

Signature of Research Provider Representative:



FINAL REPORT 2006

Due by 30th September 2006.

Part 1 - Summary Details Please use your TAB key to complete Parts 1 & 2. **CRDC Project Number: OR Cotton CRC Project Number:** 1.1.13 **Project Title:** Study of Alternaria leaf spot on cotton in Northern Australia **Project Completion Date:** 31/12/2005 **Project Commencement Date:** 1/01/2005 **CRDC Program:** - Please Select One -**OR CRC Program:** The Farm Part 2 - Contact Details **Administrator:** Ms Suzanne Simonato **Organisation:** Department of Primary Industry, Fisheries and Mines (DPIFM) **Postal Address:** PO Box 3000, Darwin, NT 0801 **Ph:** 08 89992300 **Fax:** 08-89992049 E-mail: suzanne.simonato@nt.gov.au **Principal Researcher:** Dr Shamsul Bhuiyan **Organisation:** DPIFM **Postal Address:** PO Box 1346, Katherine, NT 0851 **Ph:** 08-89739712 **Fax:** 08-89737777 E-mail: shamsul.bhuiyan@nt.gov.au **Supervisor:** Dr Colin Martin **Organisation: DPIFM Postal Address:** PO Box 3000, Darwin, NT 0801 **Ph:** 08-89992323 **Fax:** 08-89992043 E-mail: colin.martin@nt.gov.au

(The points below are to be used as a guideline when completing your final report.)

Background

Alternaria leaf blight of cotton occurs in most cotton growing countries in the world (Ellis 1971). At least two pathogens, *Alternaria macrospora* Zimm. and *A. alternata* (Fr.) Keissler are considered causal agents (Bashan and Hernandez-Saavedra 1992). Although, *A. macrospora* is considered the causal agent of leaf blight in Pima cotton (*Gossypium barbadense* L.), and *A. alternata* is considered the casual agent of leaf blight in Acala cotton (*Gossypium hirsutum* L.) (Bashan and Hernandez-Saavedra 1992), there is evidence that Alternaria blight is a disease complex of both pathogens (Rotem *et al.* 1988, Bashan *et al.* 1991).

The disease can occur on cotyledons, leaves, stem, flowering buds and fruits. Symptoms begin with small brown necrotic lesions, 1-2 mm diameter, surrounded by a purple halo. The lesions may extend up to 2-3 cm in diameter in severe conditions. A marked yellow halo surrounding the necrotic lesion is common in mature leaves. Under favourable conditions, the lesion takes on a black sooty appearances due to massive sporulation by the fungi. As the disease progresses, tissue at the centre of the old lesions becomes grey and dry, and necrotic tissue may crack and fall out, giving a 'shot-hole' appearance (Baird 2001, Bashan and Hernandez-Saavedra 1992) (Figure 1). Heavy infection of leaves may completely defoliate plant which may be the main cause of yield loss due to Alternaria leaf blight (Bashi et al. 1983).





Fig 1. Alternaria leaf blight affected leaves (top) and boll (bottom)

Alternaria leaf blight has been considered a minor disease of cotton in the cotton growing areas of southern Australia (Nehl and Allen 2002) but cotton grown in northern Australia (Kununurra and Katherine) had 100% of leaves infected in some years (S. Yeates

and J. Moulden per. comm.). A number of surveys confirmed that Alternaria leaf blight was the most prevalent disease of cotton in the northern Australia (Nehl *et al.* 2000, Bellgard 2001). There were no reports on the impact of this disease on cotton production in Australia but a yield loss of 37% was reported in India by Padaganur *et al.* (1989), and of 25% in Israel by Bashi *et al.* (1983). High disease severity has been reported in Katherine (NT), Kununurra (WA) and Burdekin (QLD) regions in the last few years and may be a problem for a developing cotton industry in northern Australia.

Shtienberg and Dreishpoun (1991) effectively controlled alternaria leaf blight of Pima cotton (*G. barbadense*) using two systemic fungicides, difenoconazole and tebuconazole, in Israel. In Australia, the foliar application of mancozeb is recommended for the control of Alternaria leaf blight of Pima cotton in Queensland and NSW. The efficacy of these fungicides in controlling Alternaria leaf blight on upland cotton (*G. hirsutum*) in northern Australia has not been determined.

Objectives

- To determine the efficacy of mancozeb fungicide on alternaria leaf blight disease incidence, severity, and subsequent defoliation
- To determine the affect of alternaria leaf blight on yield and quality of upland cotton (*G. hirsutum*)
- To determine the prevalence of *A. macrospora* and *A. alternata* at various of time of growing season
- To determine the survival of the pathogens in various conditions

Methods

The experiment was undertaken at Katherine Research Station (KRS) (14°28′ S, 132°18′ E), Katherine, Northern Territory. The cotton variety Sicot 289B was sown on March 30, 2005 with a no-till planter to a depth of approximately 3 cm. Eight to ten seeds per meter were sown in rows 1 m apart. The crop was irrigated twice weekly with a lateral-move irrigator. Total water applied was 5.5 megalitre /ha. Total fertilizer applied (most in the

irrigation water) per hectare was 162 kg N, 32 kg P, 72 kg K and 13 kg S. Insecticides and herbicides were applied when needed, and other crop husbandry practices were performed in accordance with normal procedures at KRS.

Treatments

The experiment was a randomized, complete block design with five treatments, control, mancozeb at 2.5kg/ha every 4 day, 2.5kg/ha every 7 day, 3kg/ha every 4 day and 3kg/ha every 7 day. Each treatment was replicated five times, and each replicate plot was 6 m x 10 m. Two buffer rows were maintained between treated plots to minimise drift of spray and inocula. Fungicides were applied in 100 L water/ha with a hand held boom sprayer at a pressure of 350 kPa. The first application of fungicide was on April 28, 2005. Sprays contained no spreader, sticker or adjuvant of any type. The trial plots were not artificially inoculated with *Alternaria* spp. isolates because the heavy and continuous disease incidence had been observed on cotton in the last few years.

Assessment of disease on leaves and bolls

Five randomly selected plants from each plot were tagged with flag tape and used for disease assessment. Disease assessment was done at weekly intervals for 15 weeks. Main stem leaves (Colson-Hanks *et al.* 2000) at three canopy levels - bottom (1-7 nodes), middle (8-14 nodes), and top (15+ nodes) - were assessed visually for disease incidence, severity and leaf shedding. The percent of disease incidence at each assessment date was determined by the following equation

$$\alpha = \frac{x}{y} \times 100$$

where, α = disease incidence (%), x = number of infected leaves and y = total number of leaves assessed

The mean percent of infected leaves for the whole plant or at each canopy level was determined by the equation

$$Incidence = \frac{(\alpha_1 + \dots + \alpha_{12})}{15}$$

where, α = percent of infected leaves at each assessment period, and 15 = total number of assessment

A visual scale, modified from Rotem *et al.* (1988), was used for the assessment of disease severity as follows: 0, healthy leaves; 1, leaves with up to five lesions; 2, leaves with six to 20 lesions; 3, leaves with more than 20 lesions without marked necrosis; 4, leaves with more than 20 lesions with marked necrosis; 5, necrotic leaves, badly damaged tendency to shed; 6, leaves fallen-off from nodes. Number of shed leaves at each canopy level was assessed by counting the abscission marks of fallen leaves on the main stem. The mean disease severity and leaf shedding for the whole plant or at each canopy level was calculated using similar procedures to those used for disease incidence.

Disease severity assessment on cotton fruits (bolls) was conducted in the middle canopy once towards to the end of the growing season on August 17, 2006. Bolls were collected from node 10 of 10 randomly selected (i.e. from the middle canopy level) plants. Disease severity (percent of fruit surface affected) was assessed from 0 to 3 as 0, no infection, 1, 1 to 5% infection, 2, 5 to 15%, and 3, >15% infection.

Effects of Alternaria leaf blight on yield and fibre quality

Eight metres from the two middle rows of each plot were harvested by hand picking. Seed cotton was bagged and weighed from each plot. The number of plants and bolls in the eight metres was also counted using a hand counter. Approximately 500g of seed cotton from each bag were sent to Australian Cotton Research Institute, Narrabri, NSW for fibre quality analysis.

Survival of Alternaria leaf blight at various conditions

Toward the end of cotton growing season, a survival trial for the Alternaria leaf blight pathogens was undertaken at KRS. Infected dried fallen leaves and symptomatic fresh green leaves from standing plants were collected from the cotton field of KRS. Samples were divided into six batches. The first batch of 50 infected dry fallen leaves was placed as single layer on the surface of soil and covered with plastic mesh (10mm×10mm) in a field at KRS approximately 100 m from the cotton area. The second batch of 50 fresh green leaves was placed on the soil surface in a similar way to the first batch. The third and fourth batches, of 50 fallen and 50 green leaves respectively, were placed in separate 5 cm deep trenches covered with fine (1mm×1mm) nylon mesh, and buried. The fifth batch, of 50 dried fallen leaves, was placed on cotton debris (containing stems, branches and leaves) and covered with plastic mesh (10mm×10mm). The sixth batch of dried leaves was placed in a paper bag and maintained inside KRS plant pathology laboratory at 22±1°C.

Fifty pieces of leaves were randomly collected from each treatment and plated onto blotting paper (Whatman no.1) moistened with sterile tap water. Plates were then incubated at 22±1°C in 12 h cycle of light and darkness. After 7 days incubation, assessment was conducted by counting the number of leaf pieces showing visible signs of sporulation with the aid of a stereo-binocular microscope (Leica Microsystems Ltd, Heerbrugg, Switzerland). Each treatment was replicated five times.

Prevalence of A. macrospora and A. alternata during the cotton growing season

Ten randomly selected symptomatic leaves were collected from the KRS cotton field each week from 23 May 2005 to 21 September 2005. Three necrotic lesions from each symptomatic leaf were observed under a stereo microscope and spore characteristics studied with the aid of a compound microscope (Olympus BH2, Olympus Pty Ltd). Alternaria species identification was performed according to Simmons (2003) and Elllis (1971).

Statistical analysis

Data were analysed using Statistica® (Release 7.1, StatSoft Inc., USA). Analysis of variance and Fisher's least significance test was performed using the General Linear Model (GLM). Response functions were then fitted to the mean data by linear or non-linear regressions.

Development of disease incidence, severity at all canopy levels and leaf shedding at bottom and middle canopy were described by using a Logistic model *viz*.

$$Y = A/(1 + e^{(B-C.X)})$$

Where Y= predicted incidence, or severity or defoliation, X=time in days after first assessment. A, B and C are estimated parameters. A is an estimate of the upper asymptote. B/C is the point in time (in days, from the sowing date) at which plants acquire 50% of maximum fitted incidence, severity and leaf shedding. The value of the estimate for incidence, severity and leaf shedding at 50% maximum infection was given by A/2.

An equation could not describe leaf shedding at the top canopy level because no, or very few, leaves were shed during the assessment periods.

The median disease severity on bolls within treatments were compared using a Kruskal-Wallis Analysis of Variance by Ranks (Kruskal and Wallis, 1952). Where a significant difference in the median ranks occurred, a multiple comparison test (Seigel and Castellan 1998) was performed to determine significantly different groups.

Results

Disease incidence

Mean disease incidence in control plots was significantly (P<0.001) higher than that of fungicide treated plots. Disease incidence significantly (P<0.0001) varied with plant growing time and canopy level. In all treatments, regardless of sprayed or control, disease incidence was significantly (P<0.05) higher at the bottom canopy level followed by middle and top canopy levels (Table 1). At the bottom of the canopy, disease incidence in 3kg/7 day plots was significantly (P<0.05) higher than that of other treated plots which were not significantly different from each other. No differences of disease incidence were observed among fungicide treated plots at middle canopy level. At the top of the canopy, disease

incidence was significantly (P<0.05) lower in the 2.5kg/4 day and 3kg/4 day plots compared to the 2.5kg/7 day and 3kg/7 day plots.

Table 1. Mean incidence of Alternaria leaf blight at various canopy levels at Katherine Research Station, 2005

Height	Control (%)*	2.5kg/4 day (%)	2.5kg/7 day (%)	3kg/4 day (%)	3kg/ 7 day (%)
Bottom	96.7 (1.39)a	85.0 (1.39)c	86.3 (1.39)c	86.0 (1.39)c	90.7 (1.39)b
Middle	89.1 (1.39)a	79.0b (1.39b)b	81.1 (1.39)b	76.3 (1.39)bc	81.3 (1.39)b
Top	69.2 (1.63)a	43.1 (1.59)c	51.0 (1.60)b	42.1 (1.59)c	50.0 (1.61)b

^{*} numbers within each column are significantly different from each other, and number followed by different letters in each row differs significantly by Fisher's least significance test (P<0.05). Standard error of means (\pm) within parenthesis

Development of incidence of Alternaria leaf blight with time at various canopy levels was described by regression equations in Table 2, 3 and 4, and results shown in Figure 2, 3 and 3.

Table 2. Regression equations with estimated parameters that described the development with time of Alternaria leaf blight incidence at bottom canopy level*

Treatment	Equation	R^2
Control	$0.989/(1+e^{(11.39-0.281x)})$	0.58
2.5kg/4 day	$1.02626/(1+e^{(2.947-0.564x)})$	0.83
2.5kg/7 day	$1.0027/(1+e^{(3.7665-0.0751x)})$	0.89
3 kg/4 day	$1.01317/(1+e^{(3.203-0.0637x)})$	0.91
3 kg/7 day	$0.984/(1+e^{(6.617-0.1444x)})$	0.79
•		

^{*}x= time from sowing date (March 30, 2005).

At the bottom canopy level, initial disease incidence was approximately 70% in control plots, and approximately 40% in fungicide treated plots (Fig 2). From the regression equations in Table 2, the estimated number of days required to reach 50% disease incidence in the bottom canopy were lowest (40.6 day) in control plots followed by the 3kg/7 day fungicide treatment (45.8 day) (Table 5).

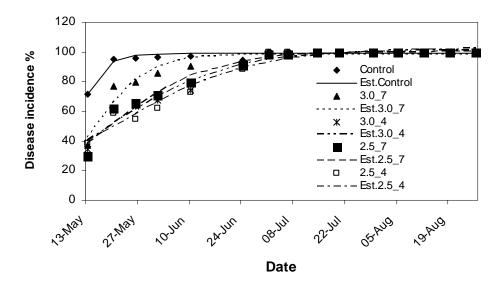


Fig 2. Actual and predicted development of Alternaria leaf blight incidence at the bottom canopy level for cotton grown in 2005 at Katherine Research Station

At the middle canopy level there was initially approximately 26% disease incidence was in control plots, and from 10 to 16% disease incidence on fungicide treated plants (Fig 3). From the regression equation in Table 3, the estimated number of days required to reach 50% disease incidence at the middle canopy level was lowest (48.9 day) in control plots followed by the 3kg/7 day fungicide treatment (58.4 day) (Table 5).

Table 3. Regression equations with estimated parameters that described the development with time of Alternaria leaf blight incidence at middle canopy level*

(7.261-0.148x)	
Control $0.9892/(1+e^{(7.261-0.148x)})$ 0.88	
2.5kg/4 day $0.981/(1+e^{(6.839-0.1138x)})$ 0.94	
2.5kg/7 day $0.9856/(1+e^{(5.785-0.1004x)})$ 0.93	
3 kg/4 day $0.987/(1+e^{(5.197-0.0816x)})$ 0.94	
3 kg/7 day $0.9966/(1+e^{(5.47-0.0937x)})$ 0.94	

^{*}x= time from sowing date (March 30, 2005).

At the top canopy level initial disease incidence was zero for all treatments including the control (Fig 4). From the regression equations in Table 4, the estimated number of days required to reach 50% disease incidence at the top canopy level was lowest (88.4 day) in control plots followed by the 3kg/7 day fungicide treatment (97.6 day) (Table 5).

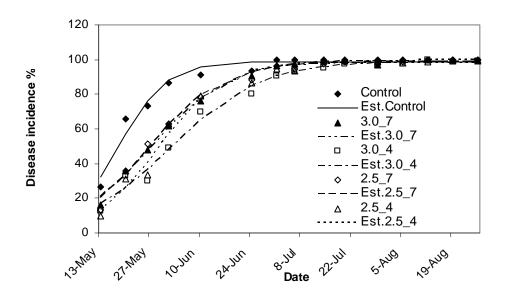


Fig 3. Actual and predicted development of Alternaria leaf blight incidence at the middle canopy level for cotton grown in 2005 at Katherine Research Station

Table 4. Regression equations with estimated parameters that described the development with time of Alternaria leaf blight incidence at the top canopy level*

Treatment	Equation	\mathbb{R}^2
Control	$0.851/(1+e^{(12.125-0.1372x)})$	0.94
2.5kg/4 day	$0.851/(1+e^{(6.775-0.0611x)})$	0.94
2.5kg/7 day	$0.891/(1+e^{(6.715-0.0636x)})$	0.92
3 kg/4 day	$0.7350/(1+e^{(6.423-0.0612x)})$	0.93
3 kg/7 day	$.7535/(1+e^{(7.379-0.0756x)})$	0.92

^{*}x= time from sowing date (March 30, 2005).

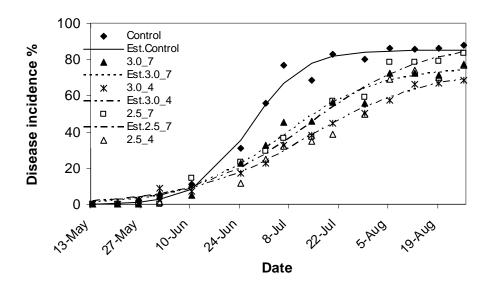


Fig 4. Actual and predicted development of Alternaria leaf blight incidence at the top canopy level for cotton grown in 2005 at Katherine Research Station

Table 5: Predicted number of days from the sowing date to reach 50% incidence of Alternaria leaf blight disease at various canopy levels.

Height	Control	2.5kg/4 day	2.5kg/7 day	3kg/4 day	3kg/7 day
Bottom	40.6 (17.04)*	52.2 (10.04)	50.2 (7.73)	50.3 (6.85)	45.8 (8.91)
Middle	48.9 (7.03)	60.1 (7.14)	57.6 (7.34)	63.7 (6.93)	58.4 (6.29)0
Top	88.4 (16.15)	110.9 (15.6)	105.5 (17.22)	105 (15.21)	97.6 (16.79)

^{*} Asymptotic standard error (±) within parenthesis

Disease severity

Mean disease severity in control plots was significantly (P<0.001) higher than that of fungicide treated plots. Disease severity were significantly (P<0.001) varied with plant growing time and canopy level. In all treatments, regardless of sprayed or control, disease severity was significantly (P<0.05) higher at the bottom canopy level followed by the middle and top canopy levels (Table 6).

At all canopy levels, disease severity on control plots was significantly (P<0.001) higher than that for fungicide treated plots but there were no differences between fungicide treatments at each level. (Table 6).

Table 6. Mean severity (0-6) of Alternaria leaf blight at various canopy level at Katherine Research Station, 2005

Height	Control*	2.5kg/4 day	2.5kg/7 day	3kg/4 day	3kg/7 day
Bottom	4.11 (0.084)a	3.57 (0.084)b	3.55 (0.084)b	3.44 (0.84)b	3.66 (0.084)b
Middle	3.19	2.09	2.17	1.89	2.21
	(0.084)a	(0.084)b	(0.084)b	(0.084)b	(0.084)b
Тор	1.55	0.57	0.69	0.49	0.65
	(0.098)a	(0.096)b	(0.096)b	(0.096)b	(0.097)b

^{*} Numbers within each column are significantly different from each other. Numbers followed by different letters in each row differ significantly by Fisher's least significance test (P<0.05). Standard error of means (\pm) within parenthesis

The development with time of Alternaria leaf blight severity on cotton leaves at various canopy levels was described by regression equations in Tables 7, 8 and 9, and shown in Figures 5, 6 and 7.

Table 7. Regression equations with estimated parameters that described the development with time of Alternaria leaf blight severity at bottom canopy level*.

Treatment	Equation	R^2
Control	$6.238/(1+e^{(3.332-0.044x)})$	0.96
2.5kg/4 day	$6.214/(1+e^{(6.411-0.0714x)})$	0.96
2.5kg/7 day	$6.298/(1+e^{(5.743-0.0634x)})$	0.96
3 kg/4 day	$6.1605/(1+e^{(6.674-0.07256x)})$	0.98
3 kg/7 day	$6.421/(1+e^{(5.023-0.0561x)})$	0.96

^{*}x= time from sowing date (March 30, 2005).

At the bottom canopy level initial disease severity was approximately 1 in control plots, but around 0.3 to 0.4 for fungicide treated plots (Fig 5). From the regression equations in Table 7, the estimated number of days required to reach 50% disease severity at the bottom canopy was lowest (75.6 day) in control plots followed by the 3kg/7 day fungicide treatment (89.5 days) (Table 10).

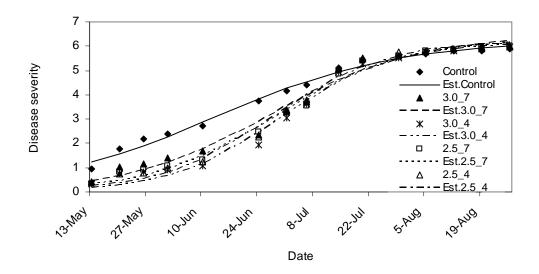


Fig 5. Actual and predicted development with time of Alternaria leaf blight severity at the bottom canopy level for cotton grown in 2005 at Katherine Research Station.

Table 8. Regression equations with estimated parameters that described the development with time of Alternaria leaf blight severity at the middle canopy level*

Treatment	Equation	\mathbb{R}^2
Control	$5.292/(1+e^{(3.182-0.03697x)})$	0.93
2.5kg/4 day	$5.8847/(1+e^{(4.8172-0.04088x)})$	0.93
2.5kg/7 day	$5.436/(1+e^{(4.336-0.0388x)})$	0.92
3 kg/4 day	$5.573/(1+e^{(4.846-0.0403x)})$	0.89
3 kg/7 day	$5.528/(1+e^{(4.336-0.0388x)})$	0.90

^{*}x= time from sowing date (March 30, 2005).

At the middle canopy, initial disease severity was approximately 0.3 in control plots, but around 0.1 to 0.2 for fungicide treated plots (Fig 6). From the regression equations in Table 8, the estimated number of days required to reach 50% disease severity in the middle canopy were lowest (86.1 (9.92) days) in control plots followed by the fungicide treatment 3kg/7 day (111.7 day) (Table 10).

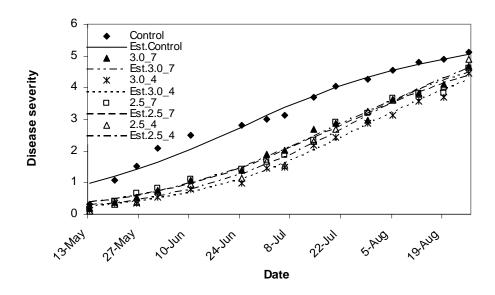


Fig 6. Actual and predicted development with time of Alternaria leaf blight severity in the middle canopy level for cotton grown in 2005 at Katherine Research Station.

Table 9. Regression equations with estimated parameters that described Alternaria leaf blight severity at the top canopy level*

Treatment	Equation	R^2
Control	$2.6229/(1+e^{(7.602-0.0724x)})$	0.92
2.5kg/4 day	$1.6333/(1+e^{(6.809-0.0533x)})$	0.94
2.5kg/7 day	$1.798/(1+e^{(6.513-0.05228x)})$	0.93
3 kg/4 day	$1.0608/(1+e^{(5.898-0.0506x)})$	0.92
3 kg/7 day	$1.4677/(1+e^{(6.123-0.0516x)})$	0.94

^{*}x = time from sowing date (March 30, 2005).

At the top canopy level initial disease severity was zero in control and fungicide treated plots, but significant (P<0.05) differences between control and treated plots were observed from June 24 onwards (Fig 7). From the regression equations in Table 9, the estimated number of days required to reach 50% disease severity at the top canopy level was lowest (105 day) in control plots followed by the 3 kg/4 days fungicide treatment (116.5 day) (Table 10).

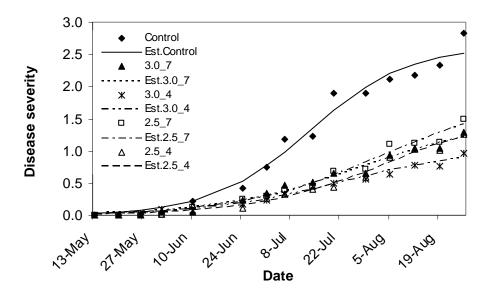


Fig 7. Actual and predicted development with time of Alternaria leaf blight severity at the top canopy level for cotton grown in 2005 at Katherine Research Station.

Table 10: Predicted number of days from the sowing date to reach 50% Alternaria leaf blight disease severity at various canopy levels.

Height	Control	2.5kg/4 day	2.5kg/7 day	3kg/4 day	3kg/7 day
Bottom	75.6 (6.47)*	89.8 (8.87)	90.6 (8.86)	92 (6.98)	98.5 (7.88)
Middle	86.1 (9.92)	117.8 (15.42)	111.9 (14.8)	120.2 (19.73)	111.7 (17.27)
Тор	105 (18.15)	127.8 (18.44)	124.6 (18.84)	116.5 (18.51)	118.7 (16.3)

^{*} Asymptotic standard error (±) within parenthesis

Leaf shedding

Mean leaf shedding due to Alternaria leaf blight was significantly (P < 0.001) higher in control plots than in fungicide treated plots. Leaf shedding significantly (P < 0.001) varied with time and canopy level. In all treatments, sprayed or control, disease severity was significantly (P < 0.05) higher at bottom canopy level followed by the middle and top canopy levels (Table 11).

At the bottom canopy level there was no difference between control and fungicide treated plots except for the 3kg/4 day plot treatment which was significantly (P=0.01) lower than the control (Table 11). At middle canopy level shedding was significantly (P<0.001) higher in control plots than the fungicide treated plots. There was no difference among fungicide treated plots. At the top canopy level there were no differences between control and fungicides treated plots, or among fungicide treatments.

Table 11. Effect of canopy level and fungicide treatments on mean leaf shedding due to Alternaria leaf blight at Katherine Research Station, 2005

Height/Treatment	Control*	2.5kg/4 day	2.5kg/7 day	3kg/4 day	3kg/7 day
Bottom	3.18	2.94	3.07	2.81	2.92
	(0.102)a	(0.102)ab	(0.102)ab	(0.102)b	(0.102)ab
Middle	1.55	0.86	0.87	0.77	1.35
	(0.102)a	(0.102)b	(0.103)b	(0.102)b	(0.102)b
Тор	0.03 (0.12)a	0.02 (0.117)a	0.02 (0.118)a	1.30e-3 (0.117)a	2.4e-5 (0.119)a

^{*} Numbers within each column are significantly different from each other, and numbers followed by different letters in each row differ significantly by Fisher's least significance test (P<0.05). Standard error of means (\pm) within parenthesis

The predicted leaf shedding due to Alternaria leaf blight at the bottom and middle canopy levels was described by regression equations in Table 12 and 13, and shown in Figure 8 and 9.

Table 12. Regression equations with estimated parameters that described leaf shedding with time at the bottom canopy level*

Treatment	Equation	R^2
Control	$6.3958/(1+e^{(10.067-0.1025x)})$	0.96
2.5kg/4 day	$6.293/(1+e^{(18.022-0.1757x)})$	0.95
2.5kg/7 day	$6.7634/(1+e^{(11.6102-0.1122x)})$	0.94
3 kg/4 day	$6.157/(1+e^{(16.054-0.155x)})$	0.97
3 kg/7 day	$6.373/(1+e^{(15.686-01518x)})$	0.97

^{*}x = time from sowing date (March 30, 2005).

At the bottom canopy level initial leaf shedding remained almost at zero in all treatment including the control for three weeks after the initial assessment date (Fig 8). From the regression equations in Table 12, the estimated number of days required to reach 50% shedding at the bottom canopy level was lowest (98.2 day) in control plots followed by the 2.5kg/4 days (102.6 days fungicide treatments (Table 14).

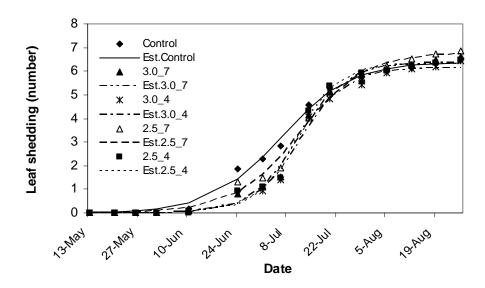


Fig 8. Actual and predicted development of leaf shedding with time at the bottom canopy level for cotton grown in 2005 at Katherine Research Station.

Table 13. Regression equations with estimated parameters that described leaf shedding with time at the middle canopy level.?

Treatment	Equation	\mathbb{R}^2
Control	$4.2701/(1+e^{(11.904-0.1053x)})$	0.87
2.5kg/4 day	$5.374/(1+e^{(11.167-0.0808x)})$	0.83
2.5kg/7 day	$3.824/(1+e^{(11082-0.0858x)})$	0.76
3 kg/4 day	$5.456/(1+e^{(11.395-0.0805x)})$	0.83
3 kg/7 day	$4.428/(1+e^{(9.676-0073x)})$	0.77

x = time from sowing date (March 30, 2005).

At the middle canopy level initial leaf shedding was almost zero in all treatments including the control for the first three weeks of assessment (Fig 9). Leaf shedding in control plots remained higher than that of fungicide treated plots from first week of June until the end of the assessment period. From the regression equations in Table 13, the estimated number of days required to reach 50% shedding at the middle canopy level was lowest (113.1 day) in control plots followed by the 2.5kg/4 days (129.2 days) fungicide treatments.

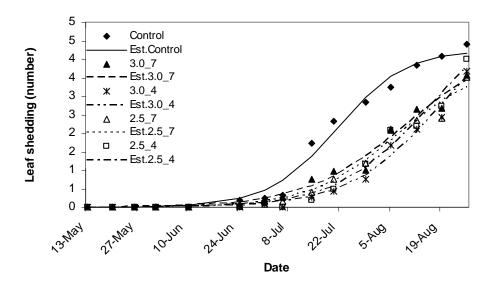


Fig 9. Actual and predicted development of leaf shedding due to Alternaria leaf blight at the middle canopy level for cotton grown in 2005 at Katherine Research Station

Table 14: Predicted number of days from the sowing date to reach 50% leaf shedding due to Alternaria leaf blight disease at bottom and middle canopy levels.

Height	Control	2.5kg/4 day	2.5kg/7 day	3kg/4 day	3kg/7 day
Bottom	98.2 (13.46)	102.6 (16.84)	103.5 (17.42)	103.5 (11.59)	103 (12.95)
Middle	113.1 (28.04)	138.2 (41.63)	129.2 (46.9)	141.5 (43.71)	132.1 (44.64)

^{*} Asymptotic standard error (±) within parenthesis

The number of leaves shed at the top canopy level remained zero until the middle of July. No leaf shedding was observed on mancozeb treatments 3kg/7 days and 3kg/4 days. The highest number of leaves shed was on control plots, where only 0.2 had been shed at the last assessment date (Figure 10).

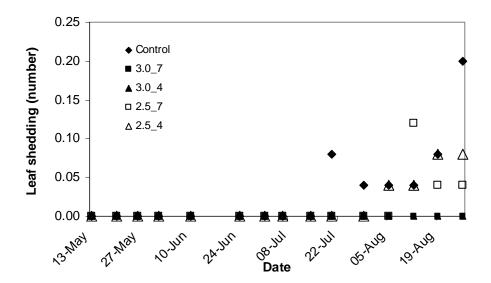


Fig 10. Leaf shedding due to Alternaria leaf blight at top canopy level for cotton grown in 2005 at Katherine Research Station

Disease severity on cotton bolls

Disease severity on cotton bolls was significantly higher (P<0.001) in control plots (2.28) than in fungicide treatments 2.5kg/4 days (0.88), 2.5kg/7 days (1.04), 3kg/4 days (0.89) and 3kg/7 days (1.02). Results are presented as catagorised histograms (Fig 11). No significance difference in median severity was observed among fungicide treated plots.

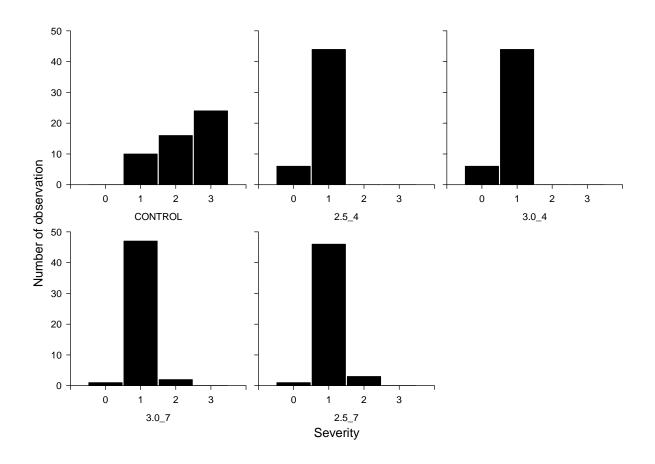


Fig 11. Catagorised histogram for comparing disease severity on cotton boll in control and fungicide treated plots using Kruskal-Wallis test. Scales (0-3) and title on X axis denote disease severity, and fungicide rate and spray interval

Effects on production and quality of cotton

No significant differences for boll production per plant and seed cotton production per plant were observed between control and fungicide treated plots (Table 14)

Table 14. Production seed cotton in control and fungicide treated plots at Katherine Research Station.

Treatment	Total no. of plants	Total no. of boll	Total weight of seed cotton (kg)	No. of boll/plant	Seed cotton/boll (gm)	Yield/plant (gm)
Control	42.1	507.7	2.12	12.06	4.17	50.29
3kg/7 day	40.4	468.5	2.02	11.60	4.31	49.99
2.5kg / 7 day	42.5	529.3	2.20	12.45	4.16	51.79
3kg/4 day	41.5	467.2	1.98	11.26	4.24	47.74
2.5kg / 4 day	44.6	487.0	2.01	10.92	4.13	45.10

Cotton fibre length was significantly (P<0.001) shorter in control plots than that of 3kg/7 day and 3kg/4 day, but no significant differences from other fungicide treated plots. There were no significant treatment effects on the fibre uniformity except between 3kg/4 day and 2.5kg/4 day treatments. There were no significant differences of fibre strength among treatments. Fibre elongation and micronaire were significantly higher on control plots than all fungicide treated plots except 3kg/4 day and 3kg/7 day plots respectively (Table 15).

Table 15. Fibre quality of cotton from control and fungicide treated plots at Katherine Research Station in 2005.

Treatment	Length (inch)	Uniformity (%)	Strength (gm/tex)	Elongation (%)	Micronaire (microgram/inch)
Control	1.084a*	78.92a	25.4	4.56ab	4.68ab
3kg /7 day	1.124b	79.56a	26.24	4.32a	4.45a
2.5kg / 7 day	1.096a	79.54a	25.92	4.12a	4.56ab
3kg/4 day	1.102a	78.70a	25.02	4.44ab	4.50a
2.5kg / 4 day	1.122b	79.82ab	26.38	4.30a	4.30a

^{*} Numbers followed by different letters in each column differ significantly by Fisher's least significance test (P < 0.05).

If there were no significant differences what are all the a's, ab's?

Prevalence of A. macrospora and A.alternata

A. macrospora was more prevalent at the beginning, whereas A. alternata was more prevalent towards end of the growing season (Fig 12).

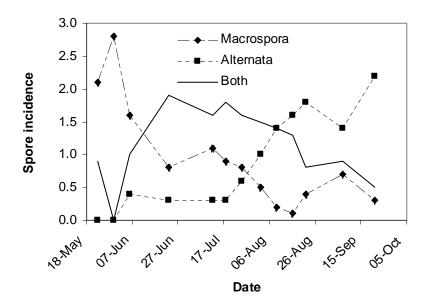


Fig 12. Prevalence of A. macrospora and A. alternata for cotton grown at KRS in 2005

Survival of Alternaria leaf blight on cotton leaves at various condition in Katherine

Alternaria sp sporulation on buried green cotton leaves was zero after two weeks, whereas, it occurred on 40% of buried dry leaves, on 78% of green leaves on soil surface and on 95% of dry leaves on soil surface with plant debris. Survival of Alternaria sp. was approximately 22% on plant debris after three and a half months from October to February. Survival was more than six months (80%) on dry leaves maintained in paper bags in laboratory.

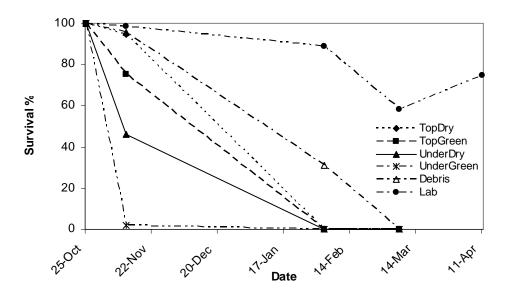


Fig 13. Survival of Alternaria spp. under different conditions at KRS in 2005.

Outcomes

- This study shows that application of mancozeb reduced disease incidence, severity and to some extent leaf shedding. Significant reduction of disease severity was also observed on fruits after spraying with mancozeb.
- Highest disease expression was observed at the bottom canopy level followed by middle, and the top canopy level was least affected..
- No differences in yield and fibre quality on fungicide treated and untreated plots were observed.
- Among the pathogens of Alternaria leaf blight, *A. macrospora* prevailed earlier in the growing season, while *A. alternata* prevailed towards end of the growing season. Both pathogens also found to occur in same lesion.
- Pathogens of Alternaria leaf blight can survive up to three and a half months with plant debris in the field and more than six months in the laboratory at about 22°C.

Conclusion

The results from this research demonstrated that manconzeb can significantly reduce the incidence and severity of Alternaria leaf blight Its effectiveness on cotton yield was not conclusive. More work for several years at various geographical locations need to be done to determine the factors affecting incidence and its effect on yield.

This was the first study to determine the prevalence of two *Alternaria* species during the cotton growing season should provide great benefits to the understanding of the epidemiology of Alternaria leaf spot and the future development of a management strategy.

Reference

Baird RE (2001) Alternaria leaf spot. In 'Compendium of cotton diseases (2nd Edition)' (Eds TL Kirkpatrick, CS Rothrock) pp. 20-21. (American Phytopathological Society, St Paul, Minnesota, USA)

Bashan Y, Hernandez-Saavedra NY (1992) Alternaria-blight of cotton: epidemiology and transmission. In 'Alternaria biology, plant diseases and metabolites. Topics in secondary metabolism'. Vol 3 (Eds J Chelkowski and A Visconti), pp. 233-266. (Elsevier Scientific Publishers, Amsterdam, the Netherlands)

- Bashan Y, Levanony H, Or R (1991) Association between *Alternaria macrospora* and *Alternaria alternata*, causal agents of cotton leaf blight. *Canadian Journal of Botany* **69**, 2603-2607.
- Bashi E, Sachs Y, Rotem J (1983) Relationship between disease and yield in cotton fields affected by *Alternaria macrospora*. *Phytoparasitica* **11**, 89-97.
- Bellgard S (2001) Northern Cotton Disease Survey. Australian Cotton Cooperative Research
 Centre
- Colson-Hanks ES, Allen SJ, Deverall BJ (2000) Effects of 2,6-dichloroisonicotinic acid or benzothiadiazole on Alternaria leaf spot, bacterial blight and Verticillium wilt in cotton under field conditions. *Australasian Plant Pathology* **29**, 170-177.
- Ellis MB, (1971) Dematiaceous Hyphomycetes. . pp 464-466, 495-496. (CAB International)
- Kruskal W.H, Wallis, W.A. (1952). Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association. 47: 583-621.
- Nehl D, Allen S (2002) Cotton Diseases: Threats and emerging threats. <u>http://cotton.pi.csiro.au/Publicat/conf/coconf02/soilhelt/087/087.htm</u>
- Nehl D, Kochman J, Bellgard S, Allen S (2000) Northern Australian cotton disease survey.

 Australian Cotton Cooperative Research Centre
- Padaganur GM, Hiremath RV, Basavaraj MK (1989) Estimation of yield loss due to Alternaria leaf spot and blight in cotton. *Journal of the Indian Society for Cotton Improvement* **14**, 144-145.
- Rotem J, Eidt J, Wendt U, Kranz J (1988) Relative effects of *Alternaria alternata* and *A. macrospora* on cotton crops. *Plant Pathology* **37**, 16-19.
- Shtienberg D, Dreishpoun J (1991) Suppression of Alternaria leaf spot in Pima cotton by systemic fungicides. *Crop Protection* **10**, 381-385.
- Siegel S., Castellan N.J. (1988). Nonparametric statistics for the behavioural sciences (2nd ed.) New York: McGraw-Hill.
- Simmons E.G. 2003. Alternaria themes and variations (310-335) species on Malvaceae. *Mycotaxon* **88**, 163-217.

Part 4 – Final Report Executive Summary

Alternaria leaf blight is the most prevalent disease of cotton in northern Australia. A trial was conducted at Katherine Research Station to determine i) the efficacy of the protectant fungicide mancozeb, ii) the effects of disease on yield and quality of cotton fibre, iii) the prevalence of Alternaria pathogen during the cotton growing season, iv) the survival of the pathogen under various conditions.

The protectant fungicide, mancozeb, significantly reduced disease incidence, severity and leaf shedding. It also reduced disease severity on cotton bolls. No significant effects of Alternaria leaf blight was observed on the yield and quality of cotton fibre.

Significantly higher disease incidence, severity and defoliation were observed in the lower canopy (1-7 nodes) level irrespective of fungicide treatment. The middle canopy (8-14 nodes) had the next highest incidence, severity and defoliation. The least disease and defoliation was observed at top canopy level (15+ nodes).

Alternaria macrospora was the most prevalent pathogen during the early growing season and diminished towards the end of the growing season. Although A. alternata did not occur at the beginning of the growing season, this pathogen was more prevalent towards end of the growing season. Both A. macrospora and A. alternata can colonise the same leaf and occur in the same lesion.

The pathogens of Alternaria leaf blight can survive up to three and a half months on plant debris in the field and more than six months in the laboratory at 22°C.