## CONTROL APPROACHES (CULTURAL AND CHEMICAL) FOR BLACK ROOT ROT OF COTTON J. E. DeVay and R. H. Garber Department of Plant Pathology, University of California, Davis, Davis, CA California Planting Cotton Seed Distributors, Shafter, CA.

#### **Abstract**

Black root rot of cotton caused by *Thielaviopsis basicola* can be effectively controlled by cultural management practices such as crop rotations with certain non-host crops and by soil flooding and soil solarization. Seed treatment with sterol-inhibiting fungicides also is effective and currently is the approach used by most growers for managing black root rot.

#### **Introduction**

One of the earliest references to black root rot of cotton appeared in a report from the U.S. Field Station in Sacaton, Arizona. However, the cause of this problem was not found until 1938 when the soilborne fungus, Thielaviopsis basicola (syn. Chalara elegans), was identified as the pathogen (King and Presley 1942). This fungal pathogen causes black rot affecting both main and lateral roots of over 137 plant species (Holtz et al.; 1994), often infecting the root through root hairs. It is mainly a pathogen of seedlings growing in cool soil (16-20 C.), destroying both the cortex tissues and mycorrhizal associations in the roots. Because T. basicola seldom rots the pericycle tissues (Mathre et al., 1966), the root can regenerate new cortex tissues and develop new lateral roots when the soil warms above 20 C. (Mauk and Hine 1988). However, persistent infections often remain in older plants and affected seedlings seldom recover their full growth and yield potential following early season stunting compared to healthy plants (Hake et al., 1985)

Management of the black root rot disease has been difficult because cotton is usually planted in cool soil and cultivars in both *Gossypium barbadense* (Pima cotton) and *G. hirsutum* (Upland cotton) have little or no resistance. Moreover, the pathogen populations in cotton field soils often consist of strains of *T. basicola* which are highly virulent and also can infect many crops and weeds (Gayed 1972; Holtz et al., 1994; Maier and Steffeldt 1960). Their inoculum density in soil can greatly increase under susceptible crops and thus become a serious threat to cotton production. In the absence of a susceptible crop, the aleuriospores of *T. basicola* can persist in soil for at least five years (Gayed 1972).

There are ways, however, of successfully managing the black root rot problem. Among these are certain crop rotations that sharply reduce the disease, seed treatments with chemicals that control the pathogen, and soil flooding and soil solarization which may eradicate the spores of *T. basicola*. These approaches for management of black root rot are the main subject of this presentation.

## Cultural Management

**Strains of Thielaviopsis basicola and their mechanisms** of pathogenicity. - The impact of black root rot on cotton production became increasingly apparent during the 1960's and was of concern since cotton lint yields in the San Joaquin Valley were on the decline (Garber et al., 1985; Hake et al. 1985; Mathre et al. 1966). About 1980, the black root rot problem became noticeably widespread and more severe in the San Joaquin Valley and none of the seed treatments, which were registered for cotton, were effective against *T. basicola*.

*T. basicola* has been isolated from diseased cotton plants grown in a wide range of soil types and the inoculum density of the fungus may vary from no detectable propagules to over 1000 propagules per gram of soil (Mathre et al., 1966; Tabachnik and DeVay 1979). Counts over 400 propagules per gram of soil are often found in cotton fields with severe black root rot (Hake and Garber 1986). The fungus inoculum tends to increase with continuous cropping of cotton, thus making it difficult to manage, since severe disease can result from inoculum densities aa low as 50 to 100 propagules per gram of soil (Holtz and Weinhold 1994; Mauk and Hine 1988; Tabachnik and DeVay 1979).

Representative strains of *T. basicola* isolated from diseased cotton plants have been compared with strains from other crops such as bean, soybean, pea, peanut, sesame, and tobacco at different inoculum concentrations. In all disease tests, with the above crops, cotton was most susceptible to black root rot (Tabachnik and DeVay 1979). Moreover, among the representative strains of *T. basicola* used in these tests, those from cotton were most virulent in cotton. A low level of host specificity of the different strains was apparent in greenhouse tests.

One of the characteristics of *T. basicola* and related fungi is their tendency to produce a mixture of fusel oils which are acetate esters of various alcohols and together have a ripe banana odor. Among fusel oil components produced by the cotton pathogen is methyl acetate which in minute amounts or physiological concentration can kill and blacken the cortex tissues of cotton. It appears that this substance is the toxigenic factor in the pathogenesis of *T. basicola* in cotton (Tabachnik and DeVay 1975, 1980).

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## Cultural practices that reduce the population density of

<u>*T. basicola* in soil and black root rot</u> - Among these practices are crop rotations with crops such as onions, garlic, grain sorghum, potato, or sunflower that are resistant to black root rot. Other practices which are more immediate and tend to lower inoculum of the pathogen from high densities to near undetectable levels are soil flooding and soil solarization.

Crop rotations. - Based on grower observations, rotations with onions and garlic have shown promise of controlling black root rot. Greenhouse experiments have confirmed the effectiveness of this practice (DeVay et al., 1990). When either onion or garlic plants were grown for one month in soil infested with T. basicola then removed, inoculum densities, initially at 198 and 317 propagules per gram of soil, were reduced more than 50% and severity of black root rot in following cotton plantings also was significantly reduced (Table 1). In field rotations with grain sorghum and/or potatoes, reductions in black root rot in following crops of cotton also were observed. When experimental plots at Shafter, CA were fallowed from June to the following April, or planted to grain sorghum (cv. Moench). or to continuous cotton, roots with black root rot in the following crop of cotton (Acala SJ-2) were reduced 93% in the fallowed plots and 75% in the sorghum plots compared with continuous cotton (Table 2). Additionally, the population of T. basicola was reduced 97% in the fallowed plots and 80% in the sorghum plots (Butterfield et al., 1978).

**Soil flooding**.- Summer flooding of fields in the Tulare Lake Basin of the San Joaquin Valley has been an effective practice for controlling black root rot of cotton. In some years as much as 40,000 acres of land have been flooded to reduce the inoculum density of *T. basicola* (Holtz and Weinhold 1994). A similar practice near Firebaugh, CA, where paddy rice is often rotated with cotton, also has been used for many years to control Verticillium wilt of cotton (Pullman and DeVay 1982).

Early observations at the Hansen Ranches at Corcoran, CA indicated that summer flooding of soil would increase lint yields and also reduce the black root rot problem (G. O'Neill, Ranch Manager, personal communication). In one trial where the propagule count was as high as 800 propagules/gram of soil in the non-flooded half of a field, lint yield was increased from three-fourths bale to over two bales per acre following flooding. In another trial, flooded and nonflooded fields with initially high infestations of Tbasicola were compared. The infestations ranged from 350 to 600 propagules/gram of soil before a 30 day flooding treatment. After flooding, propagule counts dropped from 350 to 0 and from 600 to 200 propagules/gram of soil. The following growing season, black root rot was greatly reduced in the flooded field and lint yields were increased about three-fourths bale/acre compared with the unflooded field affected by black root rot. In other trials at the Hansen Ranches, flooding soil during the winter months was ineffective for controlling black root rot.

To verify experimentally the effect of flooding on black root rot, greenhouse tests were made where soils infested with 198 and 317 propagules per gram of soil were flooded for 1, 2 and 4 weeks and compared with nonflooded controls (DeVay et al., 1990). Although significant reductions in propagule counts of *T. basicola* and the severity of black root rot of cotton seedlings occurred after 1 and 2 weeks of flooding, the greatest reduction came after 4 weeks, going from 198 to 10 and from a disease index of 4.3 to 1.6 (Table 3). In a second experiment, a significant reduction in the propagule count of *T. basicola* was observed after 3 weeks of flooding with a significant drop in the severity of black root rot in the following cotton planting (Table 3).

Holtz et al. (1996) determined the influence of soil temperature and length of flooding on naturally- occurring field populations of *T. basicola* (approximately 140 propagules/gram field soil). When soil was flooded and incubated in the laboratory at 10 or at 20 C., survival of *T. basicola* was essentially the same as that in unflooded soil. However, when soil was incubated at 30 or 40 C. the populations of *T basicola* were significantly reduced and approached 0 within 10 days at 40 C. or near 0 within 30 days at 30 C. These results explain why flooding soil in winter months does not reduce the occurrence of black root rot in following crops of cotton

In earlier studies (Pullman and DeVay 1982), soil flooding associated with paddy rice culture also controlled Verticillium wilt of cotton for 2-3 years and increased lint yields by an average of 31% compared to areas in which cotton was grown continuously. Flooding during the late fall and winter did not reduce propagule densities of *Verticillium dahliae* or the incidence of Verticillium wilt in a subsequent cotton crop. Thus, when feasible, soil flooding is an effective procedure for controlling both black root rot and Verticillium wilt.

Soil solarization. - In laboratory studies, over 90% of the propagules of T. basicola were killed within 65 min at 50 C or within 14 hr at 45 C. (Pullman et al. 1981a). These temperatures are usually achieved and often for many hours during soil solarization. This procedure not only controls black root rot but also diseases caused by other seedling pathogens. In field tests, solarization of soil for 28 days reduced the population of T. basicola from 75 propagules per gram of soil to undetectable levels to a soil depth of 46 cm at Shafter, CA while at Davis, CA, populations (approx. 75 propagules per gram of soil) were reduced to undetectable levels to a depth of 30 cm. (Pullman et al., 1981b). Costs of soil solarization are usually balanced by significant increases in lint yields and weed control. However, the use of this approach for black root rot control requires soil solarization to be done during the warm summer months. Thus, it must be used in conjunction with

a crop rotation allowing about four weeks of solarization time.

## **Chemical Seed Treatments**

Prior to 1980, few chemical seed treatments with the exception of Benomyl showed promise for control of T. basicola. Benomyl was an effective seed treatment but lack of interest by the manufacturer for its registration on cotton seed discouraged further tests. A breakthrough came in 1980, however, when several triazole compounds and an imidazole became available for greenhouse and field tests. The strong activity of these compounds was immediately apparent for control of black root rot while the triazoles also controlled damping-off caused by Rhizoctonia solani. Ciba-Geigy 64250 ((1-[2-(2,4-dichlorophenyl)-4-propyl-1,3dioxolan-2-yl methyl]-1,2,4-triazole and Ciba-Geigy 64251 (1-[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl methyl]-1H-1,2,4-triazole) were extensively tested during the early 1980's and while in early tests stunting of cotton seedlings was a major problem, this problem was overcome using lower seed dosages without reducing their effectiveness (DeVay et al. 1981, 1982). Unfortunately, Ciba-Geigy was unable to obtain registration for these triazoles later named Tilt and Vanguard for use on cotton. Meanwhile, research on Imazalil (1-[2-(2,4dichlorophenyl)-2-(2-propenyloxy) ethyl]-H-imidazole) showed that in greenhouse tests this substance was a highly effective seed treatment for black root rot (DeVay et al., 1982). Eventually, Wilbur-Ellis Co. obtained registration for this seed treatment on cotton seed, but its effectiveness under field conditions was disappointing.

Other triazoles which were tested during the 1980's included a product from Rohm and Haas named Systhane (alpha-nbutyl-alpha-(4-chlorophenyl)-H-1,2,4-triazole-1-(propanenitrile) and one from Gustafson, Inc. named Baytan 30, a product of Bayer (Germany), (beta-(4-chlorophenoxy)alpha-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol). Systhane was toxic (stunting) to cotton seedlings grown in heavy soils, whereas Baytan 30 was much less toxic. However, low dosages of both these triazoles that were nontoxic, still gave excellent protection to cotton seedlings against both T. basicola and Rhizoctonia solani (Arthur et al., 1991; Butler et al., 1996; DeVay et al., 1990). Eventually, and after a long struggle with regulatory agencies, both of these products were registered in 1994-95 for use as cotton seed treatments. The common name for Systhane (Rally) is Myclobutanil and it is sold under the trade name Nu-Flow M by Wilbur-Ellis Co. while Baytan 30 is Triadimenol and it is distributed by Gustafson, Inc. Dosage rates of Baytan 30 ranging from 1.5 to 9.0 oz/cwt of seed gave similar protection against black root rot (Table 4). However, at the 6 and 9 oz rates, stunting or rosseting of plants occurred. Current recommendations for both Nu-Flow M and Baytan 30 are in the range of 1 oz/cwt cotton seed.

Although structurally different, Triadimenol (Baytan 30) and Myclobutanil (Nu-Flow M) have a similar mode of toxicity to fungi; they inhibit sterol synthesis (Koller 1992). More specifically, they inhibit a fungal enzyme,  $14\alpha$  demethvlase which demethvlates 24methylenedihydrolanosterol, a critical step in the synthesis of fungal sterols (Benveniste and Rahier 1992). The toxicity of these triazole compounds to cotton seedlings is related to their similarity to plant growth regulating substances which inhibit gibberellin synthesis. However, there is some evidence that Triadimenol also may inhibit sterol synthesis in plants (Benveniste and Rahier 1992). The toxicity of the triazoles tested on cotton, appears only as a stunting or rosseting of the seedlings; they do not cause chlorosis or other disease symptoms.

Stunting of seedlings caused by black root rot results in weak, nonvigorous plants whereas seedlings stunted by triazole fungicides are usually sturdy and vigorous, except for shortened internodes or rosseting.

In regard to Triadimenol, a synergistic interaction with other triazoles has been observed (Gisi 1996). Moreover, when used in combination with fungicides such as Carboxin (Vitavax) (5,6-dihydro-2-methyl-N-phenyl-1,4-oxathiin-3-carboxamide) on cotton seed, its fungicidal activity is enhanced (Table 5).

# **Conclusions**

Management of black root rot, which seemed difficult a few years ago, can be done using cultural practices and chemical seed treatments. Cotton fields with low or high infestations of T. basicola can be treated to lower the infestations to undetectable levels. Caution should be exercised however, in an attempt to manage black root rot in fields with high infestations of T. basicola using only seed treatments. Under such conditions, soil solarization or fumigation may be appropriate to lessen the severity of black root rot. Most commercial recommendations involve minimal dosages of triazole compounds which under average temperature and moisture conditions are adequate to control black root rot. However, when the environmental factors greatly favor the pathogen, these seed treatments may be inadequate to manage or even lessen the severity of black root rot.

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Table 1. Effect of onion and garlic plants on the density of soilborne propagules of *Thielaviopsis basicola* and on black root rot of cotton seedlings in greenhouse tests.

Soil Treatments <sup>1</sup>	Number of Propagules per/gm Soil	Disease Index <sup>2</sup>	
Experiment I			
Fallow	198 b <sup>3</sup>	4.3 d	
White Onion	76 a	2.6 c	
Red Onion	62 a	2.2 b	
Garlic	55 a	2.0 a	
Experiment II			
Fallow	317 b	4.4 b	
Red Onion	146 a	2.5 a	

Each treatment was replicated three times. Each replication consisted of 120 seedlings (Acala SJ-2) grown in metal flats containing sandy loam soil.

<sup>2</sup>Black root rot was measured on a scale of 1 to 5: 1, healthy root and 5, tap root blackened and all lateral roots rotted off.

<sup>3</sup>Values are averages of three replications; those followed by a different letter are significantly different at the 0.05 level.

Table 2. Effect of fallowing and grain sorghum compared with continuous cotton on the incidence of black root rot in cotton seedlings and on soilborne populations of *Thielaviopsis basicola* at Shafter, CA

	Soil Treatment	Diseased Roots <sup>1</sup> (%)	Colonization of Carrot Disks by <i>T. basicola</i> <sup>2</sup> (%)
1.	Fallow	2.9x	1x
2	Sorghum	10.4x	6x
•			
3	Continuous cotton	41.3y	30y

<sup>1</sup>Percentage of diseased roots out of 30 roots per replication for eight replications. Values followed by the same letter are not significantly different (P = 0.05).

<sup>2</sup>Percentage of carrot disks colonized from soil overlays. Ten disks per 5 g soil per replication, eight replications.

Table 3. Effect of flooding on the density of soilborne propagules of *Thielaviopsis basicola* and on black root rot of cotton in greenhouse tests.

Duration of Soil Flooding <sup>1</sup> (weeks)	Number of Propagules (per/gm soil)	Disease Index <sup>2</sup>
I. Nonflooded Control	198 d <sup>3</sup>	4.3 d
1	142 c	3.6 c
2	123 b	3.3 b
4	10 a	1.3 a
II. Nonflooded Control	317 b	4.4 d
1	286 b	3.9 c
2	237 b	2.5 b
3	142 a	1.6 a

<sup>1</sup>Each treatment was replicated three times. Each replication consisted of 120 seedlings (Acala SJ-2) grown in metal flats containing a sandy loam soil.

<sup>2</sup>Black root rot was measured on a scale of 1 to 5: 1, healthy root, 5, tap root blackened and all lateral roots rotted off.

<sup>3</sup>Values are averages of three replications: those followed by a different letter are significantly different at the 0.05 level.

Table 4. Comparison of different dosage rates of Baytan 30 on cotton seed for control of black root rot in greenhouse tests<sup>1</sup>

Seed Treatments (Acala SJ-2)	Dosage <u>oz/cwt</u>	Disease Index <sup>2</sup>
1. Untreated seed		4.5 c <sup>3</sup>
2. Baytan 30	1.5 oz	2.0 b
3. Baytan 30	3.0 oz	2.0 b
4. Baytan 30	6.0	1.9 b
5. Baytan 30	9.0	1.7 a

<sup>1</sup>Each treatment was replicated three times. Each replication consisted of 120 seedlings grown in metal flats containing a sandy loam soil infested with approximately 100 propagules of *Thielaviopsis basicola* per gm soil. <sup>2</sup>Black root rot was measured on a scale of 1 to 5: 1, healthy root and 5, tap root blackened and all lateral roots rotted off.

<sup>3</sup>Values are averages of three replications; those followed by a different letter are significantly different at the 0.05 level.

Table 5. Effect of combinations of chemical seed treatments for control of damping-off and black root rot of cotton (Acala SJ-2).

		Seedling survival <sup>1</sup>		
	Dosage	<i>P.u.</i>	R. s.	<i>T.b.</i>
Seed Treat.	oz/cwt	(%)	(%)	D.I. <sup>2</sup>
1.Untreated		34 a <sup>3</sup>	8 a	4.6 a
2.Apron FL	0.75			
Captan 30	1.65			
Vitavax 30	3.3	99 a	87 a	4.5 a
3.Baytan-				
Apron HB	8	95 b	81 b	2.4 b
4.Apron Fl	0.75			
Captan 30	1.65			
Vitavax 30	3.3			
Baytan-	8	96 b	88 b	1.6 c
Apron HB				

<sup>1</sup>Each treatment was replicated three times. Each replication consisted of 120 seedlings grown in metal flats containing a sandy loam soil which was infested with *Pythium ultimum* (65 propagules/gm soil), *Rhizoctonia solani* (approx. three prop./gm soil), or *Thielaviopsis basicola* (100 prop./gm soil).

<sup>2</sup>Black root rot was measured on a scale of 1 to 5: 1, healthy root and 5, tap root blackened and all lateral roots rotted off.

<sup>3</sup>Values are averages of three replications: those followed by a different letter are significantly different at the 0.05 level.