Cotton ***Research and Development Corporation***

FINAL POST DOCTORAL PROJECT REPORT

CODE: US37C

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Proposed actual	Anticipated	
Actual start date: 1.7.97	Actual completion date: 31.6.99	Date of receipt: ....

Project title: Induced resistance to cotton diseases as part of integrated pest and disease management

CRDC Project Code **US37C**

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Materials and methods

Plants and their growth

For challenge inoculation with *A. macrospora*, seeds of *G. hirsutum* cv. Siokra 1-4, supplied by Dr. S. J. Allen, Australian Cotton Research Institute, Narrabri, NSW, Australia, were sown in 150 mm diameter pots containing a 1 : 1 sand : peat mixture, with fertiliser incorporated on potting. Following emergence, seedlings were thinned to two or three per pot. Plants were grown in a glasshouse with successive 12 h diurnal periods at 18°C and 30°C.

For ß-1,3-glucanase assays, seeds were sown in 100 mm diameter pots containing potting mix (Hortico Aust. Pty Ltd., NSW, Australia) and seedlings were thinned to five per pot following emergence. Seedlings were grown in a growth cabinet under 16 h periods from 5 x 1000 watt Tungsram® lamps and 8 x 60 watt Airm® long life incandescent bulbs with a photosynthetically active radiation of 575 ± 75 µM / m^2 / sec at 32°C followed by 8 h dark periods at 25°C.

Aquasol® was applied weekly to all pots at 1 g / L.

Pathogens and their culture

A. macrospora isolated from a leaf spot on Siokra cotton at Anderson, Queensland, in 1994 by Dr. J. F. Kochman, Queensland Department of Primary Industry, Toowoomba, Australia, was sub-cultured routinely on V8 juice agar in 250 mL conical flasks.

Verticillium dahliae, isolate 1013, was obtained from Dr. B. Lyon in the School of Biological Sciences, The University of Sydney. The isolate was originally collected from cotton at Boggabilla, New South Wales in 1994. The isolate was sub-cultured routinely onto PDA in glass Petri dishes.

Fusarium oxysporum f.sp. *vasinfectum* was isolated from S189 cotton at Cecil Plains, QLD in 1996. The isolate was stored on dried filter paper following inoculation of sterile filter paper in Petri dishes with plugs of inoculum from a single spore culture. Cultures were regrown by placing filter paper cultures onto PDA.

All cultures were grown under a 12 h photoperiod from cool-white fluorescent and NEC 'black light' fluorescent tubes at 20 - 24°C.

Inoculum was prepared from 10- to 14-day-old cultures by flooding with sterile deionised water and scraping the cultures with a sterile inoculating loop.

The *A. macrospora* spore suspension was filtered through cheesecloth before three repeated centrifugations at 600 **g** for 5 min with successive resuspensions in changes of deionised water. Concentrations were adjusted to 5 x 10^5 spores / mL. The *V. dahliae* mycelial and conidial suspension was adjusted to 1 x 10^7 microconidia / mL. The *F. oxysporum* f.sp. *vasinfectum* spore suspension was filtered through cheesecloth and concentration adjusted to 5 x 10^5 spores / mL.

The *A. macrospora* spore suspension was applied with a fine camel hair brush to both sides of the leaves being challenged. Following inoculation, the plants were placed in a humid chamber at 25 - 27°C for 24 h. The timing of inoculation and the actual leaves inoculated varied between experiments as stated. Four to six days after inoculation, the numbers of lesions formed on the upperside of the challenged leaves were counted.

After transfer to the 18-22°C glasshouse, plants were inoculated with *V. dahliae*. The soil was gently removed from around the roots of each plant and using a syringe (without needle), 20 mL of the acquired resistance could be used as part of an integrated disease management program to decrease disease symptoms and severity during the growing season.

Project Objectives

To investigate the SAR response in cotton and to evaluate a range of biotic and abiotic stimuli under both glasshouse and field conditions against *Verticillium* and *Fusarium* wilts and *Alternaria* leaf spot. Glasshouse trials will be used to determine effectiveness, application rates and duration of protection. A series of field trials based on the glasshouse results will be conducted for all three diseases. These are likely to be the *Verticillium* field trial in the *Verticillium* wilt nursery at the Cotton Research Institute, Myall Vale, NSW, *Alternaria* at Bourke, NSW, and *Fusarium* trial sites to be chosen in consultation with the industry.

Objectives achieved in each year of the grant

Year 1 Glasshouse trials assisting in the definition of the best of several activating stimuli for providing protection against all three pathogens. Carry out first season of field trials.

A range of stimuli were examined under glasshouse conditions. Field experiments were conducted at Bourke and Narrabri against Alternaria leaf spot and Verticillium wilt respectively testing the efficacy of BTH and silicic acid in inducing resistance to the leaf spot and wilt pathogen. A small field experiment examining the effect of BTH treatment against Fusarium wilt was conducted at Cecil Plains.

Year 2 Continue glasshouse trials. Use biochemical assays to determine the range and amount of PR-proteins and thus resistance afforded by various stimuli. Conduct second season of field trials. Develop methods to implement SAR as part of an integrated pest management scheme.

Glasshouse experiments continued, examining a number of agents and the applications required to afford the greatest protection against Alternaria leaf spot and Verticillium and Fusarium wilts. Biochemical assays were used to examine the amount of the PR-protein, ß-1,3-glucanase in various parts of cotton plants following treatment with a range of stimuli.

A second season of field experiments were conducted with the application of 3 BTH or silicic acid treatments to plants in the Verticillium experiment at Narrabri and 3 applications of BTH to plants in the Fusarium experiment at Cecil Plains. An Alternaria field experiment was not conducted due to a lack of Pima cotton in the Bourke region following severe losses the previous season after outbreaks of Alternaria and bacterial blight.

References

Allen, S.J. (1994). Proceedings of the 7th Australian Cotton Conference, Broadbeach, Queensland, pp. 247 - 252.

Allen, S.J. (1996). Proceedings of the 8th Australian Cotton Conference, Broadbeach, Queensland, pp. 645 - 649.

Colson, E.S. (1997). Systemic induced resistance in cotton. PhD thesis, The University of Sydney.

Kessmann, H., Staub, T., Hofmann, C., Maetzke, T. and Herzog, J. (1994a). *Annual. Reviews Phytopathology* **32,** 439 - 459.

Kessmann, H., Staub, T., Lignon, J., Oostendorp, M. and Ryals, J. (1994b). *European Journal of Plant Pathology* **100,** 359 - 369.

Kochman, J.K., Pegg, K.G., Davis, R.D., Moore, N.Y. and Bentley, S. (1994). Proceedings of the 7th Australian Cotton Conference, Broadbeach, Queensland, pp 265 - 270.

Kochman, J.K. (1995). *Australasian Plant Pathology* **24,** 74.

Lyon, G.D., Reglinski, T. and Newton, A.C. (1995). *Plant Pathology* **44,** 407 - 427.

Mauch, F., Mauch-Mani, B. and Boller, T. (1988). *Plant Physiology* **88**, 936 - 942.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wierhold, D.L., Alexander, D.C., Ahl-Goy, P., Métraux, J.P. and Ryals, J.A. (1991). *The Plant Cell* **3,** 1085 - 1094.

Background

Enhancing plants' natural defence systems to provide a broad spectrum resistance against a range of fungal and bacterial pathogens is possible through a process known as Systemic Acquired Resistance (SAR). Limited prior exposure of the plant to pathogens, attenuated forms of pathogens or some chemicals are required as part of the induction process of SAR.

SAR has been effectively demonstrated in many crop species including cucurbits, legumes and cereals (for reviews see Lyon *et al.*, 1995; Kessmann *et al.*, 1994a; Kessmann *et al.*, 1994b) using a wide range of activating stimuli and against many different pathogens. Trials conducted under both glasshouse and field conditions with cotton have shown effective induction of systemic resistance against a vascular wilt and a foliar pathogen (Colson *et al.*, 1997).

In cotton we had found that localised infections on leaves and application of 2,6-dichloroisonicotinic acid (INA) and components of formulation materials to leaves activated SAR under glasshouse and field conditions. These results clearly showed the sensitivity and responsiveness of cotton to activating stimuli but left uncertain the nature of the most effective activating agent to be used in crop protection.

Industry significance/impact

Repeated growth of monocultural crops like cotton causes a build up of disease inoculum in the field, resulting in disease outbreaks. Three of the major disease problems in Australian cotton at present are : *Verticillium* wilt, *Fusarium* wilt and *Alternaria* leaf spot, *Fusarium* wilt (Kochman *et al.*, 1994) being a relatively new and potentially serious problem for cotton growers.

Verticillium wilt results in extensive losses in production every season throughout the cotton growing regions of Australia. Conditions in the 1995/96 season were extremely conducive to disease development, with areas of NSW recording higher incidences of the disease than recorded previously (Allen, 1996). This root-infecting, vascular-inhabiting pathogen can currently only be controlled by crop rotation, residue management (Allen, 1994) and some cultivar resistance.

The discovery of *Fusarium* wilt in cotton in Australia in early 1993 (Kochman, 1995) is of major concern to cotton growers and scientists alike. The width of its distribution in the cotton-growing regions of Australia is unknown, and the potential for it to cause major losses in production is high. Residue management, crop rotations and the use of sanitation practices appear to be the only current methods used in an attempt to control and stop the spread of the pathogen.

The leaf spot pathogen, *Alternaria,* is a severe problem in some areas of Australia following continuous wet conditions. It causes premature defoliation of plants, reducing boll set and therefore yield. Incidence of *Alternaria* leaf spot in the commercial cotton cropping areas of New South Wales is increasing (Allen, 1994). In Bourke, New South Wales, there were major outbreaks of the disease in Pima cotton crops resulting in severe defoliation of plants in the 1994/95 season. There were also disease outbreaks in fields of the more resistant *G. hirsutum* cotton in Moree, New South Wales during the season, following conducive weather conditions.

Verticillium, Fusarium and *Alternaria* are three of the most economically important cotton diseases in Australia at present, with the potential to devastate entire fields. The only control measures currently available to growers include crop rotations and management of infested crop residues. Systemic

V. dahliae suspension was applied to the rootball area of each plant. The soil was replaced and the plant watered lightly. Four to six weeks after inoculation with *V. dahliae*, the following disease severity score was used to assess the glasshouse plants.

0	=	healthy plant with no foliar symptoms
1	=	1 - 2 leaves with mottling, chlorosis or necrosis.
2	=	3 - 4 leaves with foliar symptoms.
3	=	5 - 6 leaves with foliar symptoms.
4	=	> 6 leaves with foliar symptoms or plant dead.

The soil was gently removed from around the roots of each plant being inoculated with *F. oxysporum* f.sp. *vasinfectum* and using a syringe (without needle), 20 mL of the suspension was applied to the rootball area of each plant. The soil was replaced and the plant watered lightly. Four weeks after inoculation with *F. oxysporum* f.sp. *vasinfectum* the disease severity score above was used to assess the glasshouse plants.

The length of the hypocotyls was measured 4 days after application of the seed treatment in the seed coating experiments.

Chemicals and their preparation

INA (CGA 41396) was supplied by Dr. H. Kessmann, Ciba-Geigy AG, (now Novartis AG), Basle, Switzerland, as WP25 comprising 25 g active ingredient (a.i.) in 75 g wettable powder (WP) formulation material. The composition of WP25 was disclosed as 5 g silicic acid K320, 60 g kaolin (inert), 5 g Ultravon W300 (a wetting agent), 5 g Attisol II (a detergent) and 25 g INA. Dr. H. Kessmann supplied Ultravon W300, Attisol II and pure INA. Kaolin (~$Al_2Si_2O_5(OH)_4$)) and silicic acid (as approximately H_2SiO_3, <20 micron) were obtained from Aldrich Chemical Company Inc., Milwaukee, USA. BTH (CGA245704, benzothiadiazole) was supplied by Dr. M. Oostendorp, Novartis AG, Basle, Switzerland, as WG50 comprising 50 g a.i. in 50 g of wettable granule (WG) formulation material.

Suspensions of each of the WP components were prepared in deionised water. Pure INA was insoluble in water and was dissolved in 0.2 mL of ethanol and quickly diluted with 400 mL of deionised water to make a 20 ppm solution, using a technique to dissolve a crystalline compound of similar molecular weight and structure that was water-insoluble (Daly & Deverall, 1963). A control solution of 0.2 mL ethanol in 400 mL deionised water was also used.

For routine use, aqueous solutions of BTH at 0.07 g / L were prepared, thus yielding a 35 ppm concentration (a.i.), and WG at 0.035 g / L.

Suspensions or solutions were applied to the cotyledons or first leaves to run-off using a hand-held atomiser or a fine camel hair brush. The application method and the leaves treated varied between experiments as stated.

The timing of chemical application and the actual leaves treated varied between experiments as stated.

For seed coating experiments 16 Siokra 1-4 seeds were coated with 0.2 or 0.5 g of BTH respectively before being placed on moist filter paper in Petri dishes and incubated at 24°C in the dark.

Procedures for measuring ß-1,3-glucanase activities

The cotyledons of 14- to 17-day-old plants were treated in all experiments. At varying times after treatment, tissues were harvested and the fresh weight (FW) recorded, with mass (approx. 3 g) and number of leaves or stem segments (usually five to ten) in each sample varying slightly between treatments and experiments. In each experiment there were three replicate samples for each treatment.

In Experiments 1a and 1b, the first and second leaves, respectively, were harvested 120 h after application of the cotyledonary treatments. Stem segments between the cotyledons and first true leaves were harvested 72 h after treatment of the cotyledons in experiment 2. For experiments 3, 4 and 5, the first leaves were harvested 72 h after the cotyledonary treatments. In experiment 6, the first and second leaves were harvested 7 days after cotyledonary treatment application. Samples were frozen at -80°C in individual plastic bags.

Frozen plant material was ground using a mortar and pestle on ice at the rate of 1 g FW, 1 g sand / 5 mL of 50 mM potassium acetate buffer at pH 5.0 containing 7.5 mM polyethylene glycol 8000 (Sigma Chemical Co., St. Louis, USA) and 5 mM reduced glutathione.

Extracts were centrifuged at 12,500 **g** for 15 min at 4°C and the supernatants were collected with a Pasteur pipette and frozen at -10°C for subsequent assays and total protein determinations. Samples (2.5 mL) were filtered through pre-packed PD10 Sephadex G-25 M columns (Pharmacia Biotech AB, Sweden) using 50 mM potassium acetate buffer for equilibration and elution.

Total protein for each extract was determined using the Bio-Rad Protein Microassay (Bio-Rad Laboratories Pty. Ltd., North Ryde, NSW, Australia), based on the method of Bradford (1976), with bovine serum albumin (BSA) as the standard.

The ß-1,3-glucanase assay involving release of a measurable dye from the substrate azurine-cross linked pachyman (from Megazyme Pty. Ltd., Warriewood, NSW, Australia) was as described by Dann *et al.* (1996), except that the reaction was stopped after 10 min at 30°C. Activities were expressed as milliunits per g FW and milliunits per mg protein, a milliunit being the amount of enzyme releasing one nanomole of reducing sugar equivalents per minute.

Field experiment materials and methods

Six field experiments were carried out in commercial cotton crops over two seasons. Details of the experimental site locations, treatment applications and sowing, treatment and assessment dates are summarised in Table 1.

Prior to sowing, seeds were treated with the fungicides pentachloronitrobenzene (Terraclor®) and metalaxyl (Apron®) for seedling disease control, and with a blue polymer dye (Peridiam®) and the insecticide thiodicarb (Semevin®). The field experiments were managed using standard commercial practices including pesticide applications and flood irrigation as necessary.

All sites were naturally infested with *A. macrospora, V. dahliae* or *F. oxysporum* f.sp. *vasinfectum.*

1. Application of chemicals

BTH was supplied by Dr. M. Oostendorp, Novartis AG, Basel, Switzerland, as a 50% a.i. wettable granule formulation. Aqueous solutions of BTH were prepared 0.05 g a.i. / L. Treatments were applied to the entire foliage of treated plants using a knapsack sprayer. Control plots remained untreated.

Treatments were applied in each experiment following early expression of foliar *V. dahliae, F. oxysporum* f.sp. *vasinfectum* or *A. macrospora* symptoms in the field, except experiments in 6 and 7 when the first treatment was applied prior to the development of any foliar symptoms in plants.

2. Assessment

Alternaria experiments

Mainstem leaves harvested from six randomly selected plants within each plot were assessed by comparison with a standard pictorial key developed using squared graph paper for percentage leaf area infected by *A. macrospora.* In experiment 3a, the percentage leaf area infected by *X. campestris* pv. *malvacearum* was also assessed using the pictorial key.

The degree of defoliation was determined by counting the number of mainstem leaves remaining on the six randomly selected plants within each plot.

Verticillium experiments

Ten plants per plot were randomly selected and the disease severity assessed visually. Each plant was scored for foliar symptoms on a 0 to 4 scale. In experiment 6, due to poor disease development, the scale was 0 = no symptoms, 1 = 1-3 leaves showing symptoms (chlorosis or necrosis), 2 = 4-6 leaves showing symptoms, 3 = 7-10 leaves showing symptoms and 4 = > 10 leaves showing symptoms. In experiment 7, 0 = no symptoms, 1 = 1/4 of the leaves showing symptoms (chlorosis or necrosis), 2 = 1/2 of the leaves showing symptoms, 3 = 3/4 of the leaves showing symptoms and 4 = all leaves showing symptoms or plant dead.

Fusarium experiments

The height of ten plants per plot were measured and recorded. Plants were examined for vascular discolouration by cutting the stems.

Statistical analyses

Analyses of variance were performed and standard errors calculated for all the data obtained using the software package Minitab®. Values obtained from the analyses of variance were used in a Tukey's least square difference test (Steel and Torrie, 1960) to determine significant differences.

Table 1. Field experiment locations, cultivars, treatments and dates of planting, treatments and assessment.

Experiment no.[A]	3	3a	6	7	8	9
Season	97/98	97/98	97/98	98/99	97/98	98/99
Trial location	Darling Farms, Bourke, NSW	Darling Farms, Bourke, NSW	ACRI[B], Narrabri, NSW	ACRI, Narrabri, NSW	G.Clapham's, Cecil Plains, QLD	G.Clapham's, Cecil Plains, QLD
Planting date	11/10/97	11/10/97	13/10/97	29/10/98	19/10/97	29/10/98
Cultivar[C]	Pima S7	Pima S7	Siokra 1-4	Siokra 1-4	S189	S189
Pathogen	*A. macrospora*	*X. campestris* pv. *malvacearum*	*V. dahliae*	*V. dahliae*	*F. oxysporum* f.sp. *vasinfectum*	*F. oxysporum* f.sp. *vasinfectum*
Replicates[D]	5	5	7	3	6	6
Treatments	BTH, untreated	BTH, untreated	BTH, untreated	BTH, untreated	BTH, untreated	BTH, untreated
Treatment date(s)	12/1/98	12/1/98	27/11/97 15/1/98	1/12/98 14/1/99 25/2/99	26/11/97 13/1/99	16/11/98 21/12/98 22/2/99
Assessment date	16/3/98	16/3/98	10/3/98	30/3/99	16/3/99	21/4/99
No. of plants sampled / treatment	30	30	70	90	60	60

[A] Experiments 1, 2, 4 and 5 were conducted during PhD research so are not included in the above table.

[B] ACRI = Australian Cotton Research Institute, Narrabri, NSW.

[C] *G. hirsutum* cvs. Siokra 1-4, CS50 or *G. barbadense* cv. Pima S7.

[D] All plots measured 1 row x 3 m, except in the Fusarium experiments where they measured 1 row x 2 m and the 98/99 Verticillium experiment where they measured 1 row x 10 m.

Glasshouse Results

Effects of treatments on disease development

Cotton seedlings treated on the cotyledons with formulated INA developed significantly ($P \leq 0.05$) fewer lesions than water-treated seedlings following challenge inoculation with *A. macrospora* on the first or second leaves (Table 2). Disease development was less at concentrations providing 5 - 25 µg than 2.5 µg of INA / mL. Seedlings treated with formulation material (WP) without INA also had significantly ($P \leq 0.05$) fewer lesions on the first or second leaves compared with water-treated seedlings. Formulation material without INA at 15 and 37.5 µg WP / mL allowed significantly ($P \leq 0.05$) more lesions to develop than the corresponding concentrations of formulation material with INA that provided 5 and 12.5 µg INA / mL respectively.

A sample of WP used in these experiments was sent to Dr. H. Kessmann (Ciba-Geigy Ltd., Basle, Switzerland) to test for the possible presence of INA as a contaminant. No INA was detected in the sample, therefore, WP was confirmed as being active in the absence of INA.

Cotyledonary treatment with formulated BTH resulted in significantly ($P \leq 0.05$) reduced lesion formation on the first or second leaves compared with the water-treated controls (Table 3). No reductions in lesion numbers were observed on the first and second leaves of plants following application of WG formulation material without BTH to cotyledons relative to water-treated controls (data not shown).

Systemic effects of formulated INA and its components and of BTH on ß-1,3-glucanase activities

ß-1,3-Glucanase activities in the first and second leaves of plants treated 120 h earlier on the cotyledons with formulated INA were significantly ($P \leq 0.05$) higher than in water-treated controls (Table 4). Plants treated with formulation material (WP) without INA also had significantly ($P \leq 0.05$) higher ß-1,3-glucanase activities in the first leaves, though not the second leaves, 120 h after treatment of the cotyledons compared with water-treated controls. The highest ß-1,3-glucanase activities were observed in the first and second leaves of BTH-treated plants, the levels being significantly ($P \leq 0.05$) higher than in the water-treated plants (Table 4).

Significantly ($P \leq 0.05$) higher ß-1,3-glucanase activities were found in the stem tissue of plants treated on the cotyledons 72 h earlier with formulated INA or BTH compared with water-treated controls (Table 5).

Individual components of formulated INA were applied singly by brush or spray to cotyledons and ß-1,3-glucanase activities were measured in the first leaves 72 h later. Results were variable between experiments but at least one of the components caused several-fold increases in ß-1,3-glucanase activity in each experiment. Table 6 shows the results of three such experiments, in which Attisol, silicic acid, INA, formulated INA and brush-applied kaolin significantly ($P \leq 0.05$) elevated ß-1,3-glucanase activities in the first leaves relative to the water-treated controls.

Systemic effects of *A. macrospora* inoculation on ß-1,3-glucanase activities

Seven days after cotyledonary inoculation with an *A. macrospora* spore suspension significantly ($P \leq 0.05$) higher ß-1,3-glucanase activities were observed in the first or second leaves compared with water-treated control plants (Table 7).

Effect of some individual components of formulation material with INA on disease development

The effects of five individual components of formulated INA on subsequent Alternaria disease development were compared with controls in separate experiments. The experiments were each repeated three times, and data from a representative set of experiments are presented in Table 8. Each component resulted in significantly ($P \leq 0.05$) fewer lesions on first leaves of treated plants relative to control plants. All components except Ultravon significantly ($P \leq 0.05$) reduced lesion formation on the second leaves compared with the control plants.

Field experiment results

Alternaria experiments

During the 97/98 season, a severe outbreak of Alternaria leaf spot and bacterial blight occurred in Pima cotton at Bourke, NSW (experiments 3 and 3a). Symptoms on the leaves and bolls included typical *A. macrospora* lesions ranging from isolated lesions on the leaves and bolls to coalescence of lesions and complete necrosis of leaves as well as water-soaked lesions and water-soaking along the veins due to *X. campestris* pv. *malvacearum* infection of the plants. Plants were assessed for *A. macrospora* symptoms (experiment 3) and *X. campestris* pv. *malvacearum* symptoms (experiment 3a) separately.

The mean percentage leaf areas infected with *A. macrospora* or *X. campestris* pv. *malvacearum* in experiments 3 and 3a were significantly ($P \leq 0.05$) lower in the BTH-treated plants or the silicic acid treated plants compared with the untreated plants (Figures 1 and 2). Defoliation of the BTH-treated plants was significantly ($P \leq 0.05$) reduced in treated plants compared with the untreated plants (Table 9). BTH treated plants also had significantly less defoliation than the silicic acid treated plants (Table 9).

Verticillium experiments

Hot and dry conditions in December 1997 and January 1998 of the 97/98 season (experiment 6) resulted in very little disease development in the experiment. Symptoms in the *Verticillium*-susceptible cultivar Siokra 1-4 consisted mainly of mild interveinal chlorosis.

Disease severity was significantly ($P \leq 0.05$) lower in BTH-treated plants compared with untreated plants (Figures 3 and 4) in experiment 6. Silicic acid treated plants had significantly ($P \leq 0.05$) less foliar symptoms than untreated plants in the *Verticillium*-susceptible cultivar Siokra 1-4 (Figure 3) but bot in the *Verticillium*-tolerant cultivar Sicala V2.

Favourable conditions for disease development in the 98/99 season resulted in severe foliar symptoms in untreated plants in experiment 7. Symptoms ranged from substantial interveinal chlorosis to plant death in the untreated plants, with significantly ($P \leq 0.05$) less severe foliar symptoms observed in the BTH-treated and silicic acid-treated plants compared with the untreated plants(Figures 5 and 6).

Fusarium experiments

Favourable conditions for disease development in the 97/98 and 98/99 seasons resulted in severe symptoms of Fusarium wilt in the field experiments. Symptoms ranged from stunting and vascular discolouration to plant death. Significantly ($P \leq 0.05$) less stunting was observed in the BTH-treated plants compared with the untreated plants(Figures 7 and 8) compared with untreated plants. In the 97/98 season disease incidence (based on observations of vascular discolouration following stem cutting) was 25% in the treated plants compared with 35% in the untreated plants. In the 98/99 season the treated plants had a substantially lower incidence of disease (70%) compared with the untreated plants (88%).

Discussion

INA is interpreted to act by moving rapidly into and systemically through plants, as shown by Métraux *et al.* (1991) using a radioactive form. The subsequent increases in the PR protein, chitinase, and in levels of resistance in parts of the plant remote from the point of application were concluded to be consequences of the accumulation of INA in these parts. This conclusion is supported here by the action of INA alone in raising activities of the PR protein, ß-1,3-glucanase, and resistance to leaf spot formation in distal parts of cotton seedlings when contrasted with the effects of a control solution of solvents. In cotton seedlings, however, each of the other components of the formulation material tested was also able to raise activities of the enzyme and resistance in the parts distal to the point of application. Some of these components may be able to move systemically in cotton. Although this was not tested, it seems most unlikely that all can do so.

Some of the active components may bring about systemic responses by activating the natural signalling processes in cotton. These processes are activated in and around local lesions in many plants (Hammerschmidt & Kuć, 1995). The activating step normally involves localised cellular disruption in the lesions and consequent metabolic perturbation. The step results in the systemic movement of signals from the local lesions. Signals, for example in tobacco, may include salicylic acid (Shulaev *et al.*, 1995), but there is controversy about their chemical nature (Vernooij *et al.*, 1994). The detergent Attisol II and the wetting agent Ultravon W300 may cause chemical damage to cuticles and epidermal cells, and silicic acid and kaolin, especially where the latter was brush-applied, may cause minor physical damage at points of application in the present work, thereby activating signalling in cotton.

Silicates have previously been reported to decrease susceptibility of plants to foliar and root pathogens (Samuels *et al.*, 1991; Chérif *et al.*, 1994) after water-soluble potassium silicate was supplied to roots. Silicon was shown to accumulate in bases of trichomes on leaves in uninfected plants and in the epidermis around sites of powdery mildew infection, implying mobility of silicon from roots (Samuels *et al.*, 1991). Supply of potassium silicate to roots was also deduced to accelerate apparent defence-related metabolic changes at infection sites in both leaves and roots (Menzies *et al.*, 1991; Chérif *et al.*, 1994). Movement of silicon in order to bring about such changes seems impossible in the present work with cotton, unless solubilisation of normally water-insoluble silicic acid and kaolin (hydrated aluminium silicate) occurred on the surfaces of treated cotyledons and leaves.

Treatment with formulated BTH has been shown to result in decreased disease in a range of plant species including tobacco (Friedrich *et al.*, 1996), green bean (Siegrist *et al.*, 1997), soybean (Daun *et al.*, 1998) and cucumber (Benhamou and Bélanger, 1998), but it has not previously been reported to act in cotton. Accumulation of PR proteins in distal tissues has been reported in *Arabidopsis* (Lawton *et al.*,

1996) and tobacco (Friedrich *et al.*, 1996) following BTH application. Siegrist *et al.* (1997) observed a three-fold increase in ß-1,3-glucanase activity in the trifoliate leaves of green bean plants 8 days after BTH application to the primary leaves. The decreased formation of lesions and the enhanced ß-1,3-glucanase activities observed in distal leaves following BTH treatment of cotton plants in the current study support the above reports and strongly suggests that systemic resistance was induced by the application of BTH.

The elevated activities of the PR protein, ß-1,3-glucanase in the first or second leaves of cotton following INA, BTH or *A. macrospora* treatment of the cotyledons noted in the current work substantiate the claim by Brock *et al.* (1994) that systemic resistance can be induced in cotton by treatment with INA or *A. macrospora.*

Many other PR proteins are likely also to have been induced to form in cotton leaves at least by INA, which induced for example the formation of nine PR protein families in tobacco (Ward *et al.*, 1991). Some of these proteins have *in vitro* activity against fungal pathogens (Mauch *et al.*, 1988b; Woloshuk *et al.*, 1991; Niderman *et al.*, 1995). Decreased susceptibility of cotton leaves to *A. macrospora* is likely to have been caused finally by the action of these and other products (Uknes *et al.*, 1992; Wasternack *et al.*, 1994; Van Kan *et al.*, 1995; Feussner *et al.*, 1997; Schweizer *et al.*, 1997) of the process of systemic induction.

Activator application to the foliage of cotton plants in the field had no observable effect on the development of the plants, except for a decrease in the susceptibility of leaves to Alternaria leaf spot and bacterial blight and a decrease in the development of *V. dahliae* and *F.oxysporum* f.sp. *vasinfectum* infection in plants. The common decrease in susceptibility to the pathogens is interpreted as an induction of systemic and partial resistance to *A. macrospora, X. campestris* pv. *malvacearum. V. dahliae* and *F. oxysporum* f.sp. *vasinfectum* in cotton by BTH or silicic acid applications as reported for biological agents and chemical activators with other pathogens and plants (Kuć, 1980; Kessmann *et al.*, 1994).

Cotton appears to have an arsenal of resistance mechanisms that are initiated following infection. These mechanisms include lignification, vessel occlusion, vessel wall coatings, PR-proteins and phytoalexin synthesis (Bell, 1969; Bell and Presley, 1969a; Shi *et al.*, 1992; Durbery and Slater, 1997; Smit and Durbery, 1997). It is unclear which of these mechanisms would be activated in cotton following induction of systemic resistance with synthetic activators. However, in some plant species a number of these mechanisms, including lignification (Hammerschmidt and Kuć, 1982), PR-proteins (Redolfi, 1983; Ward *et al.*, 1991) and phytoalexin synthesis (Schnathorst and Mathre, 1966) have been associated with SAR.

The durability and effectiveness of SAR against leaf infections under conditions conducive to *A. macrospora* and also to *X. campestris* pv. *malvacearum* were clearly demonstrated during the 97/98 cotton-growing season. BTH was applied, and resistance induced, following the initial outbreaks. The protection provided was, therefore, most probably against secondary infections. The percentage leaf area showing lesions was substantially lower on the treated plants, most likely due to a combination of reduced successful infections and delayed symptom expression, all characteristics of SAR (Dean and Kuć, 1985). Disease pressure during the experiments was extreme as the plants were mature and entering the post-flowering stage when the plant is highly susceptible to *A. macrospora* infection

(Shtienberg *et al.*, 1995) and the closure of the canopy had created an ideal microclimate for disease development.

Premature defoliation, a major symptom of Alternaria leaf spot in cotton (Shtienberg, 1991), was also significantly lower in the treated plants, compared with the untreated plants. The observed reduction in defoliation is important as the premature shedding of leaves decreases the photosynthetic area of the plant, the primary cause of yield losses in cotton crops severely affected by Alternaria leaf spot or bacterial blight.

In the 97/98 season (experiment 6), BTH was applied twice to treated plants, the first, prior to any foliar symptom expression and the second, seven weeks later. Disease severity was extremely low in the 97/98 season making it difficult to determine whether application of the activator at this earlier stage with a booster application, resulted in a more substantial decrease in disease severity than the single treatment applications later in the outbreak that occurred in previous experiments (Colson, 1997). However, three applications of BTH to treated plants in the 98/99 season (experiment 7), with the first treatment applied prior to foliar symptoms, resulted in significant reductions in disease severity in treated plants compared with untreated plants even though the disease outbreak was severe. Therefore, application of the activator prior to foliar symptom expression and with booster applications appears to decrease disease severity even when severe disease development occurs in the field. Complete protection against infection by *V. dahliae* is unlikely to be achieved, as even genetic resistance of plants does not prevent initial infection of the vascular system (Bell, 1992).

The reduced susceptibility of plants to Alternaria leaf spot, bacterial blight and Verticillium and Fusarium wilts is therefore attributed to the induction of systemic resistance following application of BTH. This work demonstrates that SAR has potential for minimising effects of three major cotton pathogens under field conditions. With further field studies plus a greater understanding of the process of SAR, this method of plant protection could join the integrated pest management ensemble in an effort to control these pathogens under commercial conditions.

References

Bashan, Y. and Hernandez-Saavedra. N.Y. (1992) – Alternaria-blight of cotton: epidemiology and transmission. In: Chelkowski J, Viscount A, eds. *Alternaria biology, plant diseases and metabolites*. New York, USA: Elsevier, 233-266.

Bell, A.A. (1969) – Phytoalexin production and *Verticillium* wilt resistance in cotton. *Phytopathology* **59**, 1119-1127.

Bell, A.A. (1992) – Verticillium wilt. In *Cotton Diseases* (Ed. R.J. Hillocks), Chapter 3, CAB International, Wallingford.

Bell, A.A. and Presley, J.T. (1969a) – Temperature effects upon resistance and phytoalexin synthesis in cotton inoculated with *Verticillium albo-atrum*. *Phytopathology* **59**, 1141-1146.

Benhamou, N. and Bélanger, R.R. (1998) – Induction of systemic resistance to *Pythium* damping-off in cucumber plants by benzothiadiazole: ultrastructure and cytochemistry of the host response. *The Plant Journal* **14,** 13-21.

Bradford, M.M. (1976) – A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248-254.

Brock, P.M., Inwood, J.R.B. and Deverall, B.J. (1994) – Systemic induced resistance to *Alternaria macrospora* in cotton (*Gossypium hirsutum*). *Australasian Plant Pathology* **23**, 81-85.

Chérif, M., Asselin, A and Bélanger, R.R.(1994) – Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. *Phytopathology* **84**, 236-242.

Colson, E.S. (1997) – Systemic induced resistance in cotton. PhD thesis, The University of Sydney.

Dann, E.K. and Deverall, B.J. (1995) – Effectiveness of systemic resistance in bean against foliar and soilborne pathogens as induced by biological and chemical means. *Plant Pathology* **44**, 458-466.

Dann, E.K. and Deverall, B.J. (1996) – 2,6-dichloroisonicotinic acid (INA) induces resistance in green beans to the rust pathogen, *Uromyces appendiculatus*, under field conditions. *Australasian Plant Pathology* **25**, 199-204.

Dann, E.K., Meuwly, P., Métraux, JP. and Deverall, B.J. (1996) – The effect of pathogen inoculation or chemical treatment on activities of chitinase and β-1,3-glucanase and accumulation of salicylic acid in leaves of green bean, *Phaseolus vulgaris* L.. *Physiological and Molecular Plant Pathology* **49**, 307-319.

Dann, E., Diers, B., Byrum, J. and Hammerschmidt, R. (1998) – Effect of treating soybean with 2,6-dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) on seed yields and the level of disease caused by *Sclerotinia sclerotiorum* in field and greenhouse studies. *European Journal of Plant Pathology* **104,** 271-278.

Dean, R.A. and Kuć, J. (1985) – Induced systemic protection in plants. *Trends in Biotechnology* **3**, 125-129.

Feussner, I., Fritz, I.G., Hause, B., Ullrich, W.R. and Wasternack, C. (1997) – Induction of a new lipoxygenase form in cucumber leaves by salicylic acid or 2,6-dichloroisonicotinic acid. *Botanica Acta* **110**, 101-108.

Friedrich, L., Lawton, K., Ruess, W., Masner, P., Specker, N., Gut-Rella, M., Meier, B., Dincher, S., Staub, T., Uknes, S., Métraux, J-P., Kessmann, H. and Ryals. J. (1996) – A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *The Plant Journal* **10**, 61-70.

Hammerschmidt, R. and Kuć, J. (1982) – Lignification as a mechanism for induced systemic resistance in cucumber. *Physiological Plant Pathology* **20**, 61-71.

Hammerschmidt, R. and Kuc, J. eds (1995) – *Induced resistance to disease in plants*. Dordrecht, Netherlands: Kluwer.

Kessmann, H., Staub, T., Ligon, J., Oostendorp, M. and Ryals. J. (1994) – Activation of systemic acquired resistance in plants. *European Journal of Plant Pathology* **100**, 359-369.

Kuć, J. (1980) – Plant immunization-mechanisms and practical implications. In *Active defense mechanisms in plants* (Ed. R.K.S. Wood), pp. 157-178 Plenum Press, New York

Lawton, K., Friedrich, L., Hunt, M., Weymann, K., Delaney, T., Kessmann, H., Staub, T. and Ryals, J. (1996) – Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *The Plant Journal* **10**, 71-82.

Mauch, F., Mauch-Mani, B. and Boller, T. (1988b) – Antifungal hydrolases in pea tissue. II Inhibition of fungal growth by combinations of chitinase and β-1,3-glucanase. *Plant Physiology* **88**, 936-942.

Menzies, J.G., Ehret, D.L., Glass, A.D.M. and Samuels, A.L. (1991) – The influence of silicon on cytological interactions between *Sphaerotheca fuliginea* and *Cucumis sativus*. *Physiological and Molecular Plant Pathology* **39**, 403-414.

Métraux, J.P., Ahl Goy, P., Staub, T., Speich, J., Steinemann, A., Ryals, J. and Ward, E. (1991) –Induced systemic resistance in cucumber in response to 2, 6-dichloroisonicotinic acid and pathogens. In: Hennecke H, Verma DPS, eds. *Advances in Molecular Genetics of Plant-Microbe Interactions. Proceedings of the 5th International Symposium on the Molecular Genetics of Plant-Microbe Interactions, Interlaken, Switzerland, 1990.* Dordrecht, The Netherlands: Kluwer Academic Publishers, 432-439.

Niderman, T., Genetet, I., Bruyère, T., Gees, R., Stintzi, A., Legrand, M., Fritig, B. and Mösinger, E. (1995) – Pathogenesis-related PR-1 proteins are antifungal. *Plant Physiology* **108**, 17-27.

Redolfi, P. (1983) – Occurrence of pathogenesis-related (b) and similar proteins in different plant species. *Netherlands Journal of Plant Pathology* **89**, 245-254.

Samuels AL, Glass ADM, Ehret DL, Menzies JG, 1991. Mobility and deposition of silicon in cucumber plants. *Plant, Cell and Environment* **14**, 485-492.

Schnathorst, W.C. and Mathre, D.E. (1966) – Cross-protection in cotton with strains of *Verticillium albo-atrum. Phytopathology* **56**, 1204-1208.

Schweizer, P., Buchala, A. and Métraux, J.P. (1997) – Gene-expression patterns and levels of jasmonic acid in rice treated with the resistance inducer 2,6-dichloroisonicotinic acid. *Plant Physiology* **115**, 61-70.

Shi, J., Mueller, W.C. and Beckman, C.H. (1992) – Vessel occlusion and secretory activities of vessel contact cells in resistant or susceptible cotton plants infected with *Fusarium oxysporum* f.sp. *vasinfectum. Physiological and Molecular Plant Pathology* **40**, 133-147.

Shtienberg, D. (1991) – Effects of leaf susceptibility and fungicide seed treatment on leaf spot of Pima cotton caused by *Alternaria macrospora. Plant Pathology* **40,** 415-421.

Shtienberg, D., Blachinsky, D., Kremer, Y., Ben-Hador, G. and Dinoor, A. (1995) – Integration of genotype and age-related resistances to reduce fungicide use in management of Alternaria diseases of cotton and potato. *Phytopathology* **85**, 995-1002.

Shulaev, V., León ,J. and Raskin, I. (1995) – Is salicylic acid a translocated signal of systemic acquired resistance in tobacco? *The Plant Cell* **7**, 1691-1701.

Siegrist, J., Glenewinkel, D., Kolle, C. and Schmidtke, M. (1997) – Chemically induced resistance in green bean against bacterial and fungal pathogens. *Journal of Plant Diseases and Protection* **104,** 599-610.

Smit, F. and Durbery, I.A. (1997) – Cell wall reinforcement in cotton hypocotyls in response to a *Verticillium dahliae* elicitor. *Phytochemistry* **44**, 811-815.

Steel, R.G.D. and Torrie, J.H. (1960) – *Principles and procedures of statistics. A biometrical approach. Second edition.* McGraw-Hill International Book Co., Singapore.

Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E. and Ryals, J. (1992) – Acquired resistance in Arabidopsis. *The Plant Cell* **4**, 645-656.

Van Kan, J.A.L., Cozijnsen, T., Danhash, N. and De Wit, P.J.G. (1995) – Induction of tomato stress protein mRNAs by ethephon, 2,6-dichloroisonicotinic acid and salicylate. *Plant Molecular Biology* **27**, 1205-1213.

Vernoiij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H. and Ryals, J. (1994) – Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *The Plant Cell* **6**, 959-965.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Métraux, J.P. and Ryals, J. (1991) – Co-ordinate gene activity in response to agents that induce systemic acquired resistance. *The Plant Cell* **3**, 1085-1094.

Wasternack, C., Atzorn, R., Jarosch, B. and Kogel, K.H. (1994) – Induction of a thionin, the jasmonate-induced 6 kDa protein of barley by 2,6-dichloroisonicotinic acid. *Journal of Phytopathology* **140**, 280-284.

Woloshuk, C.P., Meulenhoff, J.S., Sela-Buurlage, M., Van den Elzen, P.J.M. and Cornelissen, B.J.C. (1991) – Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. *The Plant Cell* **3**, 619-628.

Watkins, G.M. (1981) – *Compendium of cotton diseases.* American Phytopathological Society.

Table 2. The effect of formulation material with and without INA applied to cotyledons on challenge inoculation of the first or second leaves of cotton with *A. macrospora*.

	Mean numbers of lesions[a,b] on	
Spray treatments to cotyledons[c]	Leaf 1	Leaf 2
Water	36.33[A]	30.00[A]
7.5 µg WP / mL	24.50[B]	18.66[BD]
15 µg WP / mL	11.50[C]	7.66[CE]
37.5 µg WP / mL	9.66[CD]	6.50[CEF]
75 µg WP / mL	5.33[CDE]	4.83[CEFG]
2.5 µg INA + 7.5 µg WP / mL	22.50[B]	12.66[CD]
5 µg INA + 15 µg WP / mL	4.30[DE]	4.00[EFG]
12.5 µg INA + 37.5 µg WP / mL	2.00[E]	2.66[FG]
25 µg INA + 75 µg WP / mL	3.50[E]	1.66[G]

[a] The first or second leaves were challenged with *A. macrospora* 4 or 10 days respectively after treatment of cotyledons. Lesions were counted 5 days after challenge. Six plants were used for each treatment and time of challenge.

[b] Means for each leaf followed by the same letter are not significantly different ($P \leq 0.05$) within a column.

[c] Of 10-day-old plants of cv. Siokra 1-4 using a hand atomiser.

Table 3. The effect of formulation material with BTH applied to cotyledons on challenge inoculation of the first or second leaves of cotton with *A. macrospora*.

	Mean numbers of lesions[a,b] on	
Spray treatments to cotyledons[c]	Leaf 1	Leaf 2
Water	11.17[A]	12.33[A]
35 µg BTH + 35 µg WG / mL	1.37[B]	0.90[B]

[a] The first or second leaves were challenged with *A. macrospora* 5 days after treatment of cotyledons. Lesions were counted 6 days after challenge. Six plants were used for each treatment and time of challenge.

[b] Means for each leaf followed by the same letter are not significantly different ($P \le 0.05$) within a column.

[c] Of 21-day-old plants of cv. Siokra 1-4 using a hand atomiser.

Table 4. β-1,3-glucanase activities in first and second leaves of cotton 120 h after spray treatment of the cotyledons with formulations of INA and BTH.

			Milliunits of glucanase activity[a,b]	
Experiment[c]	Treatment[d]	Tissue	/ g fwt	/ mg protein
1a	water	1st leaves	12.98[A]	0.48[A]
1a	60 µg WP / mL	1st leaves	17.65[B]	0.66[B]
1a	20 µg INA + 60 µg WP / mL	1st leaves	32.63[C]	1.19[C]
1a	35 µg WG / mL	1st leaves	10.41[A]	0.38[A]
1a	35 µg BTH + 35 µg WG / mL	1st leaves	41.64[D]	1.54[D]
1b	water	2nd leaves	0.70[A]	0.03[A]
1b	60 µg WP / mL	2nd leaves	1.06[A]	0.05[A]
1b	20 µg INA + 60 µg WP / mL	2nd leaves	8.78[B]	0.36[B]
1b	35 µg WG / mL	2nd leaves	1.58[A]	0.03[A]
1b	35 µg BTH + 35 µg WG / mL	2nd leaves	25.53[C]	0.96[C]

[a] Means within an experiment followed by the same letter are not significantly different ($P \leq 0.05$).

[b] Nine replicates per treatment.

[c] Experiments 1a and 1b cannot be compared directly as the assays were conducted on different days.

[d] Treatments were applied to 14-day-old plants of cv. Siokra 1-4 using a hand atomiser.

Table 5. β-1,3-glucanase activities in the stem of cotton 72 h after spray treatment of the cotyledons with formulations of INA and BTH.

			Milliunits of glucanase activity[a,b]	
Experiment	Treatment[c]	Tissue	/ g fwt	/ mg protein
2	water	stem	0.24[A]	0.01[A]
2	60 µg WP /mL	stem	0.39[A]	0.02[A]
2	20 µg INA + 60 µg WP / mL	stem	1.73[B]	0.08[B]
2	35 µg BTH + 35 µg WG / mL	stem	1.74[B]	0.08[B]

[a] Means within an experiment followed by the same letter are not significantly different ($P \leq 0.05$).

[b] Nine replicates per treatment.

[c] Treatments were applied to 14-day-old plants of cv. Siokra 1-4 using a hand atomiser.

Table 6. Effects of each component of formulated INA applied to cotyledons on β-1,3-glucanase activities in first leaves of cotton 72 h later.

			Milliunits of glucanase activity[a,b]	
Experiment[c]	Treatment[d]	Tissue	/ g fwt	/ mg protein
3	water spray	1st leaves	7.40[A]	0.30[A]
3	Ultravon (4 µg / mL) spray	1st leaves	9.88[A]	0.40[AB]
3	Attisol (4 µg / mL) spray	1st leaves	11.02[B]	0.43[B]
3	kaolin (4.9 µg / mL) spray	1st leaves	8.67[A]	0.35[AB]
3	silicic acid (4 µg / mL) spray	1st leaves	10.42[B]	0.41[B]
3	20 µg INA + 60 µg WP / mL spray	1st leaves	15.98[C]	0.63[C]
4	water spray	1st leaves	8.33[A]	0.33[A]
4	water brush	1st leaves	8.14[A]	0.33[A]
4	kaolin (4.9 µg / mL) spray	1st leaves	6.70[A]	0.27[A]
4	kaolin (4.9 µg / mL) brush	1st leaves	12.60[B]	0.51[B]
5	water spray	1st leaves	3.30[A]	0.13[A]
5	0.5 µL ethanol /mL water spray	1st leaves	6.05[B]	0.23[B]
5	20 µg INA + 0.5 µL ethanol / mL spray	1st leaves	15.43[C]	0.61[C]

[a] Means within an experiment followed by the same letter are not significantly different ($P \leq 0.05$).

[b] Nine replicates per treatment.

[c] Experiments 3, 4 and 5 cannot be compared directly as the assays were conducted on different days.

[d] Treatments were applied to 14-day-old plants of cv. Siokra 1-4 using a hand atomiser or a fine camel hair brush.

Table 7. β-1,3-glucanase activities in first and second leaves of cotton 7 days after inoculation of the cotyledons with *A. macrospora*.

			Milliunits of glucanase activity[a,b]	
Experiment[c]	Treatment[d]	Tissue	/ g fwt	/ mg protein
6a	water	1st leaves	14.34[A]	0.38[A]
6a	*Alternaria*	1st leaves	28.99[B]	0.74[B]
6b	water	2nd leaves	2.65[A]	0.07[A]
6b	*Alternaria*	2nd leaves	23.63[B]	0.57[A]

[a] Means within an experiment followed by the same letter are not significantly different ($P \leq 0.05$).

[b] Nine replicates per treatment.

[c] Experiments 6a and 6b cannot be compared directly as the assays were conducted on different days.

[d] Seventeen-day-old plants of cv. Siokra 1-4 were challenged with an *A. macrospora* spore suspension, 5×10^5 spores / mL using a fine camel hair brush.

Table 8. Effects of each component of formulated INA applied to cotyledons on challenge inoculation of the first and second leaves of cotton with *A. macrospora*.

	Mean numbers of lesions[a,b] on	
Treatment[c]	Leaf 1	Leaf 2
Water	19.05[A]	16.20[A]
Silicic acid (16 µg / mL)	5.30[B]	4.93[B]
Water	13.05[A]	9.87[A]
Kaolin (9.8 µg / mL)	7.68[B]	6.45[B]
Water	12.27[A]	6.00[A]
Attisol (16 µg / mL)	6.05[B]	3.18[B]
Water	12.25[A]	6.25[A]
Ultravon (16 µg / mL)	6.50[B]	4.48[A]
0.5 µL ethanol / mL water control	13.18[A]	10.75[A]
20 µg INA / 0.5 µL ethanol / mL water	6.95[B]	5.70[B]

[a] The first and second leaves were challenged with *A. macrospora* 6 days after treatment application. Lesions were counted 5 to 7 days later.

[b] Means for each leaf (within an experiment) followed by the same letter are not significantly different ($P \leq 0.05$).

[c] Treatments were applied to the cotyledons of 11- to 16-day-old plants using a hand atomiser, except for kaolin which was applied using a fine camel hair brush. 40 plants of cv. Siokra 1-4 were used per treatment in each of the five experiments.

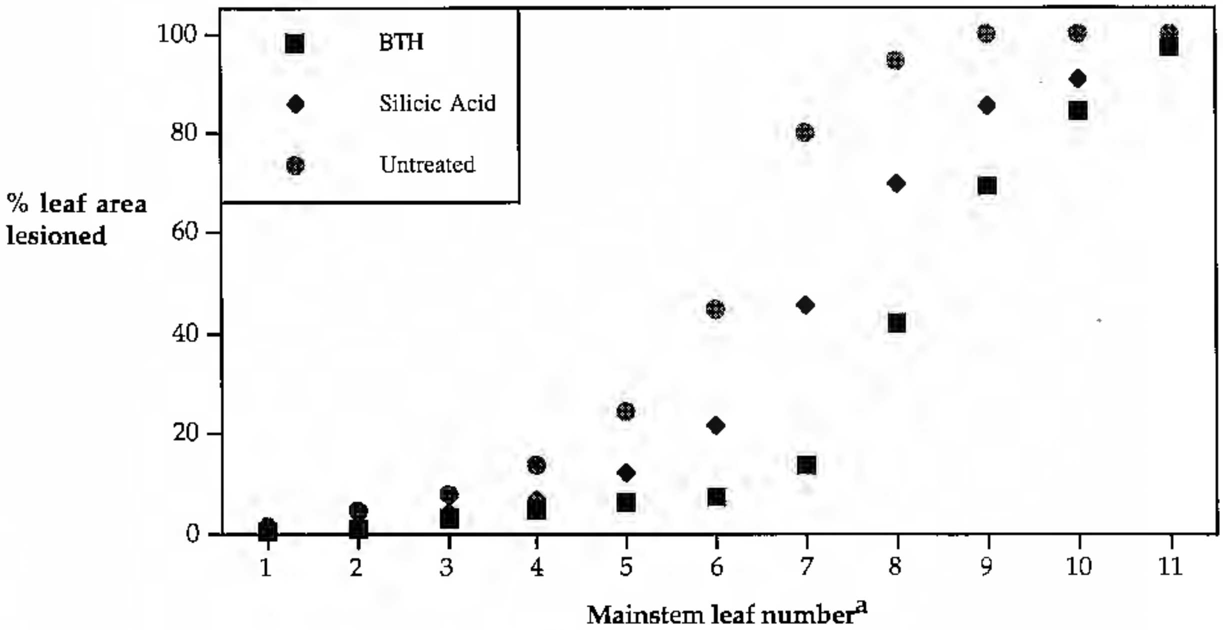

Figure 1. Alternaria field trial, Bourke, NSW 97/98 season, cv. Pima S7.

[a]The mainstem leaf number refers to the leaves left on the main stem at assessment, the higher the number the further down the stem.

Statistically significant differences ($P \leq 0.05$) occurred between the BTH treated plants and the untreated plants on main stem leaves 1 to 9. Statistically significant differences ($P \leq 0.05$) occurred between the silicic acid treated plants and the untreated plants on main stem leaves 1 to 8.

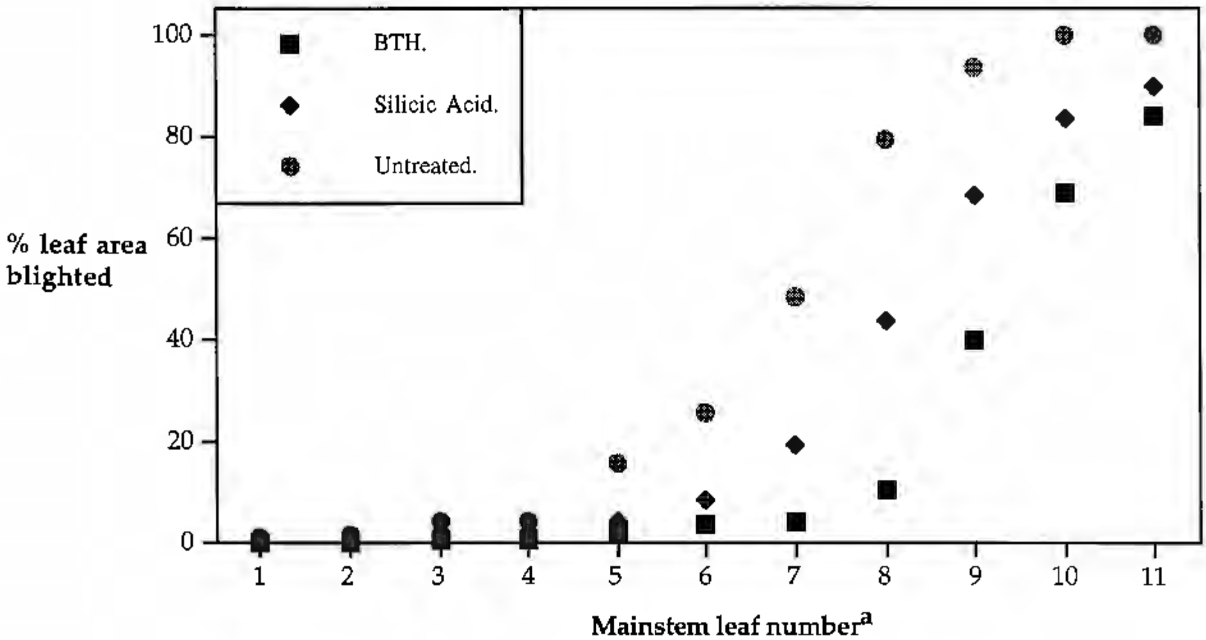

Figure 2. Bacterial blight field trial, Bourke 97/98 season, cv. Pima S7.

[a]The mainstem leaf number refers to the leaves left on the main stem at assessment, the higher the number the further down the stem.

Statistically significant differences ($P \leq 0.05$) occurred between the BTH treated plants and the untreated plants on main stem leaves 2 to 10. Statistically significant differences ($P \leq 0.05$) occurred between the silicic acid treated plants and the untreated plants on main stem leaves 2 to 7.

Table 9. The mean number of mainstem leaves remaining on Pima S7 cotton plants, following treatment with BTH or silicic acid in a field severely affected by Alternaria leaf spot and bacterial blight in Bourke, NSW, 97/98 season.

Treatment	Mean number of remaining mainstem leaves (standard errors of means)[a,b]	
Untreated control	5.77[A]	(0.21)
Silicic acid	7.03[B]	(0.28)
BTH	8.10[C]	(0.23)

[a] On the main stem at assessment of 24 plants for each treatment.

[b] Means followed by the same letter are not significantly ($P \leq 0.05$) different.

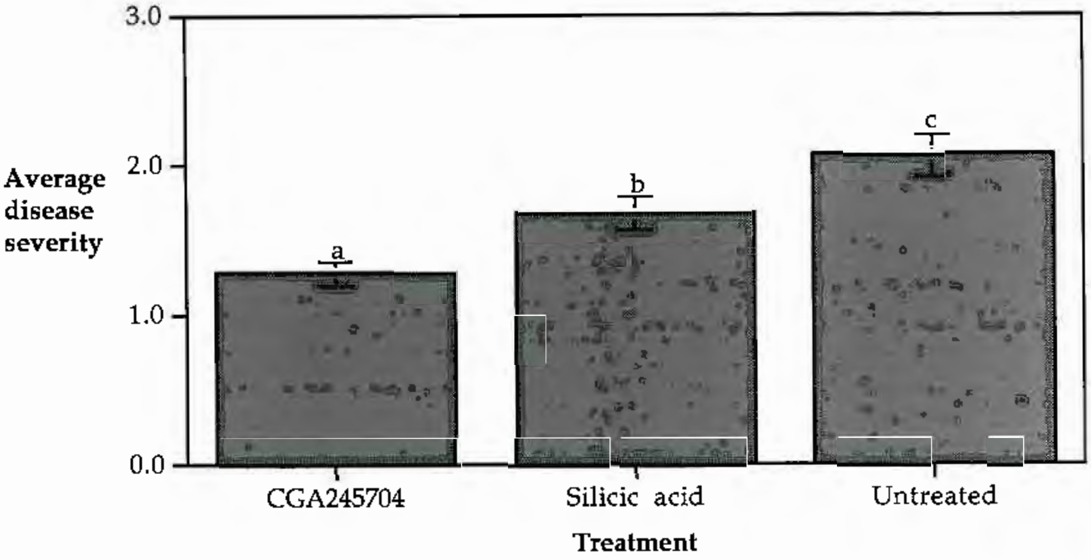

Figure 3. Verticillium field trial, Narrabri, NSW 97/98, cv. Siokra 1-4.

Histograms surmounted by the same letter represent average disease severity scores that are not significantly different ($P \leq 0.05$).

The first treatments were applied to 7 week old plants and the second treatments applied 7 weeks later. Plants were assessed 8 weeks after the second treatment.The severity score ranged from 0 for a healthy plant with no symptoms to 4 for a plant with greater than 10 leaves showing symptoms.

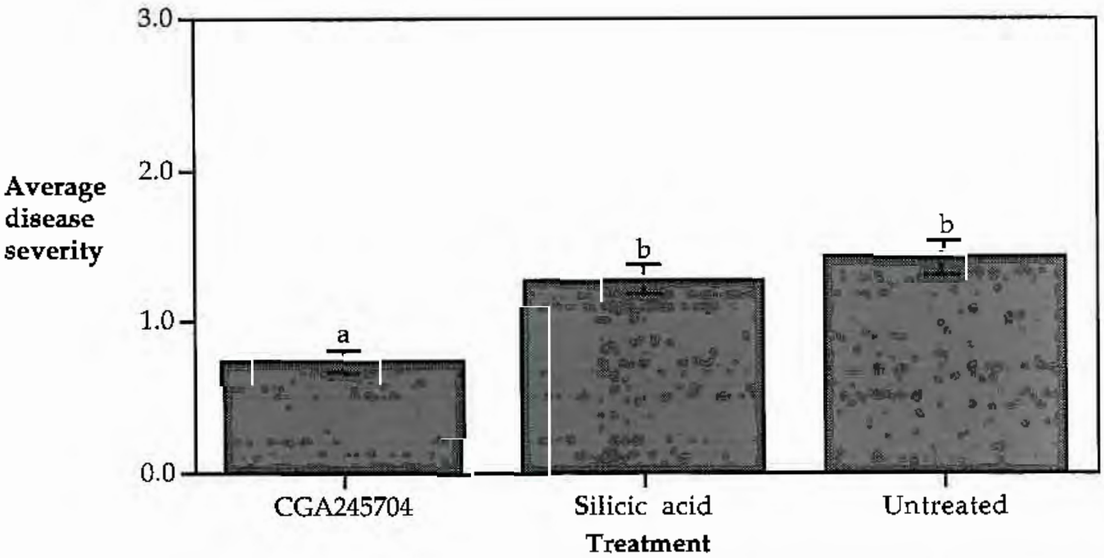

Figure 4. Verticillium field trial, Narrabri, NSW 97/98, cv. Sicala V2.

Histograms surmounted by the same letter represent average disease severity scores that are not significantly different ($P \leq 0.05$).

The first treatments were applied to 7 week old plants and the second treatments applied 7 weeks later. Plants were assessed 8 weeks after the second treatment. The severity score ranged from 0 for a healthy plant with no symptoms to 4 for a plant with greater than 7 leaves showing symptoms.

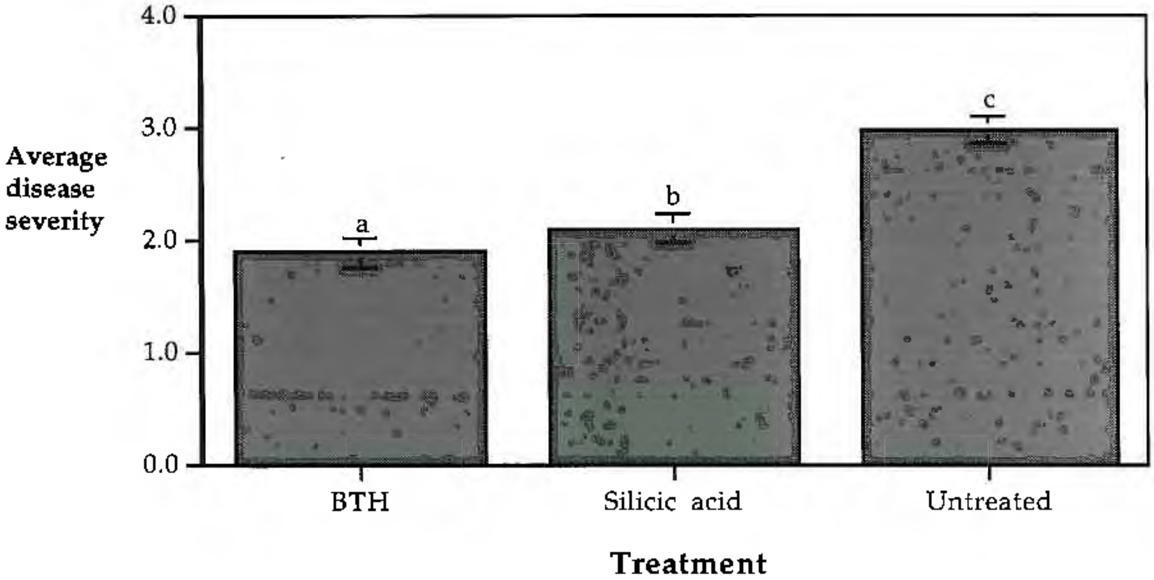

Figure 3. Verticillium field trial, Narrabri, NSW 98/99, cv. Siokra 1-4.

Histograms surmounted by the same letter represent heights that are not significantly different ($P \leq 0.05$).
The first treatments were applied to 4 week old plants, the second applied 6 weeks later and the third application occurred 6 weeks after the second. Plants were assessed 4 weeks after the final treatment. The severity score was 0=healthy plant with no foliar symptoms, 1=1/4 of plant with symptoms, 2=1/2 of plant with symptoms, 3=3/4 of plant with symptoms and 4 = whole plant with symptoms or dead.

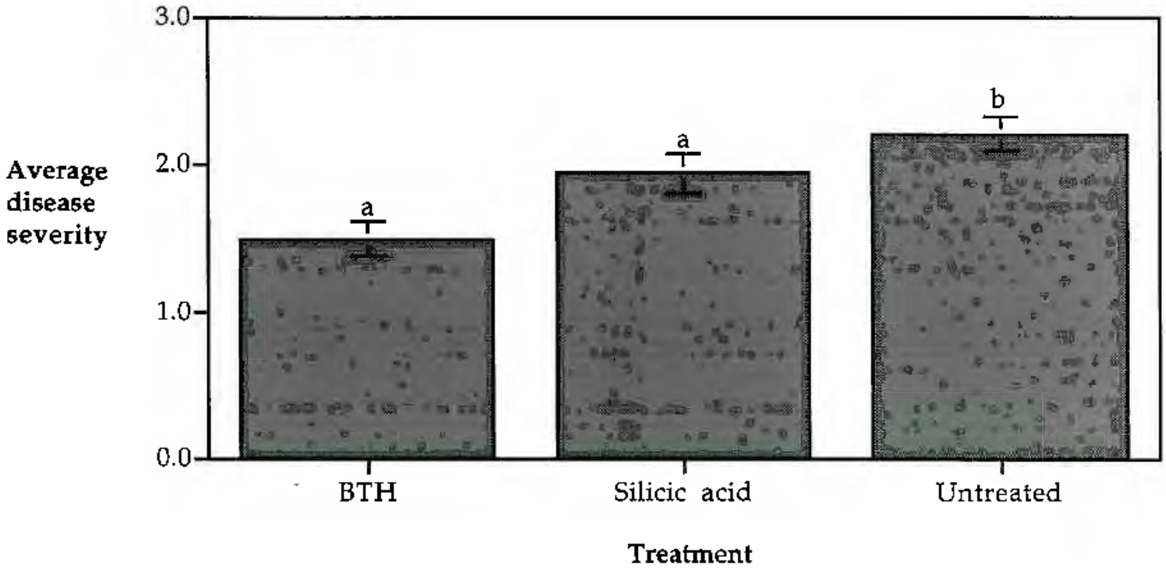

Figure 4. Verticillium field trial, Narrabri, NSW 98/99, cv. Sicala V2.

Histograms surmounted by the same letter represent heights that are not significantly different ($P \leq 0.05$).
The first treatments were applied to 4 week old plants, the second applied 6 weeks later and the third application occurred 6 weeks after the second. Plants were assessed 4 weeks after the final treatment. The severity score was 0 =healthy plant with no foliar symptoms, 1=1-3 leaves with symptoms, 2=4-6 leaves with symptoms, 3=7-10 leaves with symptoms and 4=>10 leaves with symptoms.

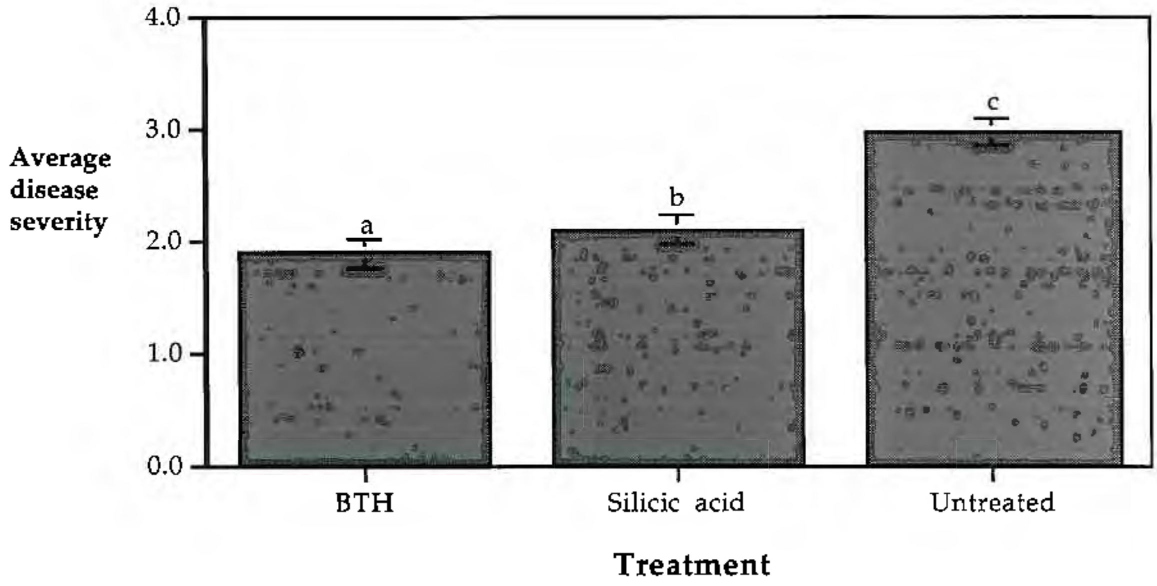

Figure 5. Verticillium field trial, Narrabri, NSW 98/99, cv. Siokra 1-4.

Histograms surmounted by the same letter represent heights that are not significantly different ($P \leq 0.05$).
The first treatments were applied to 4 week old plants, the second applied 6 weeks later and the third application occurred 6 weeks after the second. Plants were assessed 4 weeks after the final treatment. The severity score was 0=healthy plant with no foliar symptoms, 1=1/4 of plant with symptoms, 2=1/2 of plant with symptoms, 3=3/4 of plant with symptoms and 4 = whole plant with symptoms or dead.

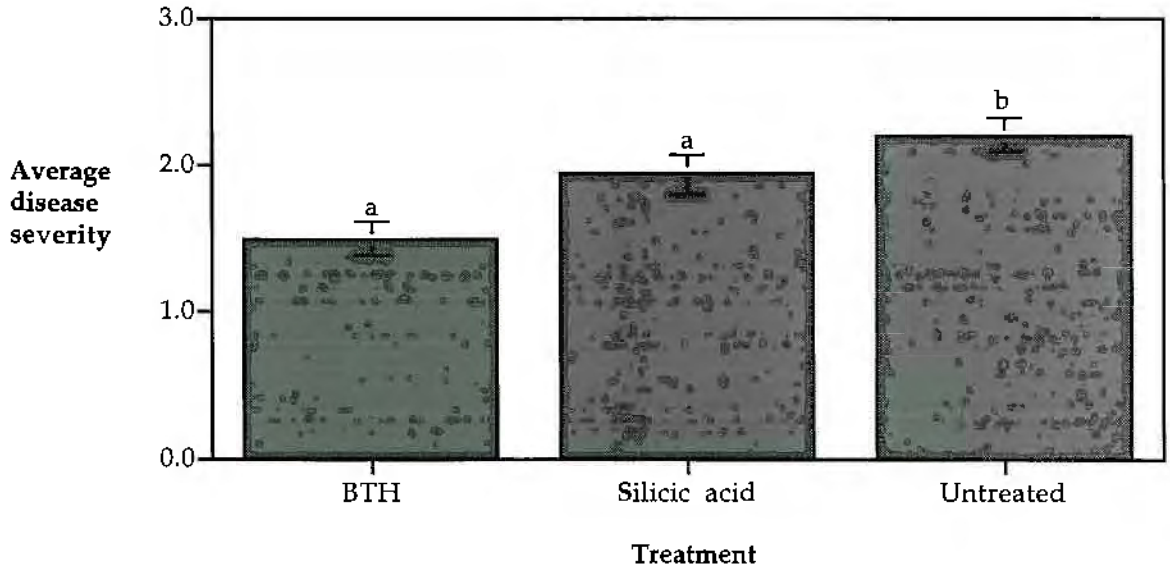

Figure 6. Verticillium field trial, Narrabri, NSW 98/99, cv. Sicala V2.

Histograms surmounted by the same letter represent heights that are not significantly different ($P \leq 0.05$).
The first treatments were applied to 4 week old plants, the second applied 6 weeks later and the third application occurred 6 weeks after the second. Plants were assessed 4 weeks after the final treatment. The severity score was 0 =healthy plant with no foliar symptoms, 1=1-3 leaves with symptoms, 2=4-6 leaves with symptoms, 3=7-10 leaves with symptoms and 4=>10 leaves with symptoms.

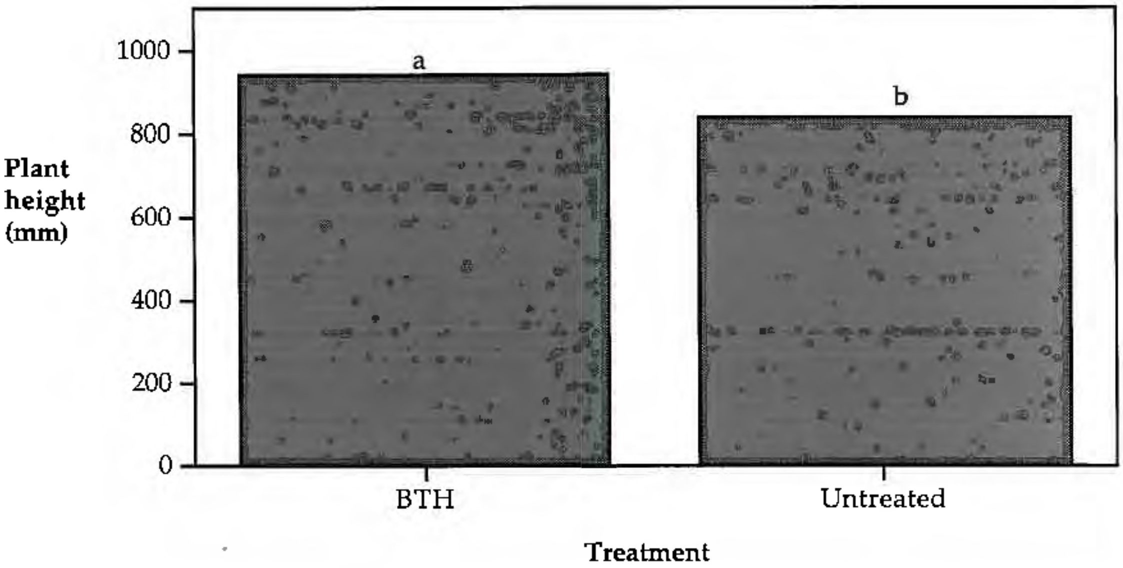

Figure 7. Fusarium field trial 97/98, Cecil Plains, QLD, cv. S189.

Histograms surmounted by the same letter represent heights that are not significantly different ($P \leq 0.05$).

The first treatment was applied to 5 week old plants and the second treatment applied 7 weeks later. Plants were assessed 5 weeks after the second treatment.

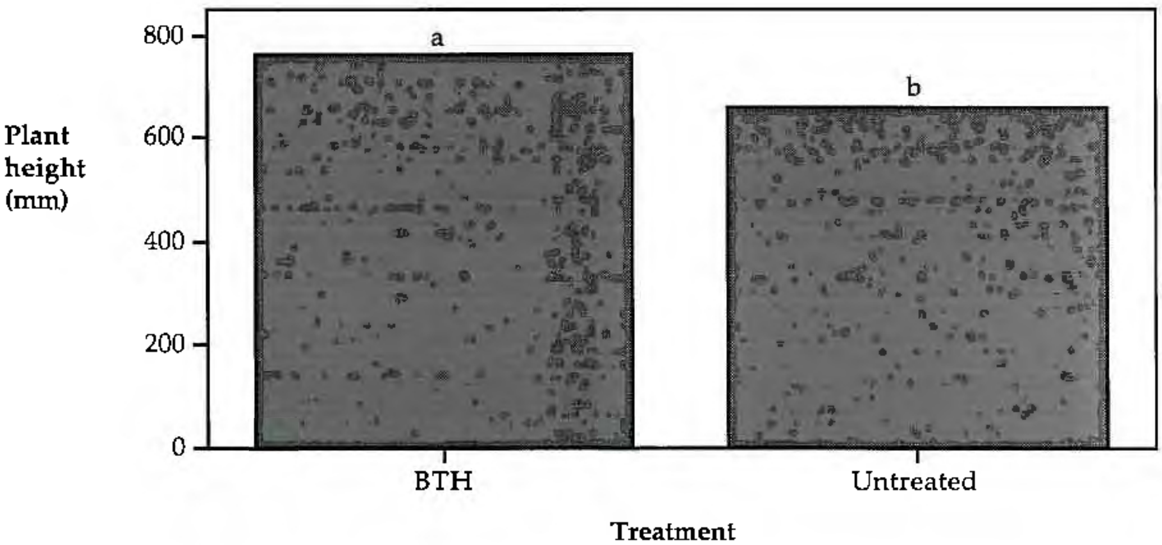

Figure 8. Fusarium field trial 98/99, Cecil Plains, QLD, cv. S189

Histograms surmounted by the same letter represent heights that are not significantly different ($P \leq 0.05$).
The first treatment was applied to 2 week old plants, the second applied 5 weeks later and the third application occurred 9 weeks after the second. Plants were assessed 8 weeks after the final treatment.

Publications arising from the postdoctoral project.

- E Colson (1998) "Systemic induced resistance helps natural cotton plant defences" The Australian Cottongrower **19** (4) pp 30 - 34.

- ES Colson-Hanks and BJ Deverall (1999) "Effect of 2,6-dichloroisonicotinic acid, its formulation materials and benzothiadiazole on systemic resistance to Alternaria leaf spot in cotton", Plant Pathology (in press).

- ES Colson, SJ Allen and BJ Deverall (1999) "Effect of 2,6-dichloroisonicotinic acid or benzothiadiazole on Alternaria leaf spot, bacterial blight and Verticillium wilt in cotton under field conditions" currently being refereed by Australasian Plant Pathology.

- "Systemic Induced Resistance in cotton", poster presented by E.S. Colson and B.J. Deverall at the 11th Biennial Australasian Plant Pathology Society conference, Perth, 1997.

- "Systemic Acquired Resistance in cotton", poster presented by E.S. Colson and B.J. Deverall at the 7th International Congress of Plant Pathology, Edinburgh, UK, 1998.

- "Systemic Acquired Resistance in cotton", poster and workshop presentation by E.S. Colson-Hanks, B.J. Deverall and S.J. Allen at the 12th Biennial Australasian Plant Pathology Society conference, Canberra, 1999.

PLAIN ENGLISH SUMMARY

It is possible to enhance plants' natural defence systems to provide a broad spectrum resistance against a range of fungal, viral and bacterial pathogens. The process, known as systemic acquired resistance (SAR), requires prior exposure of the plant to certain biological or chemical agents that 'sensitise' the plant leading to a rapid resistance response on subsequent pathogen attack. This postdoctoral project examined the SAR response in cotton and to evaluate a range of biotic and abiotic stimuli under both glasshouse and field conditions against *Verticillium* and *Fusarium* wilts and *Alternaria* leaf spot. Activity of the PR-protein, ß-1,3-glucanase, a marker of SAR, was also examined in cotton seedlings following treatment with various stimuli.

The durability and effectiveness of SAR against leaf infections under conditions conducive to *A. macrospora* and also to *X. campestris* pv. *malvacearum* were clearly demonstrated during the 97/98 cotton-growing season. BTH was applied, and resistance induced, following the initial outbreaks. The protection provided was, therefore, most probably against secondary infections. The percentage leaf area showing lesions was substantially lower on the treated plants, most likely due to a combination of reduced successful infections and delayed symptom expression, all characteristics of SAR.

Premature defoliation, a major symptom of Alternaria leaf spot in cotton, was also significantly lower in the treated plants, compared with the untreated plants. The observed reduction in defoliation is important as the premature shedding of leaves decreases the photosynthetic area of the plant, the primary cause of yield losses in cotton crops severely affected by Alternaria leaf spot or bacterial blight.

In the Verticillium field experiments, 3 applications of BTH or silicic acid to treated plants in the 97/98 and 98/99 seasons, with the first treatment applied prior to foliar symptoms, resulted in significant reductions in disease severity in treated plants compared with untreated plants even though the disease outbreak was severe. Therefore, application of the activator prior to foliar symptom expression and with booster applications appears to decrease disease severity even when severe disease development occurs in the field.

A significant decrease in stunting was observed in the Fusarium field experiments in the 97/98 and 98/99 seasons following BTH treatment of plants. Reductions in disease incidence were also noted in treated plants.

The reduced susceptibility of plants to Alternaria leaf spot, bacterial blight and Verticillium and Fusarium wilts is therefore attributed to the induction of systemic resistance following application of BTH and silicic acid. This work demonstrates that SAR has potential for minimising effects of three major cotton pathogens under field conditions. With further field studies plus a greater understanding of the process of SAR, this method of plant protection could join the integrated pest management ensemble in an effort to control these pathogens under commercial conditions.