

INDUCED RESISTANCE CAN PROTECT COTTON AND LEGUMES FROM BLACK ROOT ROT

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Introduction

Plants can utilise an array of biochemical mechanisms to protect themselves against viral, bacterial, fungal and nematode pathogens. Systemic induced resistance can be achieved by using an activator, either an organism or a chemical, at the early stage of a crop. The activator 'switches on' the host plant's defense mechanisms. There are few reports of induced resistance against soilborne and vascular pathogens. A significant reduction in the severity verticillium wilt in cotton disease was observed under field conditions after the use of isonicotinic acid (Colson-Hanks and Deverall, 2000). Application of the Novartis product Bion 50WG (a.i. benzothiadiazole 50%) to grapevines resulted in a reduced the incidence of root knot nematodes. The rate of maturity of nematodes and egg production appearing to reduced (Owen et al., 1999). Colonisation of roots by mycorrhizal fungi (VAM) may also induce systemic resistance in plants and protect them from pathogens (Dassi et al., 1998).

Black root rot (BRR) is a common disease of more than 230 plant species in 49 families caused by the fungus *Thielaviopsis basicola*. *T. basicola* is a soilborne pathogen that causes significant damage to crops, especially at the seedling and early growth stages. *T. basicola* does not kill cotton seedlings by itself but it may make them more prone to post-emergent damping off caused by *Rhizoctonia* or *Pythium*. Typical black root rot symptoms in cotton are blackening of the taproot and stunted growth. The root system may have few healthy lateral roots and its ability to absorb nutrients is reduced. The fungus infects the outer part of the root (the cortical tissue) but not the inner part of the root (water-conducting, vascular tissue). The infected tissue collapses and sloughs off when the roots eventually expand. The fungus develops abundant thin-walled spores (**endoconidia**) and thick-walled spores (**chlamydospores**) a few days after infection. Both types of spore can cause disease. While the endoconidia are short lived (up to seven months in soil), the chlamydospores remain viable for many years. Consequently, black root rot is particularly difficult to control.

Rotation with non-host crops (eg. cereals) does not prevent the build-up of spores of *T. basicola* in the soil with each cotton crop (see paper by Nehl et al., this proceedings). Rotation with susceptible hosts, such as certain legumes, may add to the build-up of spores in cotton fields. Hence, the increasing interest in rotation with legumes presents a challenge to management of black root rot in cotton farming systems. In this paper we examine the potential for induced resistance to decrease the severity of black root rot in both cotton and legumes.

Materials and Methods

Several experiments were conducted to test the potential of benzothiadiazole (BTH) to induce resistance against *T. basicola* in a number of cotton cultivars and legumes. Seed treatments were applied in the dark at room temperature by soaking in either water, as a control, or in BTH solutions (25 or 50 ppm benzothiadiazole). Legumes were soaked for times ranging from 2.5 to 18 hours (see Table 2). Cotton was soaked for 5h except for the cultivar DP 90 which was soaked for 18h. The treated seeds were planted into pots containing soil collected from a field, near Wee Waa NSW, in which the population of *T. basicola* was approximately 427 spores per g of soil. The pots were transferred to either a growth room or a glasshouse. The plants were assessed for black root rot symptoms after washing the roots. The disease severity index was determined by rating the length of the tap root with dark brown to black discolouration, on a scale from 0-10 where 0 = no disease and 10 = 100% tap root blackened. The presence of chlamydospores of *T. basicola* was determined by viewing roots under a microscope at 20 × magnification. The intensity of chlamydospore production on roots was rated qualitatively as high, medium or low.

The potential for BTH to reduce disease severity in legumes and subsequent carry-over of inoculum to cotton was examined by sowing treated and untreated faba bean (cv. Fiord) seeds in infested Wee Waa soil. Subsequently, the faba bean roots were washed, assessed for black root rot severity, cleaned again carefully to remove all dirt, shaken vigorously in sterile distilled water for 10 minutes, air dried in the shade, cut into small pieces and mixed with sterile Wee Waa soil. Untreated cotton seed was sown and disease severity assessed.

Results

Seed treatment with BTH at 50 ppm reduced the severity of black root rot in DP 90 (Table 1). In Sicala V2 and Siokra 1-4 there was a trend for less black root rot with BTH treatment but this was not significant. However, tap root length of Siokra 1-4 treated with the BTH was more than three fold that of control plants because the lower part of the root system in the controls had rotted away by the time roots were assessed. This suggests that although seed treatment with BTH did not reduce the ultimate spread of the fungus along the tap root of Siokra 1-4 (i.e. assessed at 35 days) it did reduce the intensity of the infection at an earlier stage.

Table 1. Reduced severity of black root rot of cotton following seed treatment with BTH.

Treatment ^A	Disease severity index			Tap root length (cm)
	DP 90	Sicala V2	Siokra 1-4	Siokra 1-4
water	3.5a	3.3a	9.7a	5b
BTH 25 ppm	2.9ab	1.9a	8.6a	19a
BTH 50 ppm	2.4b	1.4a	8.9a	17a
Probability	$p < 0.01$	Not significant	Not significant	$p < 0.01$

^A Seeds of DP 90, Sicala V2 and Siokra 1-4 were soaked for 18, 5 and 5 hours respectively, sown in soil infested with *T. basicola*, and plants were assessed for black root rot at 21, 18 and 35 days respectively.

The legumes examined here varied in their susceptibility to *T. basicola*. Consequently, BTH decreased the severity of black root rot to varying degrees (Table 2). BTH treatment was most effective in faba bean, field pea, cowpea and soybean. In general, the disease severity in the legumes was relatively low in comparison to cotton. In vetch, no root blackening was observed in any treatment but the roots were slightly brown to yellow in control plants.

Table 2. Decreased severity of black root rot in legumes following seed treatment with BTH.

Cultivar	Decrease in BRR severity (%)	BTH concentration (ppm)	Soaking period (hours)
Faba bean			
'Barkool'	78*	50	5
'Icarus'	45*	50	5
'Fiord'	42*	25	5
Field pea			
'Greenfeast'	93*	50	5
Cowpea			
'Poona'	68*	25	3
Soybean			
BFC 205	90*	25	2.5
Desi chickpea			
Amethyst	71	25	2.5
Mung bean			
Berken	30*	25	2.5
Green bean			
Redlands' Pioneer	48	50	5
Vetch			
'Namoi'	No visible symptoms		

* Values significant ($p \leq 0.05$). Data summarise the results of several separate experiments.

On a few occasions BTH did not reduce the severity of black root rot but it did reduce the production of chlamyospores on the roots. Fiord was the most susceptible of the faba bean cultivars but chlamyospore production was totally prevented by BTH treatment even though Fiord roots became infected. Similarly, in cowpea chlamyospore production was prevented by BTH seed treatment. In other legumes chlamyospore production was not prevented by BTH even though disease severity was decreased. This indicates that even if black root rot severity can be reduced by BTH treatment production of chlamyospores may not be stopped equally in all legumes. However, the density of chlamyospores usually appeared to be less following BTH treatment, although this was not assessed quantitatively.

In order to compare the progress of disease on a relative basis and the ability of *T. basicola* to produce chlamyospores, cowpea, pea, cotton and lablab seeds were sown in the Wee Waa soil and assessed at 14 and 21 days. Black root rot progressed faster and chlamyospore production was greater in cotton than in the legumes (Table 3). At 14 days after sowing, chlamyospores were observed on cotton roots but not on the roots of the legumes, in keeping with the greater disease severity on cotton.

Table 3. Relative progression of black root rot and production of chlamydo spores in cotton and legumes

	Disease severity index (0-10 scale) ^a		Chlamydo spores
	After 14 days	After 21 days	
Cotton (Siokra 1-4)	4.6a	7.8a	High
Lablab	2.0c	2.6b	Low
Cowpea cv. Poona	3.0b	2.4b	Medium
Field pea	0.5d	1.7c	Zero

^a Values followed by the same letter in columns are not significantly different ($p < 0.001$).

Black root rot severity increased between 14 and 21 days after sowing in all legumes except cowpea. The severity of black root rot in cotton was three times higher than in cowpea and lablab, and four times higher than in field pea. Microscopic studies revealed the presence of chlamydo spores in cowpea, lablab and cotton but not in field pea at 21 days (Table 3). This result indicates that disease progression and production of chlamydo spores is relatively much higher in cotton than in legume roots.

The disease severity of BRR cotton was reduced significantly in a treatment where cotton was grown in a mixture of sterile soil and infested Fiord roots emerged from seed treated with BTH 50 ppm a.i.. Cotton grown on a mixture of sterile soil and Fiord infested roots emerged from BTH 25 ppm treatment also had lower disease severity than control but was not significant.

Table 4. Reduced severity of black root rot in cotton (cv. Sicala V2) grown in steamed soil inoculated with roots from BTH treated faba bean (cv. Fiord) grown in unsteamed Wee Waa soil.

Faba bean seed treatment	Disease severity (0-10 scale) ^a	
	Faba bean, prior to cotton	Cotton, after faba bean
Water	3.96a	6.29a
BTH 25 ppm	2.29b	4.75ab
BTH 50 ppm	1.77b	3.87b

^a Values followed by the same letter in columns are not significantly different ($p < 0.05$).

Discussion

Control of seedling diseases is a major priority in many crop systems. Seed treatments that induce systemic resistance after seedling emergence may be an ideal way to provide protection against seedling diseases like black root rot during establishment of the crop. BTH has no antifungal activity against *T. basicola* spores *in vitro*. This provides clear evidence that our treatments with BTH induced disease resistance against *T. basicola* in both cotton and legumes.

Currently there are no completely effective control methods available for black root rot in cotton. BTH treatment of cotton did not induce complete resistance (immunity) to black root rot. However, induced resistance could be integrated with other control methods, such as biofumigation, fungicides or summer flooding (see paper by Nehl et al., this proceedings). BTH has been used successfully in combination with fungicides to reduce root disease caused by *Phytophthora cinnamomi* in nursery plants (Ali et al., 2000). Further investigation of the use of BTH in cotton, including integration with other control methods, is warranted.

Most of the legumes examined here were susceptible to *T. basicola* and treatment with BTH reduced black root rot severity considerably. Even when the reduction in disease severity was not statistically significant, spore production on legume roots was reduced. This suggests that BTH may be useful for management of black root rot in legumes. However, *T. basicola* in roots of faba bean (cv. Fiord) survived desiccation and was able to act as inoculum for subsequent plants, even though there were no visible chlamydospores on the faba bean roots. It is not known how long *T. basicola* can survive in infested root fragments under field conditions.

While reproduction of *T. basicola* was much higher in cotton, this fungus was clearly able to multiply well in many of the legumes examined here. Inclusion of susceptible legumes as rotation crops with cotton is likely to increase, or at least maintain, the population of *T. basicola* in soil. Therefore, effective management of this disease will require its control in all the crops that are susceptible. Treatment of faba bean seed with BTH reduced the amount of inoculum carried over and the subsequent development of black root rot in cotton in the glasshouse. This observation, suggests that similar treatment of susceptible legumes in the field could make an important contribution to management of black root rot in cotton farming systems.

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