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AGROBACTERIUM CONCENTRATIONS AND SEVERITY OF BRONZE WILT SYMPTOMS IN COTTON CULTIVARS TREATED WITH FUNGAL BIOCONTROL AGENTS AT PLANTING

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Abstract

The fungi, *Trichoderma virens* (Isolates GV4 and GV6), *Trichoderma koningii* x *T. virens* fusant #12, *Gliocladium catenulatum*, *Gliocladium roseum*, *Fusarium oxysporum*, and *Fusarium solani* were tested for their ability to colonize roots, to affect *Agrobacterium tumefaciens* colonization and bronze wilt severity and to affect yield. The fungi were tested with both 'Paymaster 1220 BG/RR' and 'Stoneville 373' cultivars grown with low and optimal phosphorus fertilization and with and without inoculation of soil with *Agrobacterium tumefaciens* isolate 25A. Stable concentrations of all fungi developed in roots within a few weeks after planting, indicating the potential for season-long effects on rhizoplane populations of other microorganisms. Some of the rhizoplane inhabiting fungi, especially *Fusarium* species, significantly increased yields suppressed establishment of *Agrobacterium* from infested seed and decreased bronze wilt severity when phosphorus levels were low. The treatments, however, were not effective when *A. tumefaciens* isolate 25A was added to soils. Under conditions of optimal phosphorus availability *Gliocladium* and *Fusarium* species often caused yield losses and increased *Agrobacterium* concentrations in roots. *T. virens* isolates GV4 and GV6 did not significantly affect yield, *Agrobacterium* concentrations or bronze wilt severity. The rhizoplane inhabiting fungi do not appear to be desirable for controlling *Agrobacterium* root rot or bronze wilt under recommended cultivar practices.

Introduction

Agrobacterium root necrosis and bronze wilt have been shown to be important causes of yield decline in cotton (Bell, 2002). In this study various fungi that have been isolated from cotton roots and shown to control other soilborne pathogens were tested for their ability to suppress *Agrobacterium* and bronze wilt and, thereby, increase yields.

Methods and Materials

Experimental Design

The experiment included eight biocontrol treatments: the seven fungi shown in Table 1 and sterile sorghum seed as a control. Spore suspensions from the fungi grown on potato dextrose agar (PDA) were added to whole sorghum seed wetted with an equal volume of water and sterilized twice with a 48 hour interval at 15 p.s.i. for 20 min. Sorghum cultures were grown at 28 °C and shaken at 3, 5, and 7 days to keep seed freely dispersed. Three infested seed from 7-day-old cultures were placed 2 cm away from seed and 1 cm deep in a triangular pattern at planting to inoculate pasteurized soils (8 hours at 165 °C, repeated after 48 hours). The fungi were evaluated on both 'Paymaster 1220 BG/RR' and 'Stoneville 373' cultivars grown with low and high phosphorus fertilization rates (150 mg 15-5-25 or 15-16-17 complete fertilizers added to 450 gm soil per week). Seeds of both cultivars came from plants inoculated with *Agrobacterium tumefaciens* isolate 25A, and had greater than 80% of the acid-delinted seed infested with *Agrobacterium*. This was the only source of the bacterium in one series of treatments. In a second series, 1 ml of *A. tumefaciens* isolate 26 suspension was added to soil surrounding a seed at planting. The suspension was prepared by suspending 16 10-µl loops full of bacteria from 24-hour-old cultures on PDA chalk agar in 1 liter of sterile water. The experiment was performed in the greenhouse starting in March with cooling fans set at 30 °C and heaters set at 25 °C. There were 20 replications of each treatment with five plants sampled as seedlings (5 weeks), five at initial flower (9-11 weeks), and 10 after boll maturity (15-18 weeks).

Data Recorded

The data recorded at all samplings were shoot weight (gm), root weight (gm), root necrosis (1-3), fungal concentrations in root (cfu/gm), and *Agrobacterium* concentrations in root (cfu/gm). The data recorded at final sampling only were: bronze wilt severity (1-5), seed cotton yield (gm), and percentage of fiber. Bacterial and fungal concentration were determined in root comminutions using procedures described previously (Bell, 1999; 2002). Four drops from the 1:200 dilution of tissue suspension were spread on PDA containing 50 mg tetracycline, 100 mg chloramphenicol, and 7 ml ethanol per liter to enumerate fungi. The data were analyzed using the student t-test to determine least significant differences (5% level). Correlation analyses were used to identify possible causes of yield variation.

Results and Discussion

Mean fungal and *Agrobacterium* concentrations in roots at seedling, first flower, and open boll stages of development are shown in Tables 2, 3 and 4. All fungi readily colonized the cotton root surface developing concentrations in excess of one

thousand per gm fresh root already in the seedling stage. Significant differences occurred among the fungi with *G. catenulatum* and *Fusarium* species giving the highest concentrations and the *T. konigii-T. virens* fusant and *G. roseum* giving the lowest concentrations in roots (Table 2). Cultural variables had only minor effects on fungal colonization (Table 3). Concentrations were slightly higher in PM than STV roots. Optimal phosphorus rates and inoculation with *Agrobacterium* also favored fungal development in most comparisons. In comparison, *Agrobacterium* concentrations were highest in STV roots but also were favored by optimal phosphorus rates and inoculation of soil with *Agrobacterium* (Table 4).

The effects of fungal treatments on *Agrobacterium* concentrations in roots and on bronze wilt severity are shown in Tables 5 and 6, respectively. *Fusarium* species suppressed the development of *Agrobacterium* from infested seed (-A.t.) but not from infested soils (+A.t.). In contrast, *T. virens* isolates did not reduce *Agrobacterium* concentrations from infested seed but did reduce those in infested soils. *Gliocladium* species increased *Agrobacterium* concentrations in both situations. All fungi suppressed bronze wilt severity at low phosphorus rates and with *Agrobacterium* coming only from the seed. These results may be due to enhanced phosphorus availability, i.e., a mycorhizal effect, because additional phosphorus fertilizer (16P) had an even greater suppressive effect on bronze wilt. Under all other conditions, fungi did not affect bronze wilt severity or, in the case of *Fusarium*, slightly increased severity.

Mean seed cotton yields associated with various treatments are shown in Table 7. Most fungi caused yield increases when phosphorus was deficient (5P) and *Agrobacterium* originated only from infested seed. In all comparisons, yields were significantly greater with 16% P than with 5% P. Thus, the significant increases caused by fungi at 5% P were probably due to increased phosphorus availability caused by fungi. With optimal phosphorus (16P) and inoculation of soil with *A. tumefaciens*, fungal treatments, with one exception, caused only yield decreases.

The possible causes of yield variation were studied using correlation analyses and calculation of coefficients of determination (Table 8). Bronze wilt had the greatest negative correlation with yield at the low phosphorus rate and in the absence of *Agrobacterium* inoculation of soil. When soils were inoculated with *Agrobacterium* isolate 25A, *Agrobacterium* concentrations had the greatest negative correlations with yield, regardless of phosphorus rate. Fungal concentrations also were negatively correlated with yield in thee of four comparisons. This effect can only be explained partially by their stimulation of *Agrobacterium* concentrations. *Fusarium* spp. and *Gliocladium catenulatum,* especially, appeared to be direct causes of yield losses when optimal phosphorus was available.

The results here further support the hypothesis that bronze wilt is primarily a physiological (or genetic) disorder that can be aggravated by *A. tumefaciens* infections of the root (Bell, 2003). *A. tumefaciens* also causes root damage and yield losses when conditions are not favorable for bronze wilt symptoms (e.g., with high P rates). Thus, the bacteria may be more important than bronze wilt in causing yield decline in the field.

Conclusions

Stable microbial populations developed within a few weeks of planting. Thus, seed treatments have potential for season-long effects on rhizoplane populations of *Agrobacterium*. Some rhizoplane inhabiting fungi were beneficial for yield and suppressed establishment of *Agrobacterium* from seed when phosphorus levels were low. The treatments however, were not effective against soilborne *Agrobacterium*. Under conditions of optimal phosphorus availability, rhizosphere fungi often caused yield losses and increased *Agrobacterium* concentrations in roots.

References

Bell, A. A. 1999. Agrobacterium bronzing and wilt: Cultivar reactions and effects of temperature. Proc. Beltwide Cotton Conf., p. 117-120.

Bell, A. A. 2002. Bronze wilt as a cause of yield stagnation. Proc. Beltwide Cotton. Conf. CD-ROM.

Bell, A. A. 2003. Effects of bacterial blight resistance genes and *Agrobacterium tumefaciens* virulence on development of bronze wilt symptoms and cotton yield losses. Proc. Beltwide Cotton. Conf. CD-ROM.

Table 1. Fungal species and isolates used as biocontrol agents.

- *Trichoderma virens*, isolate GV-4 (GV4)
- *Trichoderma virens*, isolate GV-6 (GV6)
- *Trichoderma koningii-T. virens* fusant #12 (TKV)
- *Gliocladium catenulatum* (GC)
- *Gliocladium roseum* (GR)
- *Fusarium oxysporum* (FO)
- *Fusarium solani* (FS)

Table 2. Mean fungal concentrations in roots inoculated with different fungal species at different stages of plant development.

	Development Stage of Cotton			
Fungus	**Seedling**	**1st flower**	**Open Boll**	**Mean**
	(thousands cfu/gm fresh root)			
CK (None)	5.0	5.7	3.6	4.8
GV4	11.3	14.1	19.6	15.0
GV6	8.3	21.2	20.4	16.6
TKV	6.2	4.3	4.6	5.0
GC	38.3	25.5	80.5	48.1
GR	12.4	8.6	6.2	9.1
FO	40.6	20.8	22.5	28.0
FS	31.5	19.1	20.2	23.6

Table 3. Mean fungal concentrations in roots of Paymaster 1220 BG/RR (PM) and Stoneville 373 (Stv) cultivars grown at low (5P) and optimal (16P) rates of phosphorus with (+A.t.) and without (-A.t.) *Agrobacterium tumefaciens* inoculated into soil.

Cultural	**Development Stage of Cotton**			
Conditions*	**Seedling**	**1st flower**	**Open Boll**	**Mean**
-A.t.:	**(thousands cfu/gm fresh root)**			
5P, PM	16.5	16.8	9.8	14.4
5P, Stv	16.3	10.7	8.0	11.7
16P, PM	15.1	10.0	20.9	15.3
+A.t.:				
5P, PM	14.4	14.4	30.6	16.5
5P, Stv	14.3	11.0	17.1	14.1
16P, PM	18.3	15.0	19.0	17.4
16P, Stv	12.1	8.4	11.4	10.6

*With (+) or (-) *Agrobacterium tumefaciens* isolate 25A added around seed in soil at planting; 5 or 16% phosphorus in 150 mg fertilizer applied weekly; 'Paymaster 1220 BG/RR' or 'Stoneville 373' cultivars.

Table 4. Mean *Agrobacterium* concentrations in roots of Paymaster 1220 BG/RR (PM) and Stoneville 373 (Stv) cultivars grown at low (5P) and optimal (16P) rates of phosphorus with (+A.t.) and without (-A.t.) *Agrobacterium tumefaciens* inoculated into soil.

Cultural	Development Stage of Cotton			
Conditions*	Seedling	1st flower	Open Boll	Mean
-A.t.:	(millions cfu/gm fresh root)			
5P, PM	1.7	1.9	2.7	2.1
5P, Stv	2.3	2.3	3.0	2.5
16P, PM	2.0	3.0	3.3	2.8
+A.t.:				
5P, PM	29.2	17.8	37.4	28.1
5P, Stv	38.5	18.5	43.2	33.4
16P, PM	38.5	26.0	61.5	42.0
16P, Stv	52.5	30.1	58.6	47.1

*With (+) or (-) *Agrobacterium tumefaciens* isolate 25A added around seed in soil at planting; 5 or 16% phosphorus in 150 mg fertilizer applied weekly; 'Paymaster 1220 BG/RR' or 'Stoneville 373' cultivar.

Table 5. Mean *Agrobacterium* concentrations in roots of plants grown at low (5P) and optimal (16P) rates of phosphorus with (+A.t.) and without (-A.t.) *Agrobacterium tumefaciens* isolate 26 and various fungi inoculated into soil.

	Cultural Conditions			
	- A.t.		+ A.t.	
Fungus	5P	16P	5P	16P
	(millions cfu/gm fresh root)			
CK (None)	3.3	2.6	20.2	46.3
GV4	2.0	2.7	26.4	24.6
GV6	2.7	3.8	17.5	27.9
TKV	1.9	1.8	29.0	36.1
GC	4.7	9.1	31.2	49.7
GR	2.2	5.1	32.4	58.6
FO	0.6	2.1	21.7	51.2
FS	0.4	0.8	48.1	46.7

Table 6. Mean bronze wilt severity of plants grown at low (5P) and optimal (16P) rates of phosphorus with (+A.t.) and without (-A.t.) *Agrobacterium tumefaciens* isolate 26 and various fungi inoculated into soil.

	Cultural Conditions			
	- A.t. 25A		+ A.t. 25A	
Fungus	5P	16P	5P	16P
	(bronze wilt grade 1-5)			
CK (None)	4.00	2.40	3.05	2.30
GV4	3.50	2.20	3.10	2.25
GV6	3.65	2.35	3.20	2.35
TKV	3.20	2.30	3.15	2.35
GC	3.35	2.35	2.65	2.40
GR	3.25	2.35	3.05	2.00
FO	3.30	2.95	3.50	2.15
FS	3.00	3.00	4.05	2.15

Table 7. Seed cotton yields of Paymaster 1220 BG/RR (PM) and Stoneville 373 (Stv) cultivars grown at low (5P) and optimal (16P) rates of phosphorus with (+A.t.) and without (-A.t.) *Agrobacterium tumefaciens* and various fungi inoculated into soil.

	Without A.t. 25A				With A.t. 25A			
Fungal	5%P		16%P		5%P		16%P	
Species	PM	Stv	PM	Stv	PM	Stv	PM	Stv
None	6.1	6.0	8.6	9.4	6.7	6.9	8.9	9.3
GV4	5.9	6.2	8.7	8.1*	6.1	6.4	9.6	9.2
GV6	6.3	6.2	9.2	8.7	65.4	7.1	9.4	9.3
TKV	6.3	7.0*	8.9	8.3*	6.6	6.6	9.9*	8.3
GC	5.9	6.7	8.6	8.9	6.4	6.5	8.6	8.5
GR	6.3	6.5	9.3	8.3	6.3	7.1	9.2	8.2
FO	6.2	6.7	8.8	8.2*	6.5	6.3*	8.4	8.6
FS	6.4	6.7*	8.8	8.3*	5.9*	5.9*	8.6	8.0*

Table 8. Possible causes of yield variations. Correlation coefficients (r) and coefficients of determination (r^2).

	Cultural Conditions*							
	Without A.t. 25A				With A.t. 25A			
	5%P		16%P		5%P		16%P	
Comparisons*	PM	Stv	PM	Stv	PM	Stv	PM	Stv
	r (r^2)							
Yield/Fungi	.07(.01)		-.40(.16)		-.50(.25)		-.49(.24)	
Yield/A.t.	-.53 (.28)		.29 (.08)		-.75(.56)		-.80(.64)	
Yield/BW	-.82(.67)		-.25(.06)		-.67(.45)		.45(2.0)	
Fungi/A.t.	.47(.22)		.24(.06)		.32(.10)		.21(.04)	
Fungi/BW	-.17(.03)		.58(.33)		-.05(.01)		.20(.04)	
A.t./BW	.52(.27)		-.43(.18)		.51(.26)		-.55(.30)	

*Fungal concentrations (cfu/gm), *Agrobacterium* concentrations (cfu/gm), and bronze wilt grades were compared with yield (gm/plant). Abbreviations defined in Table 4.