COTTON RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

CRDC Project: CRC10C
CRDC Project: CRC26C

PROJECT TITLE: **Physiological and agronomic factors affecting the efficacy of Bt in transgenic cotton**

ORGANISATION: Australian Cotton Cooperative Research Centre
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January, August & Final Reports

Part 1 - Summary Project Details **REPORTS**

Please use your TAB key to complete parts1,2,4 & 5

CRDC Project Number: **CRC10C**
CRDC Project Number: **CRC26C**

January Report: ☐ Due 29-Jan-01

August Report: ☐ Due 03-Aug-01

Final Report: ☒ Due within 3 months of project completion

Project Title: **Physiological and agronomic factors affecting the efficacy of Bt in transgenic cotton**

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CRC10C

Aims:

1) To assess in detail the impact of agronomic factors on the efficacy and expression of both one (Cry 1Ac) and two gene (Cry IAc & CryIIA or CryX) INGARD cotton varieties
2) To develop a management package that optimises the efficacy of Bt in cotton varieties
3) To assist the overall coordination of agronomic and physiological research into the efficacy of Bt cotton

Staff: Jenny Roberts, Technical Officer (grade 2)

Principal Researcher: Dr Philip Wright, NSW Agriculture

Progress:

1. Establish field experiments on the impact of a range agronomic factors on Bt efficacy
2. Train staff in bioassay and qualitative ELISA techniques for assessing Bt

Detailed studies on the impact of plant density, plant growth regulators, waterlogging and herbicides on Bt expression where made on a range of cultivars. Measurements on plant growth were made along with repeated bioassays and samples were taken for quantitative ELISA. Transitory impacts on Bt expression were evident with some herbicide and waterlogging treatments. However, the most striking impact was that of plant density. Two cultivars (CS289i and S101i) were sown at 2, 4, 8, 12 and 24 plants/m. Early in the season Bt expression was poorer in plants at low densities, however this changed as the season progressed so that by the 7th of January plants at the standard industry density of 12 plants/m were those with the poorest expression (fig. 1). When the data were examined across the season it suggests an optimum plant density for Bt expression of about 8 plants/m. These results need to be confirmed over several seasons. However, they do show that there is potential to influence and manage Bt expression with agronomic practices.

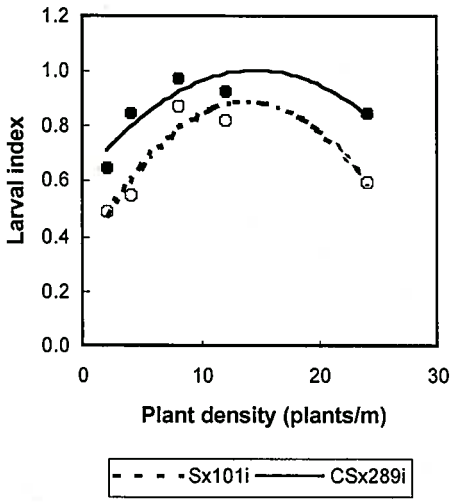


Figure 1. In both cultivars during January the poorest efficacy (high larval index) occurred at planting densities of about 10-12 plants/m.

Jenny Roberts has received training in both methods of Bt assessment.

Publications: A summary of results will be presented at the ACGRA conference

Presentations and Public Relations:

A presentation of results was made to the upper Namoi cotton growers

CRC26C

1. Background to the project.

This project follows on from project CRC10C that ceased with the loss of Dr Philip Wright from the Australian Cotton research Institute. Jenny Roberts, previously employed by NSW Agriculture and working within project CRC10C was appointed in January 2001 as a Technical Officer (CSOF3) by CSIRO Plant Industry to work on project CRC26C.

Quantitative Bt ELISA assays have only recently become available as a research tool. This research will not only assist cotton breeders in identifying lines which possess more Bt is present, but indicate where agronomic and environmental factors impact on the levels of Bt in the cotton plant. Effects of crop nutrition, time of sowing, soil condition, soil water management etc have suggested that Bt efficacy is compromised to some extent when environmental stresses are imposed on transgenic plants. Hence, productivity of some Ingard cotton crops has been substantially limited.

Various projects in the past have attempted to assess the extent to which agronomic factors influence Bt efficacy, including Project CRC3C (Dynamics of Bt protein in Ingard cotton: mechanisms of variable efficacy against Helicoverpa). Project CSE84C also conducts Bt ELISA assays to assess cotton cultivars under varying conditions.

2. Project objectives.

The primary aim of this project was to assess agronomic factors influencing the amount of Bt toxin measured using quantitative ELISA assays. This would enable identification of factors in agronomic experiments which influence Bt efficacy. Sampling of experiments involving crop nutrition, plant population and growth regulators etc. may provide a reasonable guide to indicate factors that influence the efficacy of INGARD cotton and provide a basis for recommendations to growers.

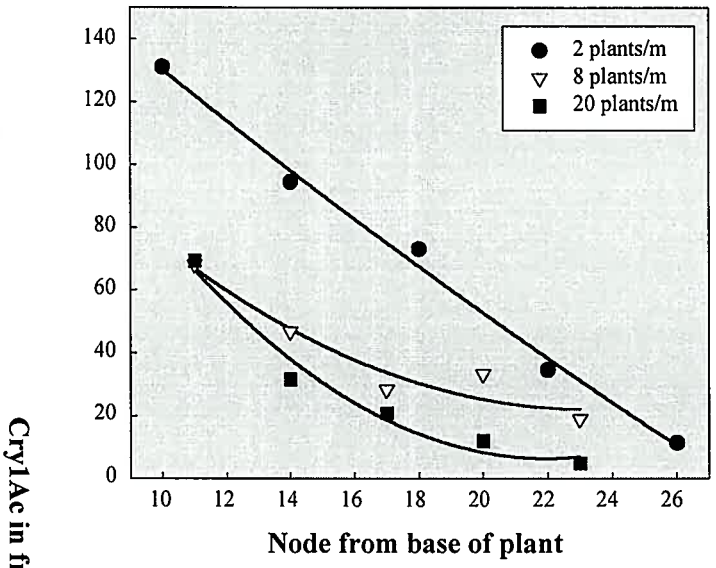
3. Methodology used.

Fresh plant material was collected from field experiments and was analyzed for Bt concentration using quantitative ELISA assays. Samples of plant material collected and dried in previous years from nitrogen and cropping systems experiments were also analyzed using quantitative ELISA assays.

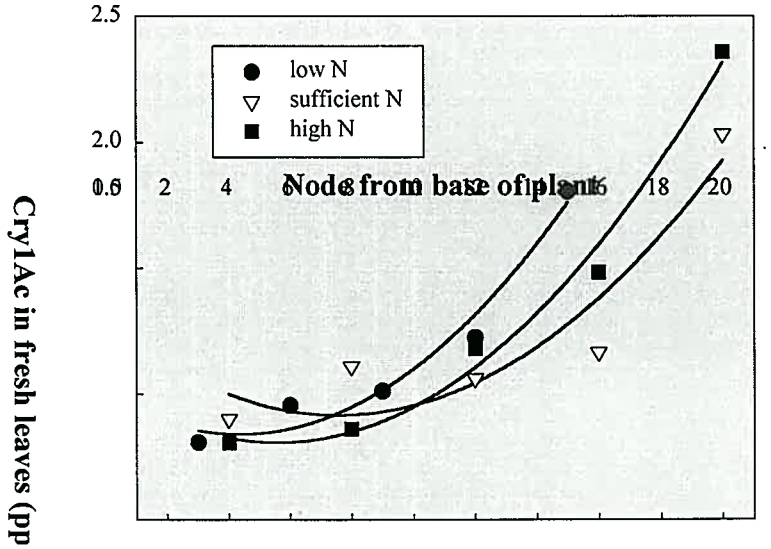
4. Detailed results.

Several experiments involving plant density, application of growth regulators and crop nutrition were sampled and Bt concentration in leaves was assayed.

Plant density had a significant effect on Bt concentration in cotton leaves, which indicates competition between plants for light, water, nutrients etc. This is being further investigated in the new project CRC34C. Plants grown at lower plant density had higher concentrations of Bt in their leaves.

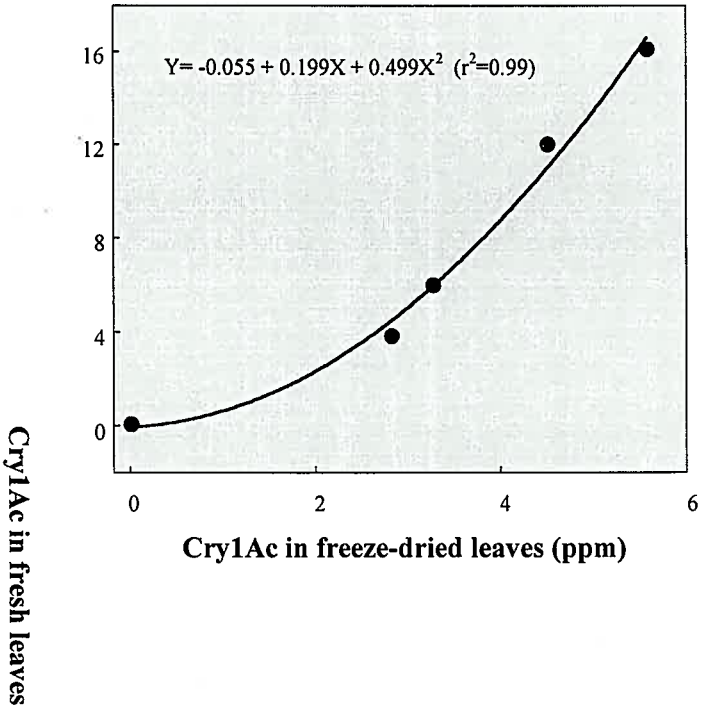


Nitrogen nutrition had little effect on Bt concentration in leaves. Lowest Bt concentrations occurred in the uppermost leaves, the opposite to N concentration, suggesting that poor N nutrition may not limit the production of Bt protein.



The application of a growth regular (mepiquat chloride) did not significantly alter the concentration of Bt in the leaves of those plants sprayed with the chemical.

Samples collected from experiments conducted in previous years may also be analyzed for Bt content using quantitative ELISA assays. This is dependent on the results of current experiments to determine the validity of using dried samples from previous year's experiments. Initial results indicate that Bt content is depressed with high temperature drying, compared with fresh or freeze dried samples, but comparative analysis of these samples from within experiments may help to determine the role of nitrogen nutrition, for example, on Bt efficacy.



5. Discussion of the results.

The results indicate that plant population affects the level of Bt contained in Ingard cotton leaves. Hence, growers should aim to establish sufficient, but not high populations of cotton plants to avoid over crowding and competition between individual plants.

Similarly, growers should aim to apply the appropriate rate of nitrogen fertilizer for each field situation, not only for the environmental reasons, but in order to maximise the utilisation of N in terms of optimising the production of the Bt protein.

Interestingly, the application of growth regulator mepiquat chloride had no significant effect on the concentration of Bt in Ingard cotton treated with this chemical.

While it is possible to use samples collected and dried from experiments conducted in previous years, substantially higher variation between replicate samples was observed. The samples indicate a similar trend as is discerned from current field experiments. Hence, it

is far more advisable to sample fresh material from existing experiments in terms of reducing sampling variation.

6. Assessment of the likely impact of the results and conclusions of the Research project.

The project has provided some evidence to indicate that some cultural and agronomic factors may reduce the effectiveness of Ingard cotton. As these factors are further researched, growers will be able to avoid situations where these circumstances occur. Further research is being conducted through project CRC34C to identify other factors that may impact on Bt efficacy.

7. Potential benefits to the Australian Cotton Industry and future research needs.

The cotton industry sees Ingard cotton as the basis for reducing the economic burden of Helicoverpa control and the environmental consequences of insecticidal sprays. Identification of means of realising the potential of Ingard cotton would assist the industry in economic terms and possibly help avoid problems of resistance to Bt genes.

8. Publications arising from the research project.

The results were presented at the CRC Research Review in July 2001.

9. Plain English summary.

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Technology is now available to assess the efficacy of Bt with the aid of commercial quantitative Bt ELISA assays. This approach has enabled assessment of various agronomic and management afctors which may have some impact on the efficacy of Bt.

This research has identified that factors such as crop nutrition, cotton growth regulators, plant desnity have small impacts on the concentration of Bt assayed in cotton leaves. Further research is required to ascertain the impacts of other factors such as temperature, solar radiation, herbicides, water management, soil quality including salinity and/or sodicity on Bt efficacy.

Identification of agronomic factors which impact on Bt efficacy will aid growers in their selection of fields for Bt planting and assist with management of those crops to realise the potential of Ingard cotton.