

Conditions favouring the multiplication of the bacteria in roots and the development of symptoms include: high clay content in soil, high soil temperature (above 30$_o$C), excess nitrogen fertilizer and/or irrigation, deficiencies of phosphorus, sulphur and/or potassium, and high soil pH (above 7.0). Prolonged dry weather followed by abundant rain or irrigation often triggers outbreaks of the disease during hot weather. Generally, the most determinant cultivars are the most vulnerable to the disease. Accordingly, disease symptoms are more severe when bolls are set at low internodes compared to high internodes and when heavy fruit loads are set compared to light fruit loads. Consequently, management systems that encourage early, heavy boll set often aggravate the disease.

Agrobacterium is a poor competitor outside root tissue. Certain isolates of soil fungi, such as *Fusarium* species, *Phoma* species, and *Talaromyces flavus* and soil bacteria such as *Bacillus* species, *Pseudomonas aeruginosa*, and *Burkholderia cepacia* are antagonistic to *Agrobacterium*. They delay disease development and decrease severity when added to pasteurized soils before planting cotton seed infested with *A. rhizogenes* biovar 1. Other microorganisms, such as nematodes, *Rhizoctonia solani* and specific isolates of *P. aeruginosa* and *B. cepacia*, enhance infection by *A. rhizogenes* biovar 1 apparently by

Control

None of the currently used seed treatments, either chemical or biological, effectively eradicate *Agrobacterium* from the cotton seed or reduce the

incidence of infections in seedlings. Cultivar resistance to the disease was evaluated by incubating seed in germination towels wet with bacterial suspension for 24 hours at 30₀C, planting in an alkaline pasteurized soil containing 25% clay, and fertilizing plants grown at 25-35₀C with 15-(2.5-5.0)-25 fertilizers containing chelated minor elements. Roots of all cultivars become extensively infected, but some cultivars apparently are less vulnerable to the pathogenic effects of the bacteria. Generally, short-season cultivars that fruited early were more susceptible to the disease than cultivars that fruited at higher nodes and were heat tolerant. Cultivars such as Deltapine 50, Deltapine Acala 90, Hyperformer HS-46, Paymaster HS 26, Paymaster 1560, Stoneville 474, Stoneville LA-887, Suregrow 125, and Tamcot Sphinx showed only slight damage from the disease in the field and when 5% P was used in the greenhouse. Damage was more severe in all cultivars when 2.5% P was used and no cultivar set fruit without P, even though soil tests showed high P levels. Susceptible cultivars should be avoided.

Preplant balanced fertilizers compared to nitrogen alone reduce disease in sterile soil in the greenhouse, but is only now being field tested. Correction of K, P, or S deficiencies is important because they increase bacterial growth in roots and disease severity. Avoidance of excess nitrogen fertilizer is even more important because of the correlation between disease severity and N fertility. Management practices that encourage early boll set should be evaluated carefully, since they may increase the severity of *Agrobacterium* bronzing and wilt.

References

Bell, A.A., H. Orta and Y. Cui. (1997): *Agrobacterium* root rot and wilt: a widespread seedborne disease of cotton and legumes. In: Proc. Beltwide Cotton Conf. P. Dugger and D. Richter, (Ed) Natl. Cotton Council, Memphis, TN.

Bouzar, H., J.B. Jones and N.C. Hode. (1993): Differential characterization of *Agrobacterium* species using carbon-source utilization patterns and fatty acid profiles. Phytopath. 83:733-739.

Cui, Y.H., A. Bell and C. Magill. (1998): unpublished data.

Cui, Y., H. Orta, A. Bell, C. Magill and C. Gonzalez. (1997): Characterization of plasmids in the *Agrobacterium* causing root rot and wilt of cotton. Phytopathology 87:S21.

Davioud, E., A. Petit, M.E. Tate, M.H. Ryder and J. Tempe. (1988): Cucumopine -- A new T-DNA-encoded opine in hairy root and crown gall. Phytochemistry 27:2429-2433.

Krieg, N.R. and J.G. Holt. (Ed). (1984): Bergey's Manual of Systematic Bacteriology, Volume 1. Williams and Wilkins, Baltimore, MD, USA.

Rodriquez, P., T.J. Burr and A. Collmer. (1991): Polygalacturonase is a virulence factor in *Agrobacteriumtumefaciens* biovar 3. Journal of Bacteriology 173:6547-6552.

Sawada, H., H. Ieki and I. Masuda. (1995): PCR detection of Ti and Ri plasmids from phytopathogenic *Agrobacterium* strains. Applied and Environ. Microbiology 61:828-831.

Schaad, N.W. (Ed). (1988): Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd Edition, APS Press, St. Paul, Minnesota.

Tarbah, F. and R.N. Goodman. (1987): Systemic spread of *Agrobacterium tunefaciens* biovar 3 in the vascular system of grapes. Phytopathology 77:915-920.

Zutra, D. and D. Orion. (1982): Crown gall bacteria (*Agrobacterium radiobacter* var.*tumefaciens*) on cotton roots in Israel. Plant Dis. 66:1200-1201.