ROLE OF *AGROBACTERIUM* IN BRONZE WILT OF COTTON

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Abstract

Species of *Agrobacterium* biovar I are endophytic parasites of the cotton plant. The bacteria occur in all seed lots produced in the United States and generally are present in more than 90% of the seed. The bacteria are primarily present in the seed coat and infect both the radicle and cotyledons during initial stages of seed germination. Movement of *Agrobacterium* to new seeds apparently occurs through xylem vessels. Symptoms associated with *Agrobacterium* infection include: 1) necrosis and browning of secondary and tertiary roots, usually beginning at their site of origin from the parent root, 2) bronzing, lesions, blight, and defoliation of leaves, 3) leaf, crown, and root galls usually in association with other microbial infections, 4) distortion and abscission of bolls, 5) reduction of seed weight and fiber length and strength, and 6) wilt. Extensive damage to the root system also destroys the ability of the plant to actively take up water and minerals such as phosphorus, potassium and iron. The bacteria also may produce toxins. Four lines of evidence indicate that *Agrobacterium* is the cause of bronze wilt: 1) many environmental variables increase both *Agrobacterium* concentrations in the root and bronze wilt severity, 2) different isolates of *Agrobacterium* show distinct differences in ability to colonize roots, cause root necrosis, and predispose plants to soreshin caused by *Rhizoctonia solani*, 3) susceptibility to bronze wilt in modern cultivars is simply inherited, and 4) single bacterial blight resistance genes (*B* genes) show significant specific effects on bronze wilt severity. Bronze wilt has increased in occurrence and severity in recent years because of the inadvertent introduction of susceptibility genes into short-season cultivars, the progressive invasion of cotton seed stocks by highly virulent strains of *Agrobacterium*, and climate changes that favor disease development.

Introduction

In plants affected by bronze wilt the bark of the taproot and stem remain healthy and there is no vascular discoloration in the wood of the lower stem and upper taproot. These characteristics readily distinguish bronze wilt from fungal wilts which exhibit dark streaks in the wood or fungal root rots which cause degeneration of the taproot bark. The bronzing and wilt symptoms apparently result from the degeneration of the fine secondary and tertiary roots. Most of the fine roots washed from core samples taken under plants showing bronze wilt are discolored and necrotic. Frequently only brown scars or lesions remain where small roots were girdled and killed at their points of origin from the parent taproot or secondary root. These root scars and lesions invariably contain high concentrations of *Agrobacterium* biovar I which frequently is accompanied by various other bacteria and fungi, none of which occur consistently in lesions from different plants or locations.

Efforts to prove that *Agrobacterium* is the cause of bronze wilt have been frustrated by the fact that *Agrobacterium* apparently is an endophytic parasite that occurs in nearly every cottonseed of any variety or seed lot. In addition, the bacterium is highly infectious and easily transmitted. Consequently, I have been unable to keep plants free of infection beyond the initial flowering stage. Because of these limitations, it has not been possible to fulfill Koch's postulates for proving that a microorganism is the cause of a disease. Several other approaches have been taken to determine whether *Agrobacterium* is essential for the development of bronze wilt. These experiments are reported in this paper.

Materials and Methods

The methods used to grow *Agrobacterium* in culture, inoculate cotton plants and enumerate bacteria in roots are described in another paper in this volume (Bell, 2000b). Only additional methods are described here.

Detection of *Agrobacterium* in Seed

Seed were acid delinted with concentrated sulfuric acid, rinsed in tap water, neutralized in water saturated with fine calcium carbonate for 30 min, rinsed again and dried at 40 °C for 1 week. Seed were then stirred in 0.5% sodium hypochlorite (10% chlorox) containing 0.1% Tween 80 for 15 min, rinsed in sterile water, and planted in a pasteurized mix of fine sand and clay soil (3:1). After 2 weeks, seedlings were pulled and 1-cm of the upper taproot, where secondary roots had emerged, was removed, washed thoroughly with tap water and crushed in four drops of sterile water in a ceramic spot plate with a rounded glass rod. The suspension was applied to D-1 medium with the rod by streaking it once over the surface of the medium. Plates were examined for the presence of *Agrobacterium* after 72 h at 28 °C.

Determination of Seed Quality

During the neutralizing step as described in the previous section, seed were divided into light and dense fractions by removing the floating seed. After drying, the seed were weighed and counted to determine mean seed weight. Fragments and broken seed were removed from the dense fraction before mean seed weight was determined.

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Development of Inoculation Techniques

The efficiency of different inoculation techniques was determined by using six different ketolactose negative (KL-) variants, two morphological variants - - one negative for extracellular polysaccharide slime (EPS-) and one that overproduced EPS (EPS+), and 2 variants that failed to grow at 38 °C (T-). These variants were used to inoculate seedlings in germination towels before transplanting to soil. After specified inoculation periods or other treatments, the percentage of the variant versus the wild type was determined in roots or other plant parts. In this way, the efficacy of techniques for displacing endogenous strains in seed with a single specific isolate could be determined.

Comparison of Pathogenicity

Surface sterilized 'Paymaster 1220 B/R' seed were chipped, and placed in 50ml of sterile water containing a single bacterial isolate. The bacterial isolates kept in water stocks were streaked on PDA medium containing 0.08% fine calcium carbonate, and after 24 h at 28 °C, three 10-µl loops full of bacteria and slime were transferred to the water to provide inoculum. The seed were placed on a shaker at 200 rpm at 30 °C for 16 h and then were rinsed with sterile water and transferred to petri dishes containing 9-cm discs of Whatman No. 3 filter paper saturated with bacterial inoculum. After an additional 24 h at 30 °C, the seedlings were each transplanted to 500g of pasteurized sand-clay (3:1) mix, and incubated in an environment chamber for 1 week at 30 °C and thereafter at 37 °C with a 15-h photoperiod. Plants were fertilized weekly with Peters 15-16-17, 150mg/plant/week.

Predisposition to Soreshin

Surgically removed embryos of 'Paymaster 1220 B/R' were washed twice for 30 min with tap water and then placed in bacterial suspensions as described previously. After shaking at 200 rpm at 30 °C for 16-20 h, the embryos were transplanted to sand:clay (3:1) mix and incubated in the greenhouse at 28-33 °C using 15-16-17 fertilizer. Two sorghum seeds infested with *Rhizoctonia solani* strain AK-33 were introduced near each plant at 19 or 30 days after planting as described elsewhere (Bell, 2000b). The percentage of plants killed was determined after 2 weeks. The entire lesion above the secondary roots was washed thoroughly with tap water and macerated to determine the *Agrobacterium* content.

Results and Discussion

Endophytic Behavior of *Agrobacterium*

A high percentage of cottonseed from any cultivar or seed lot is infested with *Agrobacterium* biovar I (Tables 1 and 2; Bell et al., 1997). Usually, *Agrobacterium* has been found in about 90% of the seed. An even higher percentage probably is infested, since other bacteria sometimes inhibit *Agrobacterium* on D-1 medium, making it impossible to find small numbers of *Agrobacterium* colonies. By the time the first flower emerges, *Agrobacterium* can be isolated from roots of 100% of cotton plants in the greenhouse. Some of these infections may have spread from other infected plants. *Agrobacterium* apparently is present in the seed coat, since the non-aggressive KL- variant isolate 14A can be used to completely displace endogenous strains by inoculating washed embryos but not by inoculating chipped seed which retain some of the seed coat (Table 3).

Agrobacterium is spread systemically through the xylem system of the plant. When plants were decapitated at the time of boll opening, *Agrobacterium* was found in xylem sap forced by natural root pressure or in xylem eluates collected by applying vacuum to elute the xylem of stem sections with water. About 10% of the plants yielded *Agrobacterium* from xylem on any given day (Table 4). By the time the cotton plant has 5-6 true leaves, *Agrobacterium* can be isolated from all tissues except the terminal stem and leaves (Table 5). This further indicates that transport is through the xylem, since vessels are not open in juvenile tissues. Presumably the bacterium also enters the seed coat through the xylem, although this has not been proven. The xylem vessels leading into the boll generally do not open until about 3 weeks after anthesis. This is the same time period required before necrosis of the bracts may occur as a symptom of bronze wilt.

Symptoms Apparently Caused by *Agrobacterium* Infections

In the absence of environmental stresses, *Agrobacterium* infections may remain confined to natural wound sites and epidermal tissue of the root. In this case few, if any, symptoms develop on any part of the plant. The bacterium still invades the entire root system by the time of flowering but concentrations of the bacteria usually remain below 1 million/g of root. Because of this situation it is difficult to determine with certainty what symptoms result from *Agrobacterium* or whether there is a negative effect on yield.

When bronze wilt is expressed, *Agrobacterium* concentrations in the whole root often exceed 10 million/g root and concentrations in necrotic or browned tissues may be as high as 500 million/g. These observations indicate that necrosis and browning of the fine roots is caused by high concentrations of *Agrobacterium*. Increased nitrogen fertilization and increased temperatures above 33°C cause marked increases in *Agrobacterium* concentrations in roots and in necrosis of fine roots (Bell, 1998a, 1999c, 2000c).

Leaf, crown, and root galls can be caused by *Agrobacterium* strains. When surgically removed embryos were inoculated with *Agrobacterium tumefaciens* strains B6 or A281, numerous tumors appeared on the cotyledons about 3 weeks after planting. Sequential inoculations of cotton with *Agrobacterium tumefaciens* strains 1A, 14A, and 34B and

Rhizoctonia solani led to a high percentage of crown galls (Bell, 1999a, 2000b). When seedlings inoculated with the same three strains were transplanted into nonsterile soils, root galls were formed (Bell, 1999b). The galls did not form in sterile soil indicating that a second pathogen, such as a nematode, is required. Crown and root galls were observed on fewer than 5% of plants with bronze wilt in the field. Thus galls are not essential for bronze wilt, but they may further debilitate the plant (Bell, 1999a, 2000b).

Plants with high concentrations of *Agrobacterium* in the root also, exhibit a number of foliar symptoms besides bronzing of the leaves. The most frequent symptom is blight of the bract followed by a blight of the leaf subtending the boll. Often these are the only foliar symptoms even when extensive root necrosis has occurred. Bracts may die completely as early as 3 weeks before the boll opens. Severe infection of seedlings usually leads to a blight of the cotyledon and lower leaves. The blight begins at the hydathodes on the leaf margins and progresses both inwards and along the margin. Once the blight affects one-third or more of the leaf, the leaf usually abscises. Blight followed by defoliation is common in cultivars carrying the B_2 or B_2,B_3 genes for blight resistance, especially when day temperatures greater than 35°C occur. Lesions occur along the major veins of old leaves near the time of boll opening. These are usually 1-5mm wide and may show zonation depending on the cultivar and the availability of potassium and sulfur nutrients. Lesions are larger and more frequent when these nutrients are deficient. *Agrobacterium* is often latent in diseased leaves but can be isolated if leaves are surface disinfected and floated on sterile water (Table 5).

When phosphorus is deficient, blight may be extremely severe (Bell, 1998b, 1999b, 2000a). This is especially true if *Agrobacterium*-inoculated plants are transplanted into pasteurized natural soils and maintained without phosphorus in the fertilizer. If all nutrients except phosphorus are supplied in fertilizers, plants appear normal and healthy at about 3 weeks after planting. Then, blight develops on the lower leaf, which defoliates. Thereafter, plants become extremely stunted as a leaf is blighted and lost for each new leaf formed. Even after many months, the plants have only a few leaves and flower buds never form. If seedlings are given a high-phosphorus starter fertilizer (e.g. 9-45-15) but then are deprived of any further phosphorus, the plants set fruit, but leaves, stems, and even whole plants may quickly collapse and die. During latter stages of fruit development sometimes the dead tissues, especially stems, turn dark brown or black in color. This indicates that phosphorus starvation also may contribute to sudden wilt or sudden death symptoms in the field.

Various boll abnormalities also occur in association with the root damage caused by *Agrobacterium*. Besides the bract necrosis, bolls may become distorted and crack and open prematurely. The cracks may allow infections by boll rot fungi such as *Fusarium semitectum* or *Aspergillus niger*. With the premature opening, locks do not fluff properly and support high populations of saprophytic fungi and bacteria. Some bolls are blighted as described in the previous paragraph and remain attached to the plant. Other bolls abscise often leaving the top of the plant devoid of fruit.

Seed and fiber also may be immature and of poor quality due to *Agrobacterium* infections and root necrosis. Table 6 shows the effects of bronze wilt on seed density and weight when the disease developed in the greenhouse from natural seed infestations. Bronze wilt caused a large increase in the percentage of light seed and resulted in a 21-29% reduction in seed weight in the three cultivars studied. In this experiment, high day temperatures and restricted phosphorus availability were maintained throughout the experiment, and considerable root necrosis had already occurred at the time of flowering.

Another experiment was performed in which four cultivars were inoculated with *Agrobacterium tumefaciens* strains 1A, 14A, and 34B and heat stress (38 °C day and 30 °C night) was applied only during the final weeks of boll maturation. Controls were maintained at 25-33 °C throughout the experiment. The late heat stress caused very few foliar symptoms and no loss in root weight. However, it resulted in about a 40% reduction in seedcotton yield in each cultivar (Figure 1). The causes of the losses with two different fertilizers, each adjusted to give 45 mg of N/plant/week, are shown in Table 7. The major cause of yield loss was a reduction in seedcotton/boll, i.e., seed weight. Bolls of heat stressed plants also opened 4-5 days earlier than those maintained at the lower temperature, which may have contributed to seed immaturity. Boll abscission also contributed to losses and was greatest when phosphorus and potassium were marginally available in the fertilizer. The combined experiments show that *Agrobacterium* infections and root necrosis with or without bronze wilt symptoms cause seed shrinkage and boll losses.

Associations of *Agrobacterium* with Recent Bronze Wilt Epidemic

In 1996 when bronze wilt occurred extensively on short-season cultivars in Louisiana, Arkansas, Texas, Missouri, and North Carolina, root samples of infected plants and soil samples from under the plants were collected from all states and potential fungal or bacteria pathogens were isolated from recently killed fine roots or lesions on taproots. The predominant fungi found were *Fusarium oxysporum*, *Fusarium solani*, *Phoma exigua*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Verticillium nigrecens*, *Fusarium equiseti*, and *Diplodia gossypina*. The bacteria encountered most frequently were *Agrobacterium* biovar I, *Pseudomonas*

aeruginosa, *Burkholderia cepacia*, and *Enterobacter agglomerans.* Several isolates of each species were used to inoculate three pasteurized soils (Weslaco sandy loam, Brazos clay, and a fine mined Brazos sand) and surface sterilized seeds of 'Hartz 1215' were planted. Plants were fertilized only with nitrogen (½ urea and ½ ammonium nitrate) for 1 month and then with Peters 15-5-25 soluble fertilizer. Greenhouse temperatures were maintained at 24-33 °C. Except for *R. solani*, the most severe bronze wilt developed on control plants grown in uninoculated pasteurized soil. The results clearly indicated that bronze wilt was caused by some factor (biological or genetic) in the seed.

In subsequent experiments, surface sterilized seed were germinated in sterile germination towels or pasteurized fine sand and seedling cotyledons and radicles, and water expressed from towels was examined for microbial content. Only *Agrobacterium* biovar I was found consistently among 43 seed lots and in both assays. Various bacteria were added to germination towels but these either stimulated growth or had no effect. Those that stimulated growth inhibited but did not eliminate *Agrobacterium* populations from roots. Only *Agrobacterium* isolates enhanced bronze wilt when used to inoculate germinating seed that were transplanted to pasteurized soils in environment chambers at temperatures above 35 °C. Thus, subsequent studies were concentrated on the probable role of *Agrobacterium* in bronze wilt.

In 1997 taproots and lower stems were collected from the Southern states, Georgia and California where local outbreaks of bronze wilt occurred. Typical results of analysis for *Agrobacterium* are shown in Table 8. The highest concentrations of *Agrobacterium* were found in lesions or callus tissues which developed on the taproot at positions where secondary roots originally formed and then were killed. *Agrobacterium* also was found in stele tissue of the root and stem, but only at 1/1000 of the concentration found in fine roots or root bark. Thus, parasitism by *Agrobacterium* is most concentrated in natural wounds and the surface of the root. When *Agrobacterium* concentrations in root lesions were compared between symptomatic and adjoining non-symptomatic plants, the bacterium was found in all plants but concentrations were higher in symptomatic plants (Table 9). The results indicated that any damage inflicted by *Agrobacterium* results from extensive colonization and damage to the root.

In 1998 severe outbreaks of bronze wilt occurred in Georgia and in Missouri, Arkansas, and Mississippi. The disease probably aggravated drought damage in other states but generally was not recognized for its contribution. For example, in the Brazos Valley of Texas, cultivars known to be susceptible to bronze wilt were more severely affected by the drought than those that are resistant to bronze wilt. Several hundred taproots were collected from more than 50 farms in Georgia and Alabama and *Agrobacterium* concentrations in the chopped root bark were determined (Table 10). Root or crown galls were found on only about 5% of the samples but all roots showed evidence of secondary and tertiary root necrosis. High concentrations of the bacterium were found in nearly all roots. Plants with the highest *Agrobacterium* concentrations were often coinfected with *R. solani*, *F. oxysporum* f. sp. *vasinfectum* or root-knot nematode, indicating possible synergistic interactions with these pathogens. The results were consistent with *Agrobacterium* being the primary cause of the disease.

In 1999, typical bronze wilt occurred in Tennessee and surrounding states. In South Carolina, Southwest Arkansas and the Upper Gulf Coast of Texas extensive yield losses due to seed and fiber immaturity occurred with few typical bronze wilt symptoms. Seed samples were collected from Texas and Arkansas to determine whether *Agrobacterium* was present in the seed and whether seed damage was consistent with that caused by bronze wilt or *Agrobacterium* inoculations in the greenhouse. In all areas where seed damage occurred with few bronze wilt symptoms, heat stress did not occur until late in the growing season, similar to the situation tested in Fig. 1 and Table 7. The results from 20 samples collected from different farms in the Texas Upper Gulf Coast Area are shown in Table 11. More than 70% of the seed in all samples were infested with *Agrobacterium* showing that the plants were indeed infected with *Agrobacterium*. Further, the percentages of light seed and reduction in seed weight were consistent with those expected for plants infected with *Agrobacterium* and subjected to late season heat stress (Fig. 1, Table 7). The disease described in South Carolina (Dr. J. Stewart, personal communication) is essentially identical to that in Texas. Thus, *Agrobacterium* infection and damage to the roots likely contributed to the loss in seed and fiber quality in 1999. In all cases of bronze wilt and seed and fiber immaturity studied during the past five years, high percentages (usually above 90%) of the seed have been infested with *Agrobacterium*, and high concentrations (usually above 10 million) of the bacterium have been found in discolored roots and lesions on taproots.

Evidence that *Agrobacterium* Causes Bronze Wilt

Three types of experimental results indicate that *Agrobacterium* strains are the cause of bronze wilt. First, at least 10 environmental and cultural variables affect *Agrobacterium* concentrations in roots and bronze wilt severity in a parallel fashion. Second, single isolates of *Agrobacterium* show significant differences in ability to colonize roots, inflict damage to cotton roots, and predispose plants to soreshin caused by *R. solani*. Finally, cultivar susceptibility to bronze wilt is simply inherited and bacterial blight resistance genes (*B* genes) show specific interactions with *Agrobacterium* strains, causing significant changes in severity of root damage and foliar blight (bronze wilt). The

effects of environmental variables and the genetics of susceptibility and resistance to bronze wilt are discussed in another article (Bell, 2000c) in this volume.

We previously reported that 86 *Agrobacterium* isolates, two each from 43 seed lots, varied in ketolactose production (two classes), relative growth at 28°C and 38°C (two classes), fatty acid profiles (at least four classes), plasmid content (nine classes), and DNA structure revealed by three primers and RAPD analyses (at least 20 classes) (Bell, 2000a; Bell et. al., 1997, 1998; Cui et. al., 1997). The 86 isolates also vary in their ability to colonize roots (Table 12), inflict damage to roots (Fig. 2, Table 13), displace endogenous strains found in the seed coats (Table 14), and predispose cotton to soreshin caused by *R. solani* (Table 13, Table15). Isolates that develop the highest populations in root also caused the greatest root damage (Fig. 3). Inconsistent relationships between bacterial concentrations below 15 million/g and root damage may be due to the fact that mutant EPS(-) forms were not counted in this experiment. Several highly virulent strains that develop only low populations of the wild-type bacterium readily form the EPS(-) variant under heat or low phosphorus stress. By 8 weeks after planting more than half of the colonies of these strains may be the variant form. Figure 2 shows only selected results from a study involving 47 isolates of *Agrobacterium*. All 47 isolates reduced root and shoot growth below that of the control which also had some infection due to endogenous strains. The reduction in shoot growth was significant at the 5% confidence level for 21 of the isolates. Although the controls also became infected, the bacterium established more quickly and developed higher populations when specific single isolates were used as inoculum. Isolates that caused the greatest root damage also were the most effective in predisposing cotton to *R. solani* soreshin development (Table 13). Collectively, these studies show that the severity of bronze wilt and soreshin are dependent on the specific *Agrobacterium* strains found in cottonseed.

Conclusions

Bronze wilt can be caused by certain strains of *Agrobacterium* biovar I that readily infect cotton roots and cause necrosis and browning of the fine roots when plants are subjected to certain stresses, such as prolonged exposure to day temperatures above 35 °C (95 °F). Necrosis of the fine roots caused by *Agrobacterium* infections reduces the ability of the plant to actively take up water and certain nutrients, such as potassium and iron. Consequently, *Agrobacterium* infections also may disrupt embryo development and fiber maturation of seed, causing marked losses in seedcotton yields and seed and fiber quality. *Agrobacterium* acts synergistically with *R. solani* to increase *Agrobacterium* populations in hypocotyl tissues, the incidence of crown gall, and the incidence of soreshin leading to death or debilitation of the plant. Similar interactions probably occur with other soilborne pathogens. Bronze wilt has increased in occurrence and severity because of three reasons: 1) recessive susceptibility genes have been introduced into many modern cultivars, especially short-season cultivars, 2) highly virulent strains of *Agrobacterium* with increased potential to cause necrosis of roots are progressively invading cottonseed stocks, and 3) global warming is causing prolonged periods of hot weather that allow *Agrobacterium* infections to become more severe and damaging in all cultivars. Detailed knowledge of variation in *Agrobacterium*, mechanism of pathogenicity, and interactions with other pathogens are needed to develop the most effective controls.

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Table 1. Percentage of 1998 field-grown cottonseed[a] infested with *Agrobacterium* biovar I.

Cultivar	No. of Seed Tested	% Infested with *Agrobacterium*
Deltapine 50	320	92
Paymaster 1220 B/R	160	93
Stoneville 373	160	88

[a]Seed grown in 1998: Deltapine 50, Lot 50-FD-8005-NT was provided by Todd Voight; Paymaster 1220 BG/RR, Lot 1220 BR-A-8028-NT was provided by Dave Albers; Stoneville 373, Lot ST 373 x 71207 BR UT_4 was provided by Roger Ward.

Table 2. Percentage of cottonseed from 20 fields from the Texas Gulf Coast in 1999 infested with *Agrobacterium* biovar I.

No. of Fields	% Infested with *Agrobacterium*[a]
8	100
6	90 – 99
5	80 – 89
1	70 – 79

[a]Based on 24 seed samples.

Table 3. Recovery of KL(-) colonies of *Agrobacterium* from roots after inoculation of chipped seed or surgically removed embryos with the KL(-) variant isolate 14A. *

	Inoculation Technique and Sampling Time		
	Chipped Seed	Embryo	
Plant number	8 weeks	6 weeks	12 weeks
	(% recovery of mutant)		
1	20	100	0
2	0	100	60
3	0	100	0
4	0	100	--
5	0	--	--

*KL(-) variants were identified by the ketolactose test (Schaad, 1988).

Table 4. Occurrence of *Agrobacterium* biovar I in xylem of 43 cultivars at time of boll maturity.

	Percentage Yielding *Agrobacterium*[a]	
Sample[b]	Cultivars	Plants (all cultivars)
Xylem Eluate	37	3.8
Xylem Fluid	51	3.4
Eluate of Fluid	67	6.0

[a]Determined for 24 to 48 plants of each cultivar.

[b]Plant stems were swabbed with 70% ethanol, severed just below the cotyledonary node, and fitted with a sterile collection reservoir. Fluids were collected for 24 h. The first internode was decorticated aseptically, fitted to a collection reservoir, and eluted with 0.5-ml sterile water under vacuum. Fluids or eluates were spread on D-1 medium to identify *Agrobacterium*.

Table 5. *Agrobacterium* biovar I concentrations[a] extracted from various foliar parts by soaking in sterile water at 23ºC[b] for 1 or 24 h.

	Soaking Time	
Plant Part	1h	24h
Leaf – 1 (Terminal)	0	0
- 2	0	133
- 3	0	133
- 4	33	2000
- 5 (Bottom)	66	3033
Hypocotyl	0	4667
Lower Stem	0	100
Upper Stem	0	0
Lower Petiole	0	33
Upper Petiole	0	0

[a]Number per ml of water. Three replications.

[b]Parts were washed with 10% chlorox containing 0.1% tween 80 and rinsed with sterile water before soaking in sterile water. Hypocotyl, stems, and petioles were cut into 1-2 mm sections.

Table 6. Bronze wilt incidence and effects on seed maturity in greenhouse test.

	Cultivar[a]		
Character	Deltapine 50	Paymaster 1220 B/R	Stoneville 373
Percent of Symptomatic Plants	35	30	40
Percent Light Seed			
With symptoms	86*	91*	73*
No symptoms	32	46	27
Mean Seed Weight (mg)			
With symptoms	54.5*	57.7*	63.3*
No symptoms	71.7	81.6	80.3

[a]Cultivars are the same as used in Table 1: 80 plants of Paymaster 1220 B/R and Stoneville 373 and 160 plants of Deltapine 50 were observed.

*Significantly different from plants with no symptoms at 1% level of confidence.

Table 7. Changes caused by late season heat stress (38-30 ºC)[a].

	Fertilizer Composition	
Character	27-15-12	15-16-17
Seedcotton Yield	-43%	-39%
Boll number	-21%	-8%
Seedcotton / Boll	-28%	-33%
First Boll Opening	5 days earlier	4 days earlier
Root Weight	0.03	-2%

[a]Mean of four cultivars; compared to same cultivars at 33 to 25ºC.

Table 8. *Agrobacterium* biovar I concentrations in different plant parts from 1997 field samples.

Cultivar	Root Parts			Stem Stele
	Tap Lesions	Tap Callus	Secondary	
	(M cfu/g fresh root)			
ST 132	70[a]	105	62	0.039
Hartz 1220	108	173	15	0.018

[a]Results are means for at least 30 plants collected in Louisiana, Texas, and Arkansas.
ST = Stoneville
H = Hartz

Table 9. *Agrobacterium* biovar I concentrations in relation to field symptoms in Texas in 1997.

Location & Situation[a]	Symptoms	
	Severe	Mild
	(M cfu/g fresh root)	
Smith Plantation Nursery: (Dead vs. Live)	28.2	6.7*
El-Zik Plantation Nursery: (Dead Center vs. Periphery)	18.7	7.2
El-Zik Upland Nursery: (Dead vs. Live Pairs)	6.8	0.9*

[a]Plantation Nurseries were on Brazos Valley clay soils (pH above 8.0), whereas Upland Nursery was on Lufkin fine sandy loam (pH 5.5-6.0). The dead center was associated with a nitrogen fertilizer spill.
*Differences based on 20 or more plants were significant at 5% level of confidence.

Table 10. *Agrobacterium* concentrations in root bark of plants showing bronze wilt in Georgia and Alabama in 1998.

Agrobacterium	Frequency of Plants in Concn Range					
Concn. Range (M cfu/g)	GA-1[a]	AL	GA-2	GA-3	GA-4	Total
< 0.01	1	0	0	1	1	3
0.01 – 0.09	17	2	3	10	2	34
0.10 – 0.99	20	6	28	25	20	99
1.00 – 9.99	10	4	30	12	10	66
10 – 99.9	9	4	18	4	5	40
> 100	1	2	6	0	0	9
Total Plants	58	18	85	52	38	251

[a]Each group was provided by a different cooperator and came from different farms. Usually, no more than five plants were examined per farm.

Table 11. Analyses of 1999 seed samples from the Texas Gulf Coast.

Sample #	Sample Wt. (g)	Percent Light Seed	Mean Seed Wt. (mg)	Percent with *Agrobacterium*[a]
1	61	60	74	100
2	67	58	74	88
3	51	74	69	96
4	37	75	73	84
5	43	65	78	92
6	36	75	67	96
7	32	67	76	75
8	45	64	78	84
9	39	73	68	100
10	106	42	76	100
11	54	71	76	100
12	100	71	77	84
13	83	63	68	100
14	114	61	70	100
15	42	34	94	96
16	73	50	95	92
17	73	52	75	96
18	73	30	74	100
19	108	50	71	100
20	45	47	73	89

[a]24 seed samples

Table 12. Specificity of *Agrobacterium* isolates for population development in root of 'Paymaster 1220 B/R'.

Cultivar Source of Isolates	Isolate	Bacterial Concn. (M cfu/g)
Hyperformer HS-44	23A	1.9 (2.3)
	23B	32.6 (11.8)*
Hartz 1560	18A	23.5 (15.3)
	18B	3.9 (3.0)*

*Significantly different from the A isolate at the 1% level of confidence. Five replications; (standard deviation in parentheses).

Table 13. Specificity of *Agrobacterium* isolates for reducing plant growth and predisposition to soreshin.

Cultivar Source of Isolate	Isolate	Plant Weight[a]	% of Plants Killed by *R. solani*[b]
Deltapine 51	8A	9.20	60
	8B	8.71	50
Deltapine 5690	12A	10.14	50
	12B	9.27	70
HY 173-90	21A	6.11	100
	21B	7.06	100
Hyperformer HS-44	23A	6.37	100
	23B	6.08	100

[a]Plant (shoot and root) weighed 8 weeks after inoculating chipped seed with *Agrobacterium* isolate and incubating at 37° C.
[b]Plant embryos were inoculated with *Agrobacterium* and 19-day-old plants with *R. solani* isolate AK-33.

Table 14. Percentage of KL(-) colonies of *Agrobacterium* recovered from roots 8 weeks after inoculation of chipped seed with KL(-) mutants.

	Mutant Isolate Used for Inoculum				
Plant No.	**1B**	**11A**	**14A**	**16B**	**19B**
	(% recovery)				
1	20	0	20	10	0
2	0	0	0	30	0
3	0	0	0	20	0
4	0	0	0	80	0
5	0	50	0	20	0

Table 15. Specificity of *Agrobacterium* isolates for predisposition to soreshin in 30-day-old plants.

Source of Isolates	Isolate	Frequency of Killed Plants[a]
Hairy Root Gall-1	HR – 7	4/5
	HR – 10	4/5
Hairy Root Gall-6	HR – 31	0/5
	HR - 34	0/5

[a]Embryos inoculated with *Agrobacterium* and 30-day-old plants with *R. solani* isolate AK-33.

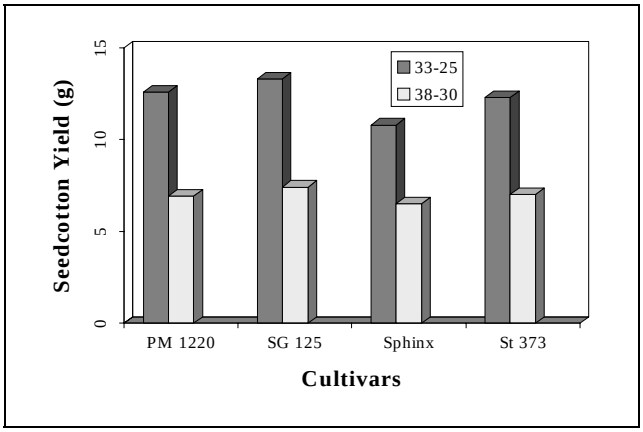

Figure 1. Effects of heat stress (38°C day and 30°C night) 3 weeks after boll set on seedcotton yield. Control plants were maintained continuously at 33 to 25°C.

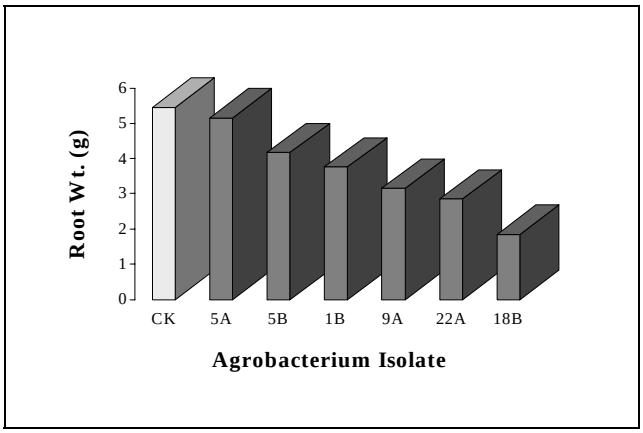

Figure 2. Inhibition of root growth of 'Paymaster 1220B/R' at 37°C by *Agrobacterium* isolates 8 weeks after planting.

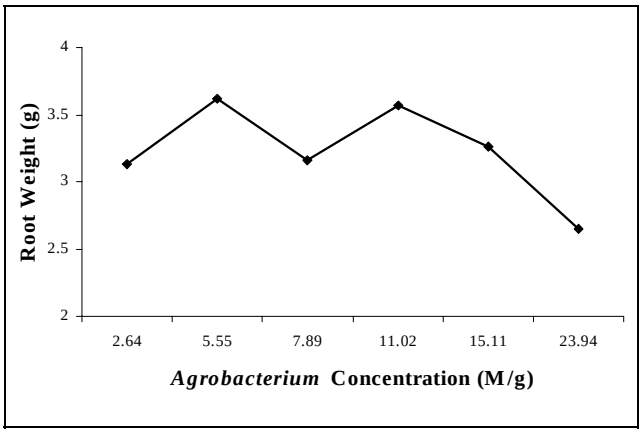

Figure 3. Relationship between *Agrobacterium* concentration and the inhibition of root growth. Points are means for each group of eight isolates obtained after data were arrayed according to concentration.