If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October.
If not, please provide a written report by 30 September.

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: UQ37

Project Title: Silicon and Bion induced defence responses in cotton infected with *Fusarium oxysporum* f. sp. *vasinfectum*

Project Commencement Date: 01/07/2005 **Project Completion Date:**

CRDC Program: 3 Crop Protection

Part 2 – Contact Details

Administrator: (Marijke Schmidt
Scholarships officer, Office of Research and Postgraduate Studies

Organisation: The University of Queensland

Postal Address: Brisbane QLD, 4072

Ph: 07) 3365 2033 **Fax:** 07) 3365 6941 **E-mail:** scholarships@research.uq.edu.au

Principal Researcher: Jennifer Whan

Organisation: The University of Queensland

Postal Address: 18 Sixth Avenue
South Townsville, QLD, 4810

Ph: 0747404639 **Fax:** **E-mail:** j.whan@uq.edu.au

Supervisor: Dr. Elizabeth Aitken

Organisation: The University of Queensland

Postal Address: School of Biological Sciences
John Hines Building
The University of Queensland
St Lucia 4072
QLD

Ph: : (0)7 3365 4775 **Fax:** (0)7 3365 4771 **E-mail:** e.aitken@uq.edu.au

Signature of Research Provider Representative: 10/9/09

Part 3 – Final Report Guide

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

Background

1. A project investigating the molecular and histological effects of silicon application on cotton infected with *Fov* was inspired by promising preliminary results obtained by Dr Linda Smith (QPI&F) in glasshouse and field trials. Silicon application has been demonstrated to effectively ameliorate or reduce the symptoms of disease caused by fungal pathogens in many plant-pathogen interactions, including those involving host species such as rice, cucumber and wheat. The mechanisms of resistance attributed to silicon treatment include the creation of a physical barrier to pathogen penetration, and the induction of defence responses associated with the increased production of defence related compounds and alterations in defence gene expression. The feasibility of applying silicon to reduce incidence or severity of Fusarium wilt in cotton was investigated utilising two forms of silicon and two cotton cultivars with different resistances to *Fov*. The following defence responses were assessed with and without silicon treatment:
    a. Disease severity and incidence, effect of silicon on nutrient acquisition
    b. Histological defence reactions including the accumulation of phenolic compounds and lignin
    c. Defence gene changes
    d. Defence enzyme activity

As Bion has recently been registered for use by the Australian cotton industry, an additional aim of the project was to ascertain the effects of this chemical resistance inducing agent on cotton infected with *Fov*. Bion is applied to cotton seed as a seed treatment for the control of both Fusarium wilt and black root rot, whilst it is also commonly applied as a foliar spray to induce defence responses in other plant species. The aim of this research was to determine if defence responses in Fusarium wilt infected cotton, including defence gene expression changes and changes in the activities of defence related enzymes, were affected by Bion treatment; and to determine if these responses were more pronounced following Bion application in the form of a seed treatment or a foliar spray.

Objectives

2. List the project objectives and the extent to which these have been achieved.

Objective 1 – Analyses of the effects of silicon on histological defence responses in silicon treated cotton

Objective 2 – Real time PCR analyses of defence/ stress gene expression changes in Sicot 189 and Sicot F-1 to determine the effects of silicon treatment

Objective 3 – Analyses of phenolics production in Sicot 189 and F1 to determine the effects of silicon treatment

Objective 4 –Assessment of the effects of Bion on defence gene expression and defence related enzyme activities in Sicot 189

Objective 5 – Analyses of defence related enzyme activity in Sicot 189 and F1 to determine effects of silicon treatment

Objective 6 – Disease assessment trials and mineral analyses of Sicot 189 and Sicot F1 treated with 2 forms of silicon to determine the effects of silicon treatments on these aspects

All of the objectives listed were achieved during the project.

Methods

3. **Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.**

Objective 1 – Analyses of the effects of silicon on histological defence responses in silicon treated cotton

Histological defence reactions were assessed using light and transmission electron microscopy (TEM). TEM is a proven technique for studying the impacts of fungal infection on plant cells and it allows visualisation of plant defence reactions such as wall apposition formation and accumulation of phenolic compounds. Novel methods were not required for these analyses, though optimisation of sample processing protocols was required. A stain specific to lignin was also utilised to determine differences in lignin accumulation between treatments.

Objective 2 – Real time PCR analyses of defence/ stress gene expression changes in Sicot 189 to determine the effects of silicon treatment

Real time-PCR is a sensitive method for determining changes in gene transcript accumulation resulting from treatment applied. This technique is versatile and widely utilised in many scientific disciplines. Real time PCR assessment of gene expression changes in plants is a popular molecular tool for the assessment of changes occurring as a result of specific treatments or other factors. This technique was extremely well suited to the objectives of the study and the reproducibility of results obtained were validated with repeat trials.

Objective 3 – Analyses of phenolics production in Sicot 189 and F1 to determine the effects of silicon treatment

The levels of phenolic compounds in cotton plants subjected to various treatments were determined using Folin-Ciocalteu reagent, which performs a colourimetric assay for the measurement of phenolic and polyphenolic antioxidants. This assay provided data to support TEM observations regarding the levels of phenolic accumulations present in plants from Si+ and Si- treatments.

Objective 4 –Assessment of the effects of Bion alone on defence/ stress gene expression and PR protein production in Sicot 189

Bion was applied as a seed soak and also as a foliar spray to assess the effects of the compound and the application method on defence gene expression and enzyme activity in cotton infected with *Fov*. Both methods have been applied in previous studies, with a seed treatment method currently preferred by seed distribution companies in Australia. The aim of this research was not to develop novel techniques, rather to assess the impacts of proven techniques on this particular interaction as the use of this compound becomes more popular.

Objective 5 – Analyses of defence related enzyme activity in Sicot 189 and F1 to determine effects of silicon treatment

Spectrophotometric assays to determine the activities of three key defence related assays were performed using modified protocols obtained from the literature and from one of my co-supervisors at the QPI&F, Dr Elizabeth Dann. These methods were effective and well suited to this objective of the study; that being to determine the *in planta* activity of these three enzymes in response to Si+ and Si- treatments.

Objective 6 – Disease assessment trials and mineral analyses of Sicot 189 and F1 treated with two forms of silicon to determine the effects of silicon treatments on these aspects

Disease assessment trials were conducted in glasshouses following methods of QPI&F Fusarium researchers. Plants were inoculated with colonised millet and scored for resistance according to extent of vascular discolouration and percent plants infected. This methodology was appropriate to meet this objective; which was to determine the effects of Si+ and Si- treatments on symptom development and rate of infection.

Results

4. **Detail and discuss the results for each objective including the statistical analysis of results.**

Objective 1 – Analyses of the effects of silicon on histological defence responses in silicon treated cotton

TEM and light microscopy analyses of histological defence responses occurring in silicon treated cotton infected with *Fov* were carried out over the course of the 3.5 year project. The effects of silicon applied as soluble potassium silicate were determined in two cultivars with different resistances to the pathogen; Sicot 189 - moderately resistant and Sicot F-1 - highly resistant. Defence responses including accumulation of osmiophillic compounds and fungal structure degradation were evident in cells of both silicon and non silicon treated plants following inoculation. Silicon enhanced observed responses in both cultivars, particularly in Sicot F-1, where responses were more rapid and of a greater magnitude. Staining, and reference to prior research, suggests that accumulated compounds were phenolic in composition, and likely to have been composed of terpenoid cotton phytoalexins such as gossypol and hemigossypol. Increased accumulations and changes in cell morphology were not observed in silicon treated plants not inoculated with the pathogen, suggesting that silicon acted to potentiate the enhanced responses observed in inoculated plants but did not immediately cause an activation of these responses. As this was a purely qualitative analysis there were no accompanying statistics.

Objective 2 – Real time PCR analyses of defence/ stress gene expression changes in Sicot 189 and Sicot F-1 to determine the effects of silicon treatment

The expression of an array of defence genes was assessed in plants subject to various silicon, potassium and control treatments. As silicon was applied as potassium silicate liquid an additional treatment incorporating potassium sulphate amendment was included to verify that effects were due to silicon rather than potassium treatment. The treatments were potassium silicate with no *Fov*, potassium silicate with *Fov*, potassium sulphate with no *Fov*, potassium sulphate with *Fov*, *Fov* with no additional treatment and mock inoculation to account for changes occurring due to inoculation trauma. Two cultivars, Sicot F-1 and Sicot 189 were assessed with material for analysis obtained at 2, 3 and 4 days post inoculation (dpi) with *Fov*. Genes assessed were peroxidase, phenylalanine ammonia lyase, β-1, 3-glucanase, chitinase, polygalacturonase inhibiting protein, cadinene synthase, osmotin like protein and 4-Coumarate Co-A ligase-like protein. Several genes were upregulated by silicon treatment in inoculated plants from both cultivars compared to inoculated plants receiving no additional treatments. Very few changes in gene expression were observed in root material at 2 dpi, which is in accordance with previous studies. In hypocotyl material at 3 dpi chitinase and osmotin were significantly upregulated in Sicot 189 and cadinene synthase, osmotin and polygalacturonase inhibiting protein were upregulated in Sicot F-1. At 4 dpi in Sicot 189, osmotin, chitinase and β-1, 3-glucanase expression was enhanced in inoculated silicon treated plants whilst in Sicot F-1 peroxidase, β-1, 3-glucanase and polygalacturonase inhibiting protein were upregulated. Gene expression of potassium sulphate treated material at 3 dpi did not result in the increased transcript accumulation of any genes assessed compared to plants inoculated with *Fov* and receiving no additional treatment. Genes induced by silicon treatment in Sicot F-1 plants were involved in lignin, phenolic compound and phytoalexin accumulation whilst genes upregulated in Sicot 189 were predominately genes from pathogenesis related gene families. No upregulation of defence gene expression was observed in silicon treated plants not challenge inoculated with *Fov*, once again providing evidence that silicon may contribute to the development of a primed state in cotton plants.

Objective 3 – Analyses of phenolics production in Sicot 189 and F1 to determine the effects of silicon treatment

Phenolics production was assessed to verify TEM observations of increased osmiophillic accumulations in silicon treated cotton infected with *Fov*. Both cultivars were assessed following treatment with potassium silicate. At 7 dpi there were significantly more phenolic compounds in root extracts from inoculated, silicon treated Sicot F-1 plants compared to inoculated plants receiving no silicon. This trend was also observed at 3 dpi but the difference was not significant in this case. In the more susceptible Sicot 189, levels between these two treatments were not significantly different from each other at either 3 or 7 dpi.

Objective 4 –Assessment of the effects of Bion on defence gene expression and defence related enzyme activities in Sicot 189

Bion was applied as a seed soak or foliar spray prior to plants being inoculated or not inoculated with *Fov*, to assess any resultant changes in defence gene expression and defence related enzyme activity. The cultivar used for this assessment was Sicot 189, the industry standard for the formulation of Fusarium resistance rankings. The expression of several key defence related genes in response to Bion treatments and fungal inoculation was assessed using real time PCR. Bion seed soak treatment combined with fungal inoculation resulted in the upregulation of chitinase, peroxidase and β-1, 3-glucanase compared to fungal inoculation alone. Fewer significant results were obtained following application in the form of a foliar spray; however osmotin-like protein was upregulated as a result of this treatment. To assess the impact of Bion treatments on production of the defence related enzymes, chitinase, peroxidase, polyphenol oxidase and β-1, 3-glucanase, a series of spectrophotometric assays were carried out with root extracts obtained at 3 and 7 dpi. The greatest increases in chitinase, β-1, 3-glucanase and peroxidise activity occurred in root and shoot tissue from Bion seed treated material inoculated with *Fov*

Objective 5 – Analyses of defence related enzyme activity in Sicot 189 and F1 to determine effects of silicon treatment

The effect of silicon nutrition on the induction of pathogenesis-related (PR) and antifungal enzymes was assessed in two cotton cultivars inoculated with the fungal pathogen *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*). Regular application of soluble potassium silicate significantly increased the activity of peroxidise in root extracts from the highly resistant cultivar Sicot F-1, at 3, 4 and 7 days post inoculation with *Fov*; and in root extracts from the moderately resistant Sicot 189 at 3 and 4 dpi. Greater peroxidise activity was observed in root rather than shoot material, and levels were significantly greater in extracts from Sicot F-1 plants compared to Sicot 189 plants. Significant increases in chitinase activity in inoculated, potassium silicate treated Sicot 189 plants were observed in root extracts at 3 dpi, and in shoot extracts at 4 dpi. Potassium silicate treatment resulted in significant increases in β-1, 3- glucanase activity in Sicot 189 root extracts at 4 dpi compared to extracts from all other treatments except those from plants grown in media amended with calcium silicate. At 7 dpi in inoculated Sicot 189 plants, β-1, 3- glucanase activity was greatest in root extracts obtained from plants treated with either calcium silicate or calcium sulphate. Few significant differences between treatments in terms of chitinase and β-1, 3- glucanase activity were detected in Sicot F-1 plants, despite there being significantly higher levels of each of these enzymes in root and shoot extracts obtained from this cultivar. No significant induction in the activities of these enzymes was observed in mock inoculated plants treated with either silicon source, suggesting the possibility of a silicon-induced priming effect.

Objective 6 – Disease assessment trials and mineral analyses of Sicot 189 and Sicot F1 treated with 2 forms of silicon to determine the effects of silicon treatments on these aspects

Two cultivars with differing resistances to *Fov* were treated either with potassium silicate (Kasil) or calcium silicate (wollastonite) prior to inoculation with the pathogen; with their subsequent response to infection rated in terms of vascular discolouration and percentage of plants infected. Additional controls were included to determine the possible effects of either potassium or calcium on disease suppression in this interaction. Vascular discolouration was significantly reduced in the more resistant cultivar, Sicot F-1 following treatment with soluble potassium silicate, compared to mock inoculated plants or inoculated plants treated with potassium sulphate. Vascular discolouration of potassium silicate treated Sicot F-1 plants was also reduced compared to that observed in plants treated with calcium silicate, though not significantly. No significant differences between treatments were observed in the moderately resistant cultivar, Sicot 189. Assessment of plants from both cultivars showed that potassium silicate and potassium sulphate treatment of both inoculated and mock inoculated plants significantly increased dry weights compared to all other treatments. Nutrient analysis of shoot tissue demonstrated that the regular addition of soluble silicon to media increased uptake, with significantly more silicon present in shoots of potassium silicate treated plants of both cultivars, compared to that observed in all other treatments, including calcium silicate. Results suggest that the addition of soluble silicon may enhance the resistance of Sicot F-1 to infection by *Fov*, and improve the growth of plants from both cultivars.

Outcomes

5. **Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.**

The information delivered by this project will contribute to future research projects involving investigations of silicon and Bion as control measures for *Fov*. The results obtained support the requirement for further research into the area of silicon application through both glasshouse and field based trials. An investigation of different sources of silicon for field application is required to determine the most feasible and effective forms for this situation. Also, an investigation of the effects of silicon on different cultivars, including transgenics, is advisable as different cultivar responses were observed in this study. The objectives of the project were to investigate the effects of silicon on histological and molecular cotton defence mechanisms. This was achieved and the results obtained support more research into this area to develop a method or product that can be effectively utilised as part of integrated disease management programmes in the future. Future research under consideration presently is to investigate the combined effects of Bion and silicon on *Fov* infected cotton, and to apply silicon prior to hill formation to address previous issues encountered with application methods. This should ensure more effective and uniform uptake of silicon by plants in the field so that this elements impact on disease development, yield and fibre quality can be adequately assessed.

6. Please describe any:-

a) Technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

N/A

b) Other information developed from research (eg discoveries in methodology, equipment design, etc.)

N/A

c) Required changes to the Intellectual Property register.

N/A

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

The effects of two forms of silicon on cotton defence responses to *Fov* were investigated glasshouse and laboratory experiments. It was determined that soluble potassium silicate, rather than wollastonite, was more effective at enhancing histological and molecular defence responses. Of the two cultivars assessed, Sicot 189 and Sicot F-1, the latter less susceptible line showed a greater response to treatment with silicon than did the former. Silicon appeared to prime defence responses in cotton, meaning cotton plants treated with silicon responded more quickly and effectively to subsequent pathogen challenge. Silicon treatment did activate defence responses prior to challenge inoculation, meaning possible trade offs in yield and growth would not be problematic if silicon was utilised as a defence activating compound. Experiments with Bion demonstrated that application as a seed soak was most effective at potentiating defence gene upregulation and defence related enzyme activities in cotton infected with *Fov*.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

- Further studies are being planned by QPI&F researchers to continue examining the effects of silicon and Bion on cotton infected with *Fov*, with an aim to develop a treatment that could be applied at the farm level as part of integrated disease management strategies.

(b) for the future presentation and dissemination of the project outcomes.

- A project summary was recently submitted towards the Fuscom 2009 proceedings.
- A paper regarding Bion related research has recently been accepted for publication
- Further publications regarding silicon associated research are being worked on presently.

(c) for future research

- Further studies planned by QPI&F staff

- Opportunity for further PhD or postdoc projects centring on silicon and Bion application.

8. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)

1. In Press (June 09) Physiological and Molecular Plant Pathology: 'Acibenzolar-S-methyl induced alteration of defence gene expression and enzyme activity in cotton infected with *Fusarium oxysporum* f. sp. *vasinfectum*'
2. Plans to submit papers based on defence gene analysis results, histological analyses and enzyme activity analyses in the future

B. Have you developed any online resources and what is the website address?

N/A

Part 4 – Final Report Executive Summary

The beneficial effects of silicon on the growth and disease resistance of important agricultural crops such as sugarcane, rice, wheat and barley are well documented; with large scale application of silicon in the form of slag or other silicon containing compounds increasingly common in some systems. Research was carried out to determine if similar effects could be induced by silicon treatment in cotton infected with *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), following on from promising results obtained in preliminary glasshouse and field disease trials. Molecular and histological analyses of silicon treated cotton infected with *Fov* showed that silicon could enhance defence responses in both Sicot 189 and Sicot F-1 plants, with silicon having a more pronounced effect on the more resistant cultivar, Sicot F-1.

In summary:

- Silicon was more effective when applied regularly as soluble potassium silicate rather than as calcium silicate powder
- Disease assessment trials indicated that silicon had more of an effect on the resistance of Sicot F-1 rather than Sicot 189
- Silicon treatment followed by fungal inoculation potentiated defence gene induction in both cultivars
  –Sicot F-1: genes related to phytoalexin biosynthesis and the production of lignin and phenolic compounds
  –Sicot 189 –pathogenesis related genes – chitinase & osmotin
- Defence enzymes were induced following silicon application and fungal inoculation –peroxidase was strongly induced in Sicot F-1
- Silicon enhanced the production of phenolic compounds & lignin –more so in the resistant cultivar Sicot F-1
- The effects of silicon treatment were not observed until after inoculation with *Fov*– strongly suggesting that silicon primes induced defence responses in cotton infected with *Fov*

An additional aim of the research project was to assess the effects of Bion applied as a seed treatment or a foliar spray on defence gene expression and enzyme activity in cotton infected with *Fov*. Results supported the use of Bion as a seed treatment with increased peroxidise, chitinase and β-1, 3-glucanase gene transcripts observed in inoculated plants cultivated from seed treated with Bion, compared to inoculated plants receiving no additional treatment. The greatest increases in chitinase, β-1, 3-glucanase and peroxidise enzyme activity was also observed in root and shoot tissue from Bion seed treated material inoculated with *Fov*. Results obtained from silicon and Bion research support the importance of further research into the use of silicon as a control measure for *Fov*, and the continued use of Bion as a seed treatment for current control of *Fov* in the field. Please contact Jennifer Whan for more information (j.whan@uq.edu.au or jen.whan@jcu.edu.au)