

# DEVELOPING BION<sup>®</sup> AS A SEED TREATMENT FOR BLACK ROOT ROT IN COTTON

Susanna A. Driessen<sup>1,2</sup>, David. B. Nehl<sup>1,2</sup>, Stephen J. Allen<sup>1,3</sup>

<sup>1</sup> Australian Cotton Cooperative Research Centre, Locked Bag 59, Narrabri, NSW, 2390

<sup>2</sup> NSW Department of Primary Industries, Locked Bag 1000, Narrabri NSW 2390

<sup>3</sup> Cotton Seed Distributors, PO Box 117, Wee Waa NSW

## Black root rot in cotton

Black root rot (caused by *Thielaviopsis basicola*), is an important seedling pathogen in the cotton industry in Australia (Nehl et al. 2004). The disease develops in the early part of the season (3-5 weeks after sowing), under favourable weather conditions, especially low soil temperatures (16-20°C). All production areas in NSW and Queensland currently have the pathogen. There are currently no practical eradication methods and prevention, by farm hygiene, is vital. The pathogen produces large quantities of thick-walled spores (chlamydospores) that persist in the soil over long periods. Movement of contaminated soil via farm machinery and vehicles has a significant contribution to transferring the pathogen within and between farms.

Several leads are currently under investigation for developing better disease control, including the impact of crop rotations on spore concentration and disease, the use of novel biofumigation (green manure) crops, and the relationship between soil types and disease severity. A further avenue of research is the use of synthetic chemicals, such as acibenzolar-S-methyl (**Bion**<sup>®</sup>, Syngenta Crop Protection), which act to induce resistance to the pathogen prior to the pathogen attacking the plant. This enhanced form of resistance is referred to as systemic acquired resistance (**SAR**).

## SAR in integrated disease management

Various biological and non-biological stimuli are able to induce plants to become more resistant to disease (Oostendorp, Kunz *et al.* 2001). When the effect occurs throughout the whole plant, it is referred to as systemic acquired resistance (in contrast to locally induced resistance, which occurs solely within the area affected). Pathogen attack is one biological factor that induces a plant defence response. However, in commercial crops it is preferable that the plant be already 'primed' to defend itself against possible pathogen attack. Several natural and synthetic inducers of resistance can enable plants to do this (Vallad and Goodman 2004).

Various synthetic chemicals have been shown to induce resistance, by means of different chemical pathways, though few have reached commercialisation stage (Gozzo 2004; Walters, Walsh *et al.* 2005). Unlike fungicides, these chemicals are not directly toxic to the pathogen (Oostendorp, Kunz et al. 2001). At high levels, some of these 'inducing' chemicals can be toxic to the plant. Acibenzolar-S-methyl, the active ingredient in **Bion**<sup>®</sup>, was the first chemical inducer to be successfully commercialised. This compound has been shown to provide a degree of resistance to many pathogens on several different plant hosts (da Rocha and Hammerschmidt 2005).

## Black root rot and SAR

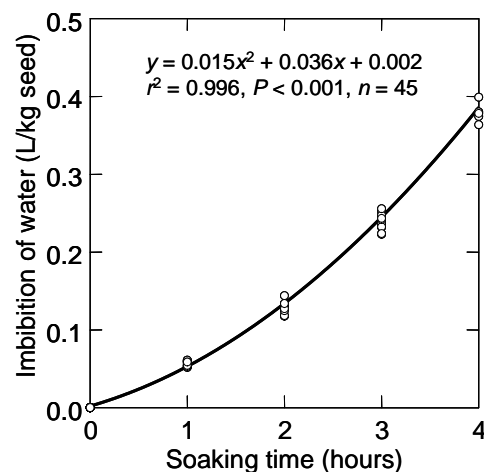
Initial field trials by NSW Department of Primary Industries highlighted the potential of **Bion**<sup>®</sup> as a tool for integrated management of black root rot in cotton. When **Bion**<sup>®</sup> was applied as a seed coating (at certain rates), as an in-furrow spray at sowing and by seed soaking just prior to sowing,

it reduced disease severity on cotton tap roots by 20 to 33% (Mondal et al., 2005). Furthermore, in the in-furrow spray experiment, the number of relatively healthy lateral roots was increased by 350%, which was accompanied by an increase in cotton growth and fruit production (Mondal et al. 2005). Although the seed-soaking method was effective, it was time consuming to soak and dry the seed prior to sowing and seed-swelling affected the rate of delivery in some planters. Seed treatment, with Bion® incorporated with the standard seed-coatings on commercial seed, would be more practical. To effectively deploy Bion® as part of an integrated management strategy for black root rot in cotton, improved methods of application were necessary.

## Development of Bion® as a cotton seed-coating

### Seed-coating rates

To determine appropriate rates for application of Bion® with the standard seed coatings, the equivalent amount of Bion® absorbed during the seed soaking process had to be calculated. Imbibition of water by cotton seed (var. Sicala V2) was determined gravimetrically in two experiments and the data pooled to fit a model for the rate of absorption of water (Figure 1).



**Figure 1.** Uptake of water by cotton seed (cv. Sicala V2) over time (data estimated gravimetrically and pooled for two studies)

In previous experiments, soaking cotton seed for three hours in a solution of acibenzolar-S-methyl (25 mg/L) was sufficient to induce heightened resistance against *T. basicola* (Mondal et al. 2005). Since 1 kg of cotton seed imbibed 0.24 L of water in three hours (Fig. 1), uptake of acibenzolar-S-methyl in those experiments was equivalent to 6 mg/kg seed.

### Checking for phytotoxicity

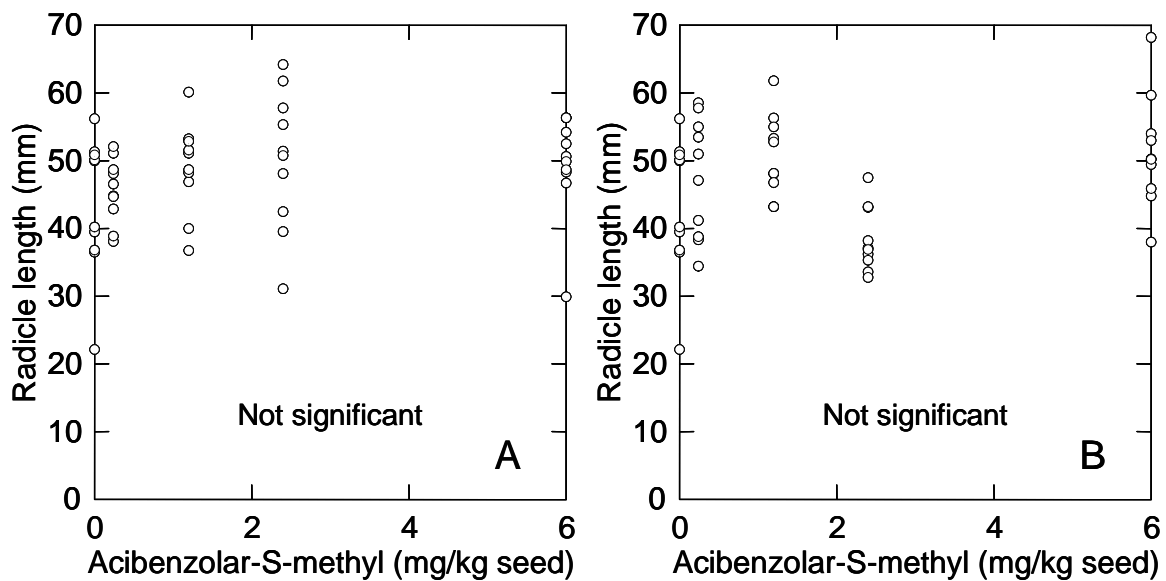
To test whether or not using Bion® as a seed coating might have a negative effect on the plant (phytotoxicity), even with a rate equivalent to seed soaking, several rates were evaluated in combination with the standard seed-coating fungicides (Table 1). Acibenzolar-S-methyl, as a granular formulation, was ground finely in a mortar and pestle before applying to 200 g of black seed (var. Sicot 189) in a plastic bag, agitating the bag until the powder was sufficiently dispersed on the seed and then adding 1.7 mL of liquid QAP (PCNB, metalaxyl-M, Peridiam™ blue), with agitation until seeds were evenly coated. Bion® was applied in a similar manner, except the Bion®

was allowed to dry on the seeds before coating with the QAP. The seeds were germinated on moist paper towelling in sterile germination chambers that allowed the root to grow downwards such that its length could be measured easily.

**Table 1.** Rates of acibenzolar-S-methyl and equivalent rates for formulations applied, with the fungicides PCNB and metalaxyl-M, as a seed coating to cotton seed to evaluate the potential for phytotoxicity in germinating seedlings

Seed-soaking equivalent (Acibenzolar-S-methyl ppm in solution)	Acibenzolar-S-methyl (mg/kg seed)	Formulation equivalent	
		Granules (mg/kg seed)	Bion® (µL/kg seed)
0	0	0	0
1	0.24	0.48	0.48
5	1.2	2.4	2.4
10	2.4	4.8	4.8
25	6.0	12	12

Application of both formulations of acibenzolar-S-methyl, in combination with the fungicides PCNB and metalaxyl-M, had no detrimental effect on germination and growth of cotton seedlings under sterile conditions (Figure 2).



**Figure 2.** Application of acibenzolar-S-methyl, formulated as granules (A) or a liquid, Bion® (B), to cotton seed (cv. Sicot 189) with the standard seed-coating fungicides (PCNB and metalaxyl-M) had no effect on germination and radicle growth under sterile conditions.

### Assessing the effect of Bion® on emergence

To test whether or not seed-coating with these formulations might affect emergence of cotton in soil, seed was treated with either the granular formulation or the liquid (Bion®) at rates equivalent to 0.24 and 1.2 mg acibenzolar-S-methyl/kg seed. For each treatment five seeds were sown in each of five replicate pots filled with soil collected from a cotton field at the Australian Cotton Research Institute. Progressive assessment of plant establishment indicated that none of the formulations affected seedling emergence (Table 2).

**Table 2.** Application of acibenzolar-S-methyl, formulated as granules or liquid (Bion<sup>®</sup>), to cotton seed (cv. Sicot 189) with the standard seed-coating fungicides (control = PCNB and metalaxyl-M) had no effect on germination in potted soil (DAS = days after sowing)

Seed treatment	Seedling emergence (%)		
	8 DAS	11 DAS	13 DAS
Control	36	60	64
Granules (2.4 mg/kg seed)	28	68	96
Granules (12 mg/kg seed)	40	64	84
Bion <sup>®</sup> (2.4 µL/kg seed)	72	88	88
Bion <sup>®</sup> (2.4 µL/kg seed)	44	80	88
	Not significant	Not significant	Not significant

The above investigation demonstrated that application of acibenzolar-S-methyl to cotton seed at the rate of 6 mg/kg is equivalent to the rate used successfully against black root rot by soaking seed. Furthermore, application of acibenzolar-S-methyl in combination with standard seed-coating fungicides had no phytotoxic effects on germination of seed and subsequent growth.

### Testing Bion<sup>®</sup> seed-coating in the field

Having established an appropriate rate for incorporation of Bion<sup>®</sup> with the standard seed-coating fungicides used for cotton, field experiments were conducted to evaluate its effectiveness.

In the 2004-05 season, cotton (var. Sicala 60BR) was sown on 5 Oct in 36-row plots, with six replicates, in a field near Narrabri that was infested with *T. basicola*. Bion<sup>®</sup> was applied to the seed, with the standard seed coating fungicides and carrier, at the rate of equivalent to 6 mg of acibenzolar-S-methyl per kg seed. Disease severity on tap roots was assessed on 2 and 29 Nov in 30 plants in each plot, taken along two transects across the plots; one transect was at a distance of 100 m from the tail drain and one was 100 m from the head ditch. Shoot dry mass was recorded for the same plants used for assessment of tap roots. Yield was assessed as module weights, with a single module being harvested from each plot. Disease severity was high in that field and Bion<sup>®</sup> decreased the severity of black root rot on tap roots by 4% at 28 days after sowing (DAS; Table 3). This effect was evident in the transect near the tail drain but not in the transect near the head ditch. Bion<sup>®</sup> decreased the shoot mass of cotton by nearly 11% at 28 DAS but the difference was around 1% at 55 DAS (Table 3). Yield with and without Bion<sup>®</sup> was 12.80 and 11.95 bales/ha, respectively, but the difference was not statistically significant ( $P = 0.054$ ).

In the 2005-06 season, Bion<sup>®</sup> was tested in 19 field trials in several localities (Table 4). Cotton seed was treated with a commercially available fungicide treatment (Dynasty<sup>®</sup>, Syngenta Crop Protection), with the addition of Bion<sup>®</sup> or without Bion<sup>®</sup> (control). These two treatments were sown in alternating plots, as wide as the planter (8 to 12 m), running the length of the field, except for the two fields at Narrabri where the plots were 36 rows wide in a randomised block design. Disease severity was assessed at three to six weeks after sowing. Bion<sup>®</sup> decreased the severity of black root rot in 13 of the 19 trials (Table 4). However, Bion<sup>®</sup> tended to be less effective when the severity of disease in the field (as observed in the control plots) was either very high or very low; 47% of the variation in the effectiveness of Bion<sup>®</sup> could be explained by disease severity (Fig. 3). The lack of effectiveness when disease was very low reflects either a variable distribution of the pathogen across the trial site or its absence altogether. At very high disease pressure, symptoms on tap roots of cotton reach a saturation level and may not be representative of the effects of Bion<sup>®</sup> on the rest

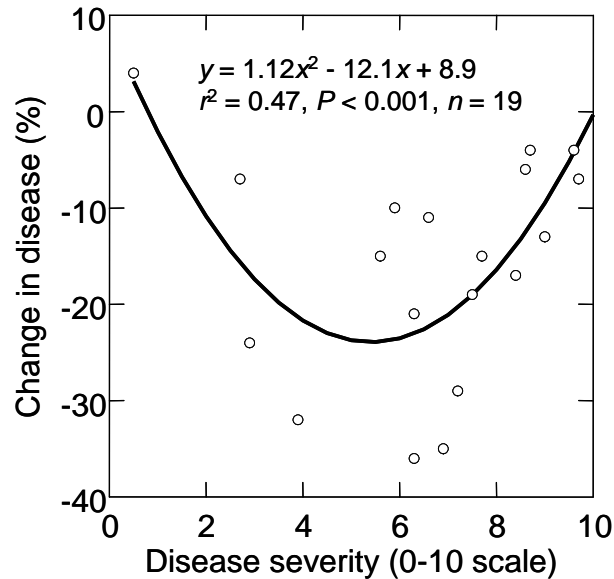
of the roots system (see paper by Nehl et al. in these proceedings). Bion<sup>®</sup> tended to be most effective at medium levels of disease severity (Fig. 3). Uneven distribution of the pathogen in the soil, local environmental conditions and different times of assessment are likely to have contributed to some of the observed variability.

**Table 3.** Application of Bion<sup>®</sup>, to cotton seed with the standard seed-coating fungicides (PCNB and metalaxyl-M) had a variable effect on the severity of black root rot and growth of cotton in an infested field near Narrabri in the 2004-05 season (DAS = days after sowing; NS = not significant; control had standard fungicides alone)

	Disease severity (0-10 scale)					
	28 DAS			55 DAS		
	Control	Bion <sup>®</sup>	Probability	Control	Bion <sup>®</sup>	Probability
<b>Head ditch transect</b>						
Disease severity (0-10 scale)	9.1	8.6	NS	0.9	0.5	$P = 0.015$
Shoot dry mass (g/plant)	0.185	0.162	$P = 0.011$	1.58	1.41	NS
<b>Tail drain transect</b>						
Disease severity (0-10 scale)	9.2	9.0	$P = 0.011$	0.9	1.0	NS
Shoot dry mass (g/plant)	0.172	0.155	$P = 0.041$	1.47	1.34	NS
<b>Combined transects</b>						
Disease severity (0-10 scale)	9.2	8.8	$P = 0.011$	0.9	0.8	NS
Shoot dry mass (g/plant)	0.178	0.159	$P < 0.001$	1.45	1.43	$P = 0.013$

**Table 4.** Application of Bion<sup>®</sup> to cotton seed decreased the severity of black root rot in 13 out of 19 field experiments in the 2005-06 season (NS = not significant; DAS = days after sowing)

Locality	Assessment (DAS)	Disease severity (0-10 scale)		Probability
		Control	Bion <sup>®</sup>	
Boggabri	40	2.7	2.5	$P = 0.003$
Carroll	20	8.4	7.0	$P = 0.003$
Gunnedah	27	9.7	9.0	$P = 0.003$
Gunnedah	27	9.6	9.2	NS
Maules Ck	20	7.5	6.1	$P = 0.001$
Merah North	21	6.6	5.9	NS
Moree	23	6.3	4.1	$P = 0.041$
Moree	22	2.9	2.2	$P = 0.003$
Moree	20	7.7	6.6	$P = 0.018$
Moree	24	8.7	8.4	NS
Moree	23	5.6	4.8	$P < 0.001$
Moree	21	8.6	8.1	NS
Moree	20	5.9	5.3	NS
Mungindi	22	0.5	0.5	NS
Narrabri	27	6.3	5.0	$P = 0.008$
Wee Waa	35	3.9	2.7	$P < 0.001$
Wee Waa	22	7.2	5.1	$P = 0.002$
Wee Waa	21	9.0	7.8	$P = 0.020$
Wee Waa	24	6.9	4.5	$P < 0.001$



**Figure 3.** Seed-coating with Bion<sup>®</sup> tended to be less effective against black root rot of cotton as disease severity increased.

### Further development of Bion<sup>®</sup>

Given the absence of cotton varieties with resistance against black root rot, the success of Bion<sup>®</sup> in inducing SAR against this disease warrants its deployment as part of an integrated disease management strategy. The application of Bion<sup>®</sup> to seed, in combination with standard seed-coatings, has proven to be an effective and practical method for delivery. However, the reasons for variation in the effectiveness of Bion<sup>®</sup> are unclear. The field experiments in 2005-06 suggest that the resistance response induced by Bion<sup>®</sup> may not be sufficient to inhibit disease when the population density of the pathogen is high. Furthermore, the impact of climatic conditions (such as temperature) on the effectiveness of Bion<sup>®</sup> is unknown. Further investigation of these factors is proceeding in a CRDC-funded project with NSW Department of Primary Industries at Narrabri. Financial support towards this future work is also being provided by Syngenta. If Bion<sup>®</sup> proves capable of slowing the reproduction of *T. basicola* then its deployment would have the added advantage of slowing the build-up and dispersal of this pathogen.

### Acknowledgments

This research was funded by the Cotton R&D Corporation, Cotton Seed Distributors Pty Ltd and NSW Department of Primary Industries. Technical assistance from Michael Dummigan, Brendon Hatton, Greg MacNamara, Anowar Mondal, Peter Lonergan, Tracey Mor and James Quinn, the generous cooperation of numerous cotton growers and provision of Bion<sup>®</sup> by Syngenta Crop Protection is gratefully acknowledged.

### References

- da Rocha AB, Hammerschmidt R (2005) History and perspectives on the use of disease resistance inducers in horticultural crops. *HortTechnology* **15**, 518-529.
- Gozzo F (2004) Systemic acquired resistance in crop protection. *Outlooks on Pest Management*, 20-23.

- Mondal AH, Nehl DB, Allen SJ (2005) Acibenzolar-S-methyl induces systemic resistance in cotton against black root rot caused by *Thielaviopsis basicola*. *Australasian Plant Pathology* **34**, 499-507.
- Nehl DB, Allen SJ, Mondal AH, Lonergan PA (2004). Black root rot: a pandemic in Australian cotton. *Australasian Plant Pathology* **33**: 87-95
- Oostendorp M, Kunz W, Dietrich B, Staub T (2001) Induced disease resistance in plants by chemicals. *European Journal of Plant Pathology* **107**, 19-28.
- Vallad GE, Goodman RM (2004) Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science* **44**, 1920-1934.
- Walters D, Walsh D, Newton A, Lyon G (2005) Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. *Phytopathology* **95**, 1368-1373.