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COTTON RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

PROJECT TITLE: **DYNAMICS OF Bt PROTEIN IN INGARD COTTON:**
MECHANISMS OF VARIABLE EFFICACY AGAINST *HELICOVERPA*

PROJECT CODE: **CRC3C**

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DYNAMICS OF Bt PROTEIN IN INGARD COTTON:
MECHANISMS OF VARIABLE EFFICACY AGAINST *HELICOVERPA*

There were at least ten commercial fields of Ingard in the 1996/97 season with low efficacy pre flowering and requiring up to three insecticide sprays. Most other commercial Ingard fields required none or one insecticide when adjacent conventional cotton has been sprayed for *Helicoverpa* five times. Variability was also evident in 1997/98 although with lower pest pressure in some districts that year, performance of Ingard was relatively better

This project set up pilot studies to investigate physiological causes of variation in Ingard efficacy. The information will assist with crop management to minimise efficacy problems and plant breeders may utilise the findings to breed for improved stability of efficacy.

It was found that shade and low temperature could affect Bt levels and efficacy against *Helicoverpa*. Those preliminary studies require further study to confirm the result and to clarify the mechanism.

CRC linkages

This project is the initiative of the CRC in response to the problems experienced in 1996/97 with Ingard cotton. The fact that a coordinated project can be put together is a positive consequence of the CRC concept. Research into physiology issues is a sub-program of the Cropping Systems Program of the CRC.

Research proposal summary

Pilot studies were done in 1997. A significant proportion of the research was done in growth cabinet, glasshouse and phytotron where particular environmental conditions could be controlled. *Targeted outcomes were hypotheses of the cause of low efficacy in Ingard cotton which would contribute to field management of future Ingard crops as well as specific plant breeding strategies.*

Background

Ingard cotton was approved for limited commercial use (30,000 ha) in the 1996/97 season. There are various proposals and ideas on increasing areas for future seasons, eg 90,000 to 150,000 ha in 1997/98. Most regulatory and research concerns with the technology are to ensure a management strategy is in place to minimise the likelihood of *Helicoverpa* developing resistance to the Bt protein.

Previous (but limited) trials with Ingard have shown very good efficacy against *Helicoverpa* except after crop cutout when larvae survival has indicated a reduction in Bt protein levels and so in some instances at least one insecticide spray was required late season. Early season efficacy has even been good in Kununurra with extreme *Helicoverpa* pressure, but one occasion in January 1996 at Narrabri, showed a reduction in efficacy which was attributed to weather conditions such as cool temperature, cloud and/or waterlogging.

There are up to ten commercial fields of Ingard in the 1996/97 season with low efficacy pre flowering and requiring up to three insecticide sprays. Most other commercial Ingard fields have required none or one insecticide when adjacent conventional cotton has been sprayed for *Helicoverpa* five times. There is generally no question that all Ingard fields will have a good yield.

This situation of varying efficacy has raised a number of concerns, not the least being the dissatisfaction by cotton growers of the value of Ingard - requiring insecticide applications when they have been charged $245/ha for the licence. Of additional concerns are:

- Bad publicity with insecticide being applied to this technology, when it has been promoted as drastically reducing pesticide use. Overall the technology may not achieve that objective if the low efficacy is common.

- The Bt resistance management strategy relies on a high dose. With such high survival of *Helicoverpa* neonate larvae occurring this season, clearly the dose does not always qualify as 'high'.

There has been no clear indication of the primary cause of the low efficacy. Following are brief comments on some of the hypotheses:

Low temperatures. Nitrogen uptake and protein synthesis are tied to photosynthesis, so factors reducing crop growth, including temperature extremes and cloudy weather, could potentially reduce protein production, including Bt protein. Chilling injury, even to seedlings, has been shown to interfere with subsequent protein synthesis. Whether Bt protein is different in these reactions to plant protein is yet to be determined.

Waterlogging. Previous research has shown that waterlogging of cotton will reduce nitrogen uptake. The reduced nitrogen status could affect protein levels.

Growth dilution. A number of the problem fields are in the western side of the cotton producing belt (Bourke, St George, Mungindi). In most cases these fields are in very fertile rotations and have less cool weather than eastern locations. The reduction in efficacy with some of these fields coincided with rapid vegetative growth in November-December. Rapid expansion of leaves and squares may dilute the Bt protein to levels which allow survival of *Helicoverpa* larvae. A combination of cool weather, followed by good conditions for rapid growth, may exaggerate the single effects of each factor.

Variety. Bioassays have shown that there are differences between varieties in efficacy. Field observations are consistent with that data, although the major problem Ingard fields include most varieties. Some experimental varieties contain different promoters; the full difference between these promoters derived from different viruses has not been fully measured. Maybe they have different reactions to climate and plant growth stage or plant part.

Objectives and achievements

Study the effect of different environmental and plant stresses on plant protein dynamics.

Methodology

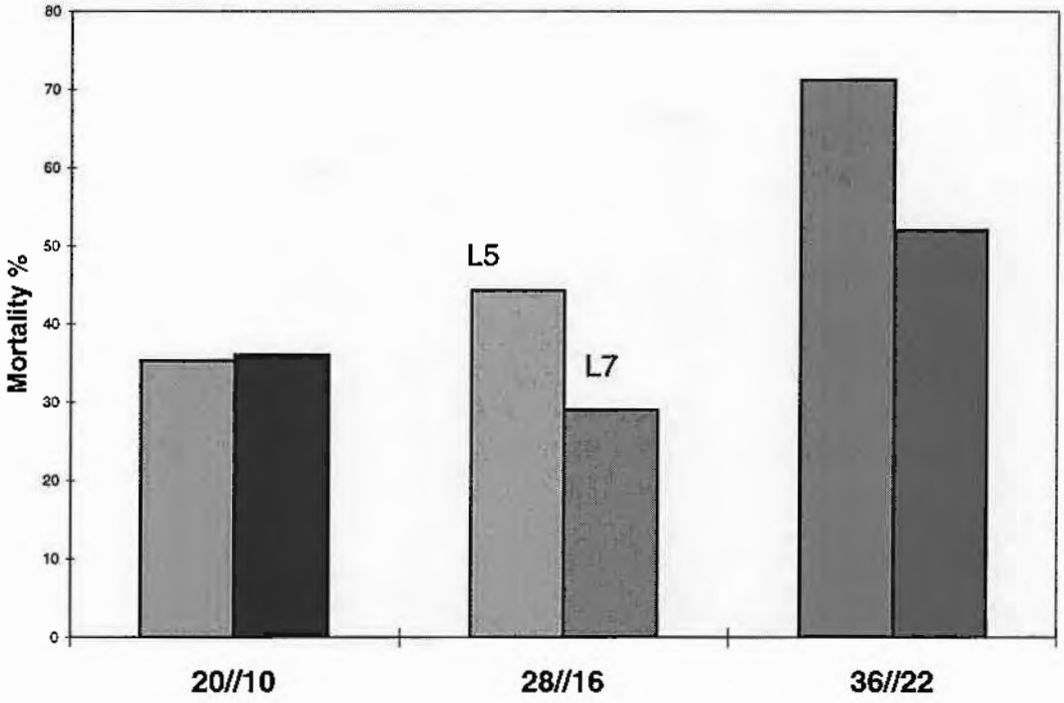
Field and controlled environment studies are required to separate the various environmental and plant interactions and their effect on Bt level/efficacy. A range of people/skills are to be integrated in the preliminary stage of this project.

- Field experiments on soil-plant nutrient status;
- Field or glasshouse waterlogging treatments;
- Cabinet studies on various combinations of temperature during germination;
- Phytotron studies on combinations of high/low temperature and light.

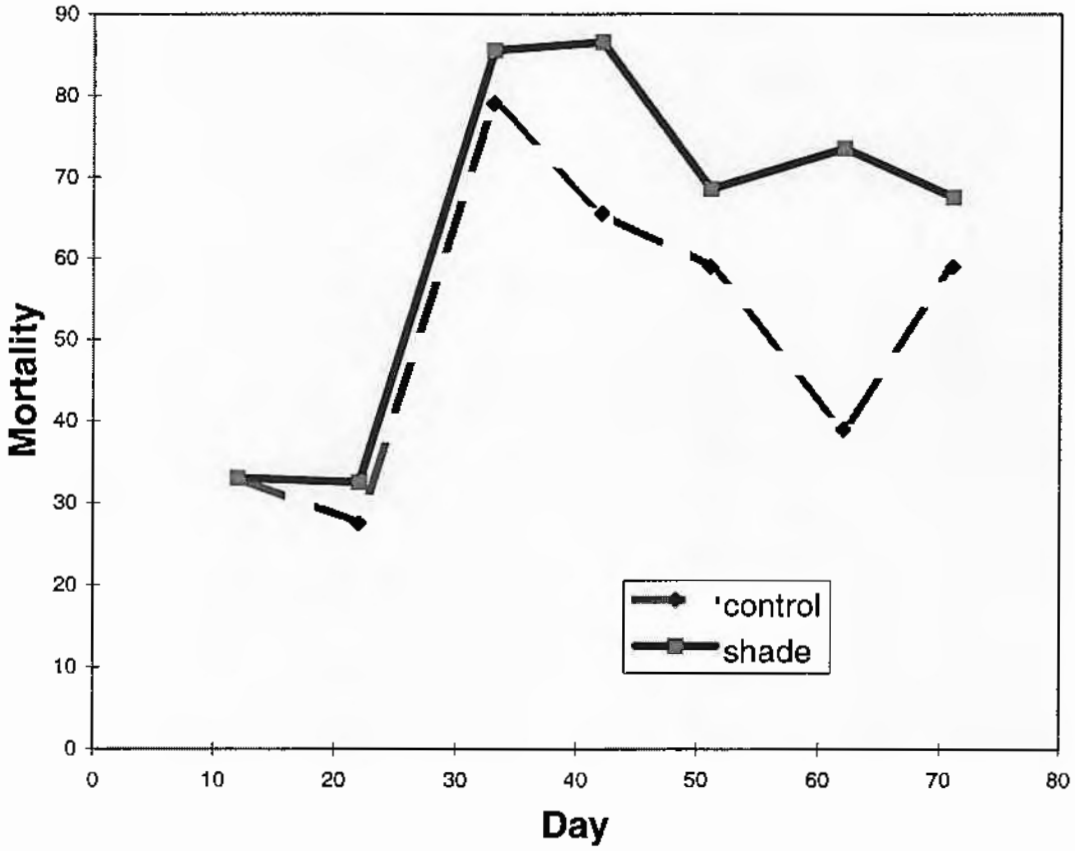
When critical combinations of environmental effects are identified, they should be repeated on the various combinations of genotype, promoter, gene and construct. Two gene Ingard should be studied as well, because we should be moving in that direction anyway. The two gene Ingard should therefore be seed increased (even if not fully developed yet for yield) to be included in extensive field trials for Ingard efficacy.

Results and discussion

Two pilot experiments were established in the phytotron in Canberra to establish known differences in light and temperature history on Ingard (Siokra V-15*i* plants). The following figures summarise the results.



Mortality of *Helicoverpa* larvae feeding on two different leaves (leaf 5 or leaf 7) for cotton plants grown at three diurnal temperatures for one week. All plants were grown at 28/16 for the rest of the experiment



Mortality of *Helicoverpa* larvae feeding on cotton leaves grown at full sun or in shade for one week up to day 10.

There was clear evidence for shade and higher temperatures to increase the subsequent efficacy of Bt in cotton leaves. These results may not be intuitive and the reduced efficacy for young leaves is not common, but the differences created by shade or temperature were clear. Explanations for the results need to consider a number of factors such as direct effects of treatment on Bt production, indirect effects such as growth dilution for rapidly growing leaves, and possible effects of treatment on activity of promoters associated with the Bt gene.

Recommendations

These data showed there was potential for single climatic events to affect Ingard efficacy. The preliminary experiments actually raised more questions than were answered and more detailed experiments are required, particularly on waterlogging effects. New experiments should be undertaken which would allow the direct effects of climatic factors to be separated from factors associated with the transgene construct-events.

The greatest limitation to progress in this research area is a coordinated approach. CRDC have funded a number of separate projects in this general area without addressing a balanced approach to research disciplines of field pest management (especially Ingard thresholds), plant physiology and molecular biology.