BRONZE WILT AS A CAUSE OF YIELD STAGNATION

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Abstract

Cultivars and breeding lines submitted for the 2000 Uniform Variety Trials were inoculated with *Agrobacterium tumefaciens* isolate 25A, grown in the greenhouse, and observed for plant development, yield, seed quality, bronze wilt, root necrosis, and bacterial concentration in roots. Five plants of each of 128 entries were sacrificed just prior to initial bloom and 10 others were allowed to develop mature bolls. Of the 1280 mature plants, 181 (14%) had severe root necrosis, 125 (10%) had severe bronze wilt, and 20 (2%) showed severe root necrosis and bronze wilt. Among the 128 cultivars and lines, 60 showed severe root necrosis and 49 showed severe bronze wilt in 10% or more of the plants. Cultivars that had 30% or more of the plants with severe root necrosis or bronze wilt yielded 14% and 15% less seed cotton, respectively, than cultivars that showed neither symptom. Within cultivars, plants that had severe bronze wilt or root necrosis yielded 10% and 14% less, respectively, than plants with no or mild symptoms. Plants with both symptoms yielded 26% less than control plants. Losses from bronze wilt were entirely due to reductions in seed weight, which were accompanied by large increases in the percentage of light seed. In contrast, losses from root necrosis were entirely due to reduction of seed number per plant with no change in seed weight or percentage of light seed. Decreased root weight occurred with both symptoms. However, the greatest reduction in root weight occurred before flowering with root necrosis, and after fruit set with bronze wilt. Cultivars and individual plants with root necrosis consistently had greater concentrations of *A. tumefaciens* in roots than those without symptoms, whereas bacterial concentrations in roots of cultivars with bronze wilt were not different or slightly less than those in cultivars with no symptoms. The results are consistent with the hypothesis that root necrosis is truly a susceptible reaction to *A. tumefaciens*, whereas bronze wilt results from toxins produced in roots and transported to leaves during fruit development. Losses from root necrosis may be a major, unrecognized cause of yield stagnation in cotton, since *A. tumefaciens* strains similar to 25A now occur in all cotton seed lots.

Introduction

Bronze wilt is a newly recognized disease of cotton characterized by bronze or red discoloration and wilting of leaves (Bell et al., 2002). It was first recognized as a problem in Mississippi and Louisiana in 1995 and in Texas, Arkansas, Tennessee and North Carolina in 1996. Some fields in Texas were so severely affected that they were not harvested. Devastating losses from bronze wilt occurred in 1998 in the Upper Mississippi River Delta and the Gulf Coast States. The disease was associated with record high temperatures in May and early June. In 1999, bronze wilt occurred in South Carolina, Tennessee and the Gulf Coast of Texas, and it was associated with high temperatures, mostly late in the season. In these cases, many plants showed excessive fruit shed or embryo abortions within bolls rather than foliar symptoms. Sporadic outbreaks occurred in 2000 and 2001 throughout the cotton belt even though many of the most susceptible cultivars have been removed from the market.

Losses from bronze wilt are difficult to estimate, in part because the symptoms are not easily distinguished from those of drought, nutrient deficiencies or several common soilborne diseases. Further, the symptoms vary depending on temperatures and fruit load. Under controlled environments 'Paymaster 1220 BG/RR' plants inoculated with *A. tumefaciens* isolate 25A developed severe foliar symptoms (bronzing, blighting and defoliation) only at 30 ºC days and 25 ºC nights (Bell, unpublished). When night or day temperatures were increased 5 ºC, yields were further reduced because of increased boll abortion or embryo abortion within bolls, but foliar symptoms were actually reduced. Thus, symptoms of bronze wilt may change dramatically depending on small changes in temperature as well as cultivar susceptibility. Finally some cultivars, when inoculated with *A. tumefaciens*, suffer extensive root necrosis but show only slight stunting and shortened internodes as foliar symptoms (Bell, 2001). For these reasons, a controlled greenhouse experiment was used to determine the extent and bases of yield losses caused by root necrosis and/or bronze wilt induced by inoculation with *A. tumefaciens* at planting.

Materials and Methods

Sources and Growth of Plants

The experiment included 128 entries: 118 cultivars and breeding lines from the 2000 Uniform Variety Trials and 10 lines containing different *B* genes for resistance to bacterial blight. Soil mixture was prepared and pasteurized, and seed were prepared, planted, and inoculated, as described previously (Bell, 2001). The experiment was performed in glass greenhouses beginning March 1, 2001. Heating and cooling thermostats were set at 20 °C and 30 °C, respectively, until boll set and at 25 °C and 35 °C, respectively after boll set. Plants received 150 mg 15-16-17 fertilizer containing chelated minor elements weekly.

Preparation and Measurements of Bacteria

Based on current nomenclature recommendations (Moore, Bouzar, and Burr, 2001) the *Agrobacterium* biovar 1 strain infecting cotton roots should be called *Agrobacterium tumefaciens*. Isolate 25A of *A. tumefaciens* from cotton was maintained and new cultures started as described previously (Bell, 2001). Wild-type behavior was confirmed on TSA, PDA chaulk, D-1 and Ketolactose test media (Moore, Bouzar, and Burr, 2001) before individual colonies were used to prepare inoculum as described previously (Bell, 2001). Bacterial concentrations in roots were determined just prior to flower in five plants and at maturity in 10 plants using procedures described by Bell (1999).

Data Collected

Days to boll set, node of first fruit set, days for boll maturity, shoot weight, root weight and seed cotton weight were recorded as measures of plant development. Percentage of fiber in seed cotton, mean weight of delinted seed, and percentage of light seed in 70% acetone were used as measures of fiber and seed quality. Foliar symptoms at three weeks (1-3 scale), bronze wilt severity at boll crack (1-5 scale), root necrosis severity at 8 weeks and after boll opening (1-5 scale), and bacterial concentrations in roots were used as measures of disease severity. Symptoms designated as severe had a rating of 4 or 5.

Results and Discussion

Frequency of Symptoms

A. tumefaciens isolate 25A readily infected feeder roots of all cotton cultivars and lines, and developed mean populations that ranged from 7.9 to 375.1 million (M)/gm fresh root in different entries just before fruit set. After boll opening, the concentrations in different entries ranged from 13.8 to 90.7 M/gm root. Mean concentrations for 38 entries sampled at both stages were 42.7 M/gm prior to boll set and 48.0 M/gm after boll opening. The frequency of infected plants showing severe root necrosis, bronze wilt or both is shown in Table 1. Of 1280 plants, 306 (24%) showed severe root necrosis, bronze wilt or both. Only 2% of the plants showed both severe root necrosis and bronze wilt. Thus, most diseased plants were affected by either root necrosis or bronze wilt but not both. Only 19 of the 128 entries had no severely affected plants among the 10 observed. Thus, both root necrosis and bronze wilt continue to occur extensively in some plants of cultivars currently being planted, even though many cultivars most susceptible to bronze wilt have been removed from the market (Bell et al, 2002).

Effects on Yield

The effects of bronze wilt and root necrosis on yield were estimated from comparisons among entries (Table 2) and among plants within entries (Table 3). The 54 entries in which 30% or more of the plants showed severe root necrosis or bronze wilt, yielded 15% less than the 15 entries which did not show either symptom. Plants that showed severe bronze wilt (4 or 5 rating) yielded 10% less than plants with mild or no symptoms within the same cultivar or line. Plants with severe root necrosis yielded 14% less than those with mild or no symptoms. Mild or moderate bronze wilt symptoms, in addition to severe root necrosis, caused no greater yield losses than those caused by root necrosis alone. This could indicate that these bronze symptoms resulted from the root damage rather than from the mechanisms usually responsible for severe bronze wilt.

Comparisons between symptomatic and asymptomatic plants within cultivars appeared to underestimate the importance of bronze wilt. Plants that shed part of their boll load often were spared from the typical foliar symptoms of bronze wilt, but these plants suffer the same losses in seed cotton yield and seed quality. Thus, plants without symptoms should have the same number of bolls as symptomatic plants for a valid comparison. In several entries this was not possible, probably because the cultivar was uniformly susceptible to bronze wilt. The comparisons among cultivars, which probably are more useful than those among plants, indicate that either disease expression (bronze wilt or root necrosis) causes yield losses of at least 15% in affected plants under normal growing conditions.

Role of Seed Development in Yield Losses

The effects of bronze wilt and root necrosis on seed development are shown in Tables 4 and 5. The affected cultivars were randomly arranged in groups of 9-10 based on the order that they were planted in the experiment. This shows the consistency of results and allowed determination of significance.

Yield losses from bronze wilt were entirely due to a reduction of mean seed weight which was accompanied by a large increase in percentage of light seed. This indicates that bronze wilt develops primarily after fruit set and disrupts normal seed development, causing many immature embryos and fibers. In contrast, yield losses from root necrosis were entirely due to a reduction in number of seed per plant. Seed weight and percentage of light seed were not affected by root necrosis. Both abortion of bolls and failure of embryos to develop in ovules contributed to the reduced seed numbers. Root necrosis affected plants at any time after seedlings were 3 weeks old and sometimes was severe even before flowering.

Effects on Root Development

The effects of root necrosis and bronze wilt on root development is shown in Table 6. Root necrosis caused early root damage resulting in a 30% reduction in root weight of susceptible cultivars compared to cultivars with no symptoms at 8 weeks after planting. In contrast, bronze wilt reduced root weight mostly after fruit set but had the greatest effect by the time that plants were mature (31% reduction in weight). Roots of plants affected by bronze wilt were only slightly discolored, whereas numerous dark brown lesions occurred on those showing root necrosis.

Associations of Symptoms with Bacterial Concentrations

Relationship between symptoms and *A. tumefaciens* concentrations in roots are shown in Tables 7 and 8. Root necrosis was consistently associated with high *Agrobacterium* concentrations indicating a causal relationship. Cultivars susceptible to bronze wilt had lower concentrations of *Agrobacterium* at first flower than cultivars that showed no symptoms. No difference between these groups occurred at maturity. This may indicate that bronze wilt is caused by bacterial toxins rather than simple tissue injury caused by bacterial enzymes and metabolism.

Associations of Symptoms with Earliness

Relationships between symptoms and mean days to flower, location of first fruit node, and days for boll maturity are shown in Table 9. Cultivars that developed bronze wilt usually flowered earlier and set the first fruit on a lower node than cultivars that had no symptoms. Plants with root necrosis did not show a delay in flowering or first fruit set, even though these cultivars had 30% less root mass just before flowering (Table 6). The early flowering, in spite of a stunted root, may lead to the reduced numbers of mature seed that occur with root necrosis (Table 5).

The apparent relationship between earliness and bronze wilt may be misleading. In a separate experiment, very few 'Paymaster 1220 BG/RR' plants grown at 30 ºC, 14-hr days and 20 ºC, 10-hr nights developed severe bronze wilt symptoms, whereas nearly all plants grown at 25 ºC nights, with 30 ºC days, developed severe bronze wilt and suffered a 42% yield loss compared to plants at 20 ºC nights (Bell, unpublished). Plants with 20 ºC nights, however, set fruit slightly earlier and had slightly more bolls per plant than those at 25 ºC nights. Thus, earliness of fruit load, per se, is not the cause of bronze wilt. Rather, temperature, especially high night temperature, may be more important in development of symptoms.

The behavior of Fibermax cultivars also indicates that earliness is not causally related to bronze wilt. Severe bronze wilt developed in 50% of the plants of both 'Fibermax 832' and 'Fibermax 989' (Table 10). Yet, the mean days to first flower were 72 and 70, respectively, and the first fruit usually set on the 7th node in these cultivars. Thus, these cultivars were not earlier than the 15 cultivars which developed no bronze wilt symptoms (Table 9).

Conclusions

Inoculations with *Agrobacterium tumefaciens* increase frequency and severity of bronze wilt (BW) and root necrosis (RN) symptoms in cotton cultivars. The two symptoms are usually expressed in different cultivars and appear to be separate disease phenomena. At least 10% of the plants in 109 of 128 cultivars and breeding lines developed severe symptoms of either BW or RN when inoculated with *Agrobacterium*. About 25% of all plants developed either severe BW or RN, but only 2% developed both symptoms. Yield losses of affected plants averaged about 15% of the potential yield with either disease expression. Yield losses from BW were due to decreased seed size, while those from RN were due to decreased seed number. Losses from *Agrobacterium* root necrosis are probably more frequent than those from bronze wilt and may be a primary cause of yield stagnation.

References

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Table 1. Frequency of bronze wilt and root necrosis among cultivars and breeding lines.

	No. of Cultivars	No. (and %) of Plants
Total Observed	128	1280
With Severe Root Necrosis (RN)	60	181 (14)
With Severe Bronze Wilt (BW)	49	125 (10)
With RN and BW	16	20 (2)

Table 2. Seed cotton yields of cultivars susceptible to bronze wilt and root necrosis compared to cultivars without symp oms.

Symptoms	No. of Cultivars	Seed Cotton Yield gm/plant)	% Difference
None	15	7.84	
Bronze Wilt[a]	31	6.69	-15
Root Necrosis[b]	23	6.78	-14

[a]30% or more of the plants within each cultivar had severe symptoms. Yield is the mean value for all 10 plants of all cultivars observed.

Table 3. Effects of bronze wilt (BW) and root necrosis (RN) on seed cotton yields of individual plants within cultivars.

Symptoms	BW Severity[a]	No. of Cultivars	Effect on Yield[b]
BW Only	2 Rating	56	+ 2%
	3 Rating	35	- 5%
	4 Rating	45	- 11%
	5 Rating	15	- 9%
RN Only (4-5 Rating)		60	-14%
BW and RN	2 Rating	17	-14%
	3 Rating	19	-12%
	4 Rating	11	-15%
	5 Rating	5	-26%

[a]Bronze wilt was rated on a scale of 1 (no symptoms) to 5 (total collapse of plant).
[b]Comparisons were made between symptomatic plants and plants with no or few symptoms within the same cultivar. Data are means for all cultivars observed.

Table 4. Effects of bronze wilt on seed development.

Cultivar	Seeds per Plant		Seed Wt. (mg)		% Light Seed[c]	
Group[a]	CK[b]	BW[b]	CK	BW	CK	BW
1	58	60	71	58	11	67
2	58	58	67	58	18	64
3	52	58	71	55	19	72
4	52	55	71	57	12	57
5	47	53	71	50	9	63
MEAN	**53**	**57**	**70**	**56****	**14**	**65****

[a]Each group has 9-10 different cultivars and the data are means for the group.
[b]CK = symptomless plants; BW = plants with bronze wilt
[c]Determined in 70% acetone.
**Significant at the 1% confidence level (Student t test).

Table 5. Effects of root necrosis on seed development.

Cultivar Group[a]	Seeds per Plant		Seed Wt. (mg)		% Light Seed[c]	
	CK[b]	RN[b]	CK	RN	CK	RN
1	57	47	73	71	13	14
2	64	53	68	69	19	22
3	59	47	68	75	22	11
4	58	48	70	68	22	22
5	51	41	72	76	12	13
6	48	42	79	77	15	16
MEAN	**56**	**46***	**72**	**73**	**17**	**16**

[a]Each group has 10 different cultivars and the data are means for the group.
[b]CK = symptomless plants; RN = plants with root necrosis
[c]Determined in 70% acetone.
*Significant at the 5% confidence level (Student t test).

Table 6. Root weights of cultivars showing root necrosis and bronze wilt, or no symptoms.

	Root Weight (g)[a]		
Plant Age	No Symptoms	Root Necrosis	Bronze Wilt
8 Weeks	8.07	5.72*	7.06
Mature	25.40	21.10	17.60*

[a]Weights are the means of 15 cultivars that showed no symptoms (control) or that showed the greatest percentages of severe symptoms as indicated.
*Significantly different from the control at 5% confidence level (student t test).

Table 7. *Agrobacterium* concentrations in roots of cultivars showing bronze wilt, root necrosis or no symptoms.

	Agrobacterium concentration (M/g fresh root)[a]		
Plant Age	No Symptoms	Root Necrosis	Bronze Wilt
8 Weeks	45.6	98.6*	27.0
Mature	41.6	63.6	38.0

[a]Concentrations are the means of 15 cultivars that showed no symptoms (control) or that showed the greatest percentages of severe symptoms as indicated.
*Significantly greater than the control at 5% confidence level (student t test after log conversion).

Table 8. *Agrobacterium* concentrations in roots of 8-week-old plants with root necrosis compared to plants without symptoms within the same cultivar.

Cultivar Group[a]	Bacteria in Roots (M/g)	
	No Symptoms	Root Necrosis
1	48.3	263.5
2	35.4	85.1
3	22.6	154.1
4	29.0	59.6
5	14.4	93.7
6	34.5	40.8
MEAN	**30.6**	**118.7****

[a]Each group contains five different cultivars.
**Significantly different at the 1% level of confidence (student t test after log conversion).

Table 9. Associations between root necrosis, bronze wilt, and earliness.[a]

	Symptom Expression		
Character	**None**	**Root Necrosis**	**Bronze Wilt**
Days to Flower	70	68	64
1st Fruit Node	5.9	5.3	4.9
Days for Boll Maturity	40	40	39

*Comparisons among 15 cultivars that showed no symptoms and 15 that showed root necrosis or bronze wilt.

Table 10. Cultivars and lines most affected by bronze wilt or root necrosis.

Bronze Wilt		**Root Necrosis**	
Cultivar	**% Affected Plants**	**Cultivar**	**% Affected Plants**
NK 2165 C	80	AP 1500 RR	80
Tamcot Sphinx	80	Nata	80
Tamcot Pyramid	70	PM 1560 BG/RR	80
SG 501 BG/RR	60	SG 821	80
Tamcot Luxor	60	DP 5415 RR	70
FM 989	50	NK 2108 SS	70
Texas 208	50	SG 125 BG/RR	70
DP 422 BG/RR	50	All Tex Excess	60
FM 832	50	PSC 355	60
HCR 7163	50	SG 215 BG/RR	60
NK 2108 SS	50	SG 747	60
PM 1560 BG	50	DP 451 BR	50
SG 125 BG/RR	50	FM 989	50
SS 904	50	HCR 7312	50