INTERACTION OF THIELAVIOPSIS BASICOLA WITH PYTHIUM SPP. AND RHIZOCTONIA SOLANI

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Abstract

The benefits of seed treatments to plant emergence and root necrosis were investigated in fields with mixtures of seedling disease pathogens and fields with one seedling disease pathogen (*Thielaviopsis basicola*). In fields with mixtures of seedling disease pathogens disease control was inadequate, while with *T. basicola* alone, control was adequate. In growth chamber experiments using naturally infested field soil with either mixtures of pathogens or *T. basicola* alone, plant emergence was unaffected by seed treatment and emergence was worse for the mixed pathogens than the single pathogen. Root necrosis was reduced in the case of *T. basicola* alone when seed was treated with Nuflow M, but not with combinations of Captan, Vitavax-PCNB, or Apron. Soil (sterilized) was infested with *T. basicola* or *Pythium* or both and emergence, root length, and root necrosis was compared with different seed treatments. An interaction between the two fungi was found with respect to root length and root necrosis, however the nature of that interaction (+/-) was inconsistent between experiments. Composite soil samples were taken during a survey from 102 fields in west Texas and seed treated with fungicides active only on *Pythium*, or *Rhizoctonia solani*, or neither were planted in the soil and held under cool, wet conditions. Field samples were divided into categories of low or high root necrosis, and emergence was compared to determine if root necrosis was associated with cotton seedling disease. There was no association of plant emergence with root necrosis.

Introduction

Testing fungicides in field studies for reduction of seedling disease, is difficult because of inconsistent weather patterns and mixtures of fungi involved in seedling disease. The consequence of seedling disease of cotton can be measured in a number of ways. The number of plants that emerge or survive/unit area; the amount of area where no plants emerge/unit area; amount of disease on roots or stems; and size (area or length) of roots can all be used to indicate seedling disease. The ultimate measures are probability of replant and/or yield loss in cotton.

Many cotton fields in west Texas have combinations of *Thielaviopsis basicola*, *Rhizoctonia solani*, and *Pythium* spp. Management of seedling disease consists almost entirely of seed treatments, with $\leq$ 1% of fields treated with in-furrow fungicides (T. A. Wheeler, unpublished). Testing of in-furrow fungicides by extension specialists in this region have rarely shown emergence or yield benefits (H. W. Kaufman, personal communication). Seed treatments are available with specific activity on *T. basicola*, *R. solani*, or *Pythium* spp. However, in-furrow fungicides, which provide both a larger quantity of fungicide and over a larger soil zone than seed treatments, are not known for activity against *T. basicola*.

Mixtures of seedling disease pathogens, particularly when *T. basicola* is involved, may be more difficult to control using seed treatments than when *T. basicola* alone. The objective of this study are to determine if seed treatments are ineffective when *T. basicola* is present with either or both *R. solani* and *Pythium* spp; if an interaction between *T. basicola* and these fungi can be found using survey techniques; and if an interaction is indicated through artificially infested soil using experimental techniques.

Materials and Methods

Effectiveness of seed treatments in field tests

Seed of the variety 'Paymaster HS-26' had the following seed treatments: none; Captan 4000 (2.5 oz/100 lb seed [C]); C+Apron Fl (0.75 oz/100 lb seed [A]); C+A+Vitavax-PCNB (6 oz/100 lb seed [V]); C+Baytan 30(1 oz/100 lb seed [B]); and C+A+B. In 1994, a field in Swisher co., TX with high levels of *Pythium* spp., *T. basicola*, and *R. solani* was selected along with another field in Lynn co. with high levels of *T. basicola*, but no other pathogens. In 1995, a field with high levels of *T. basicola* and *R. solani* was selected in Swisher co., along with the same field in Lynn co. again. Plots were four rows wide (30" or 40" centers, respectively) and 37' long. Weekly emergence counts of plants in the two center row were taken. In 1995, 10 plants/plot were rated for root necrosis. Seed treatments were arranged in a randomized complete block design with 6 and 4 blocks in 1994 and 1995, respectively. Plant emergence and root necrosis were analyzed with analysis of variance and when prob. $F \leq 0.05$ then the Waller k-ratio t test was used to determine fungicide treatment differences (P=0.05).

Effectiveness of seed treatments in growth chamber tests

Seed of the variety Paymaster HS-26 had the following treatments: C (2.5 oz/100 lb seed); C+Apron TL (2.0 oz/100 lb seed [A]); C+V (6 oz/100 lb seed); C+A+V; C+Nuflow M (1.75 oz/100 lb seed [N]); and C+A+N. Soil was collected from a field in Crosby co., TX, with a history of black root rot (caused by *T. basicola*) and from a field in Swisher co., TX with a history of high densities of *T. basicola*, *R. solani*, and *Pythium* spp. Soil was placed in conetainers (100 cm^3 soil) and planted with two seed for each of the seed treatments. Conetainers were arranged in a randomized complete block design with 6 replications and placed in a growth chamber at 18-19C for 21 days. Plant survival was determined at 21 days after planting (DAP) and root necrosis and length were determined by an image analysis system (Decagon). Each test was repeated. Analyses were conducted as described earlier.

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Survey

Seed of the variety Paymaster HS-26 had the following treatments: none; Apron TL (2.0 oz/100 lb seed); and V (6 oz/100 lb seed). Composite soil samples were collected from 102 randonly selected fields over 20 counties in the High Plains of Texas in August of 1996. Soil samples consisted of 20 cores taken at a depth of 4-8" near the stalk of cotton plants, with approximately 10 paces between each core. Three composite samples were taken per field. Soil from each sample was placed in conetainers (100 cm^3 soil) and planted with two seed from each of the seed treatments. The soil was kept under florescent lights in a room where the temperature ranged from 60-70F. After 18 days, surviving number of plants were counted and roots were rated visually for percent of root necrosis. Microscope slides were examined of necrotic root tissue for *T. basicola*. Mean values of survival with different seed treatments were compared for fields where average root necrosis was <20% and ≥20%.

Interaction tested between Pythium spp. and T. basicol in growth chamber

Pythium spp. collected from a field in 1995 were transferred on PDA and then grown on autoclaved wheat seed for 2 wk. *T. basicola* spores were grown on carrot agar (50 ml carrot juice + 1 g $CaCO_3$ + 15 g agar/l) for 6 wk. Soil (100 cm^3) was treated with the following combination of pathogens: none (3 ml of water + 5 cc autoclaved wheat seed, uninfested); *Pythium* spp (5 cc autoclaved wheat seed infested); *T. basicola* (200 spores/cc soil in 3 ml water); or both *Pythium* spp. and *T. basicola*. Seed (Paymaster HS-26) was treated with: none; Thiram 42S (3 oz/100 lb seed [T]); T+Apron Fl (0.75 oz/100 lb seed); T+A+Baytan 30 (1 oz/100 lb seed); and C+A+Nuflow M (1.75 oz/100 lb seed). Two seed of each treatment were planted in each pathogen combination with 6 replications in a randomized complete block design. Conetainers were maintained in a growth chamber at 18-19C for 21 days. Plant emergence was monitored three times and root length and % necrosis were measured with an image analysis system. Analysis of variance was conducted as described previously.

Results

Effectiveness of seed treatments in field tests

Emergence was poor for all seed treatments in both Swisher co. fields (Table 1). Root necrosis averaged greater than 70% for all seed treatments at the Swisher co. field in 1995. Both Swisher co. fields eventually were replanted with another crop. In the Lynn co. fields where only *T. basicola* was present, emergence was higher in 1994 when seed was treated with B than for seed treated with CA. Emergence was similar between all seed treatments in 1995. In Lynn co. in 1995, root necrosis was significantly reduced when seed was treated with Baytan 30 than when seed was treated with C, CA, or no seed treatment.

Effectiveness of seed treatments in growth chamber tests

Emergence at 21 DAP was not significantly different among seed treatments in the Swisher co. soil where multiple pathogens were found, nor in the Crosby co. soil, where damage was due primarily to *T. basicola* (Table 2). However, emergence among seed treatments averaged 60 and 43% in the Swisher co. soil (runs I and II) and 91 and 85% in the Crosby co. soil (runs I and II). Root length was not affected by seed treatment (Table 2). Root necrosis, however, was significantly impacted by seed treatment in the Crosby co. tests, but not in the Swisher co. tests. In the Swisher co. sites root necrosis ranged from 37 to 69%, and the seed treatments were clearly unable to prevent typical black root rot symptoms. In Crosby co., seeds treated with Nuflow M had significantly less root necrosis than seed treated with CA, CV, or CAV (Table 2). The level of root necrosis in the Nuflow M treated seed in Crosby co. (average of 19% in I and 13% in II) should not cause a replant situation. The average root necrosis with Nuflow M treated seed in Swisher co. was 37 and 67%.

Survey

There were 39 fields with levels of root necrosis ≥ 20% and 63 fields with none or minor levels of root necrosis. Altogether, 72% of the fields were confirmed to have *T. basicola*, including all the fields with root necrosis ≥ 20%. Average survival of plants with < 20% root necrosis for untreated seed, and seed treated with A or V was 33, 68, and 52%, respectively. Average survival of plants with ≥20% root necrosis for untreated seed or seed treated with A or V was 29, 67, and 46%, respectively. There was no association of reduced plant survival with root necrosis for any of the tested seed treatments.

Interaction tested between Pythium spp. and T. basicol in growth chamber

Plant survival at 21 DAP was not affected by a *T. basicola* x *Pythium* spp. interaction in either test. In test I, *T. basicola*, *Pythium* spp., seed treatment and *Pythium* x seed treatment affected plant emergence at 21 DAP and in test II, only *Pythium* affected emergence at 21 DAP. In test I, *T. basicola* density was associated with an increase in emergence (at all 3 times monitored), while *Pythium* was associated with a reduction in emergence, particularly when Apron was not included as a seed treatment (Table 3). In test II, *Pythium* was associated with a reduction in emergence over all seed treatments (Table 3).

Root length was impacted by a *T. basicola* x *Pythium* interaction in both tests. In test I, there was a 16-23% reduction in root length with the addition of *Pythium* or *T. basicola*, but no additional reduction (17%) when both pathogens were included (Table 3). In test II, there was a 34-39% reduction in root length for each pathogen, and a 47% reduction in root length for the combination of both pathogens (Table 3). Root length was also affected by *T. basicola* x seed treatment interaction. In test I, when *T. basicola* was present and *Pythium* was not, then root length

was longer where N or A was present then in untreated or T treated seed (data not shown for this *T. basicola* x *Pythium* x seed treatment interaction). In test II, when *T. basicola* was present (with and without *Pythium*), root length was longer when seed was treated with CAN than for seed treated with T, T+A, or untreated seed (Table 3).

Root necrosis was affected by interactions between *T. basicola* x *Pythium*, and *T. basicola* x seed treatment in both tests, and *Pythium* x seed treatment in test I. In test I, root necrosis was increased an average of 9% with the addition of *Pythium* and 52% with the addition of *T. basicola*, however, root necrosis was only increased 34% with the addition of both pathogens (Table 3). This less than additive affect was not seen in test II, where root necrosis was: not increased with *Pythium* alone; increased 16% with *T. basicola*; and increased 31% with both pathogens (Table 3). In this case there was a more than additive affect of both pathogens. Both tests had an interaction, but in test I it was to the benefit of the plant and in test to it was to the detriment of the plant. The interaction between *T. basicola* and seed treatment was the result of no differences in root necrosis among seed treatments when *T. basicola* was absent, but significantly less root necrosis for seed treated with CAN or TAB than the other seed treatments when *T. basicola* was present (in both tests) (Table 3).

Discussion

In field tests with different composition of pathogens, seed treatments specific for *T. basicola* and *R. solani* (Baytan 30) were more effective when *T. basicola* was the only major soil-borne pathogen. Soil tests conducted in 1994 indicated that both fields had densities of *T. basicola* of approximately 100 cfu/cm^3 soil (Wheeler et al., 1995). There were differences between these fields other than pathogen composition including soil texture, irrigation method and weather patterns. To examine multiple pathogen situations with less bias, two fine-textured soils were tested in a growth chamber under similar environmental conditions with the same source of treated seeds. Results were similar to the field situation where seedling disease (measured as root necrosis and emergence) was acceptably controlled by seed applied fungicides when *T. basicola* was the primary pathogen present, but not when multiple fungal pathogens were present. An experiment was then conducted where *T. basicola* and *Pythium* spp. were applied to sterilized soil with different seed treatments. An interaction between *T. basicola* and *Pythium* spp. was observed in two experiments with respect to root length and root necrosis. However, the qualitative nature of the interaction was inconsistent between the two experiments.

Summary

Seedling disease was inadequately controlled by seed treatments when *T. basicola* was present with other seedling disease pathogens. Seedling disease was adequately controlled when only *T. basicola* was present. Interactions between *R. solani*, *Pythium* sp. and *T. basicola* may be extremely complex and may change depending on environmental conditions and pathogen densities. Control of seedling disease in the presence of multiple fungal pathogens and favorable environment for disease, requires more management than high quality seed plus seed applied fungicides.

References

Wheeler, T. A., J. R. Gannaway, H. W. Kaufman. 1995. Cotton seedling disease: the importance of pathogen preplant density, seed treatment, and microbial activity in the soil. Proceedings Beltwide Cotton Conferences p. 223.

Table 1. Cotton emergence and root necrosis for fields with multiple seedling disease pathogens (S) and fields with *Thielaviopsis basicola* alone (L).

	1994		1995		1995	
Seed	% Emergence		% Emergence		% Root necrosis	
Trt[1]	S[2]	L	S	L	S	L
None	27 bc[3]	47 ab	41	53	85	84 a
C	25 c	49 ab	35	67	78	70 ab
CA	29 bc	44 b	39	69	76	71 ab
CAV	32 ab	50 ab	58	66	80	57 bc
CB	35 a	58 a	33	69	75	48 c
CAB	36 a	59 a	33	62	70	46 c

[1]Seed treatments were: none, Captan 4000 (2.5 oz/100 lb seed [C]), C + Apron Fl (0.75 oz/100 lb seed [A]), C+A+Vitavax-PCNB (6 oz/100 lb seed [V]), C+Baytan 30 (1.0 oz/100 lb seed [B]), and C+A+B.
[2]S is a field in Swisher co., TX and L is a field in Lynn co., TX.
[3]Means separation with Waller Duncan k-ratio t test (P=0.05).

Table 2. Cotton emergence, root necrosis, and root length in soils naturally infested with multiple seedling disease pathogens (Swisher) or *Thielaviopsis basicola* alone (Crosby)

	Swisher I			Swisher II		
Seed Trt[1]	21 DAP[2]	% Root necrosis	Root length[3]	21 DAP	% Root necrosis	Root length
C	63	67	9.4	50	39	12.8
CA	79	69	9.6	17	42	13.0
CV	50	61	11.0	50	39	11.4
CAV	58	63	9.4	42	52	13.2
CN	54	50	11.8	50	60	10.6
CAN	58	37	13.9	50	67	10.3
	Crosby I			Crosby II		
C	79	51 a[4]	13.6	75	34 bc	8.2 a
CA	96	53 a	15.9	83	46 ab	7.9 ab
CV	100	51 a	11.4	67	62 a	6.3 bc
CAV	88	41 a	12.3	92	42 ab	7.8 abc
CN	88	22 b	12.9	92	16 c	7.5 abc
CAN	96	16 b	20.0	100	10 c	6.1 c

[1]Seed treatments were: none, Captan 4000 (2.5 oz/100 lb seed [C]), C + Apron Fl (0.75 oz/100 lb seed [A]), C+A+Vitavax-PCNB (6 oz/100 lb seed [V]), C+Baytan 30 (1.0 oz/100 lb seed [B]), and C+A+B.
[2]% Emergence at 21 days after planting.
[3]Root length per plant (cm).
[4]means were separated with the Waller Duncan k-ratio t test.

Table 3. Influence of *Pythium* sp. (Py), *Thielaviopsis basicola* (Tb) and seed treatment on cotton emergence, root length (cm) and root necrosis.

Tb	Py	Seed[1] Trt	21 DAP[2]		Root length		% Root necrosis	
			I[3]	II	I	II	I	II
0	0		87	93	55	89	0	1
0	+		50	79	32	50	9	1
+	0		97	88	39	55	52	16
+	+		60	85	38	42	34	31
	0	None	79	88	29	71	45a[4]	12
	0	T	96	100	48	77	31b	14
	0	TA	96	92	53	61	28bc	13
	0	TAB	96	83	50	74	12d	1
	0	CAN	92	91	52	76	16cd	0
	+	None	4 c	82	7	40	100a	13
	+	T	17 c	88	25	34	48b	40
	+	TA	75 b	88	26	48	42b	19
	+	TAB	100 a	83	44	50	2c	1
	+	CAN	79 ab	71	38	58	14c	5
0		None	33	86	41	75	0	0
0		T	54	96	57	77	6	2
0		TA	79	92	48	67	7	1
0		TAB	100	83	46	67	1	0
0		CAN	75	73	42	64	3	0
+		None	50	83	18 b	39 bc	85 a	25 bc
+		T	58	92	32 ab	34 c	61 b	52 a
+		TA	92	88	33 ab	42 bc	62 b	32 ab
+		TAB	96	83	48 a	57 ab	14 c	3 c
+		CAN	96	88	49 a	70 a	26 c	5 c

[1]Seed treatments were: none; Thiram 42S (3 oz/100 lb seed [T]); Apron Fl (0.75 oz/100 lb seed [A]); Baytan 30 (1 oz/100 lb seed [B]); Captan 4000 (2.5 oz/100 lb seed [C]); and Nuflow M (1.75 oz/100 lb seed [N]).
[2]% Emergence at 21 days after planting.
[3]I is the first run of the experiment and II is the second.
[4]means separation with the Waller Duncan k-ratio t test.