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Environmental stress and the efficacy of Bt cotton

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Bt or Ingard cottons have been grown commercially in Australia since 1996. They produce an insecticidal protein from the naturally-occurring bacterium, Bacillus thuringiensis, and were developed to help in the control of the main insect pests of cotton — Helicoverpa armigera and H. punctigera.

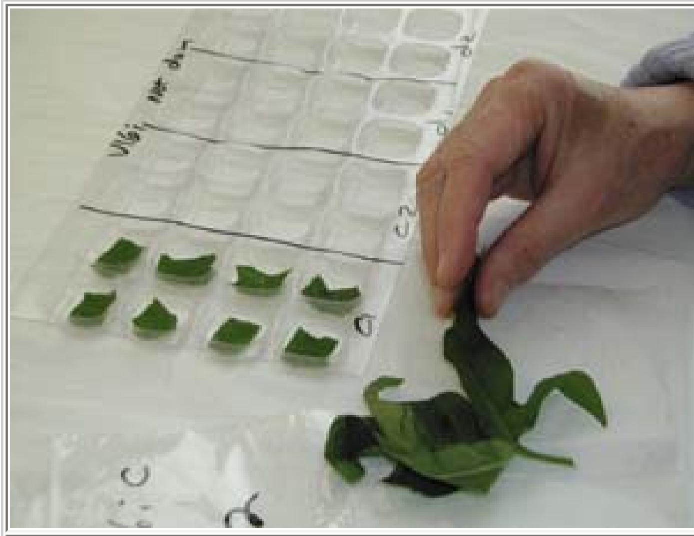
But the efficacy of Bt cotton against H. armigera has not been consistent over the growing season and scientists from the Australian Cotton CRC and CSIRO have been investigating why. A late season reduction in efficacy is observed repeatedly and variation in efficacy has also been recorded early in the season. This apparent instability of efficacy has consequences for the management of these pests.

On occasions, Bt cotton fields have unexpectedly required applications of insecticide for heliothis control in late spring or early summer in addition to the late season applications recommended as part of the resistance management strategy. This variability was documented by Gary Fitt in 1998. Reductions in efficacy could also affect the rate of evolution of resistance to Bt in H. armigera.

The variation in efficacy could be due to the inactivation of the introduced gene leading to a reduction in the amount of toxin produced. This effect has been reported in other plants as a response to environmental stresses, such as high light intensity or elevated temperatures.

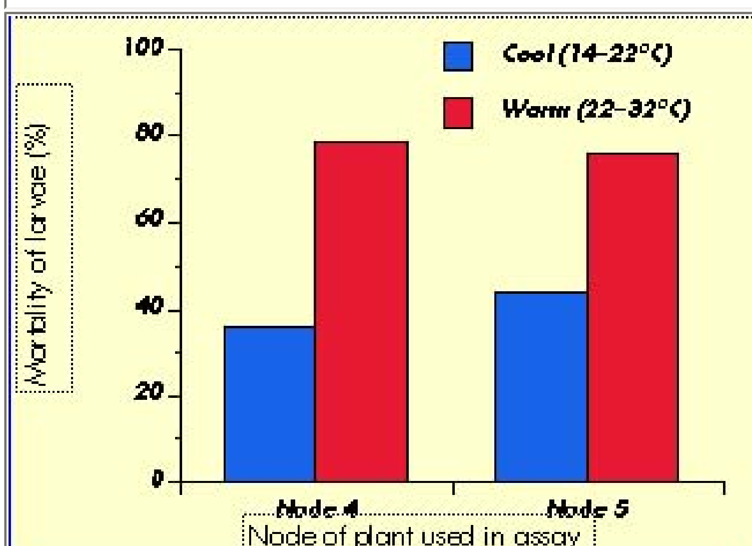
Perhaps in times of stress less toxin is produced. Scientists needed to uncover the basis for both the early season variability and late season decline in efficacy so that the information could be used as a basis for making decisions on the management of current Bt cotton lines and the development of new ones.

Gary Fitt, when discussing possible causes of variations in efficacy, had suggested that early season environmental factors could play a part but their role was unclear. It is extremely difficult to isolate and test individual stress factors under field conditions without the plants



Trays being prepared for bioassay. A portion of leaf was placed in each well which contained a little agar to keep the leaf moist. A single larva was placed in each well which was then sealed. Larvae were examined seven days later and scored as either alive or dead.

FIGURE 1: Efficacy of leaves grown under warm or cool conditions

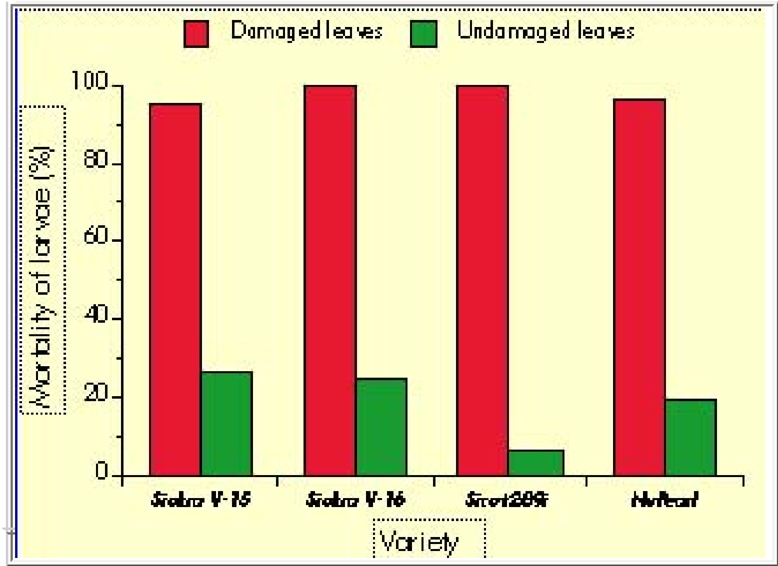


being also influenced by an assortment of other factors such as changes in light intensity, water and nitrogen availability or insect, wind and hail damage.

Therefore, experiments to test the interaction between efficacy of Bt cotton and the environment were carried out under precisely-controlled conditions in a phytotron or growth room. The help of Greg Constable in planning initial experiments to ensure that they were relevant to field conditions and current Bt cotton varieties was invaluable.

The results showed that environmental factors certainly affect the efficacy of Bt cotton plants. Temperature (high or low) during all stages of plant growth influenced the survival of H. armigera larvae on Bt cotton. It is clear that under certain environmental conditions, such as cool temperatures during germination and growth, the efficacy of Bt plants can be markedly reduced (Figure 1). But wherever an environmental stress induced a response, experiments showed that the amount of Bt toxin present in the stressed and control plants was comparable. It was concluded that the plants modified their metabolism in some manner and this change influenced the survival of larvae.

FIGURE 2: Efficacy following damage by H. armigera larvae



Plants produce many chemicals and some of these appear to limit damage from insects and other herbivores. The influence of stress is not totally unexpected as plants produce or increase the concentrations of secondary chemicals (phenolics and orthoquinones) during periods of physiological stress or physical damage.

Such secondary products have already been implicated as affecting the efficacy of Bt sprays against other moth larvae. The effect may be a direct one, where the secondary chemicals limit larval survival, or an indirect effect where the chemicals interact with the Bt toxin.

Prior to this study it was not known whether stress responses in cotton plants affected Bt toxin production as well as the production of plant chemicals, or whether the production of Bt toxin was independent of the stress response. This research has shown the latter is true.

One of the most interesting results was the response of cotton plants following damage by chewing insects. Plants were “challenged” by infesting them with either young H. armigera larvae or aphids for seven days, then the leaves were harvested and a portion fed to individual larvae. Larvae were sealed in individual cells and were scored as either alive or dead seven days later.

Prior damage by H. armigera larvae stimulated a marked improvement in efficacy — fewer larvae survived (Figure 2). Interestingly, no change in efficacy was observed following infestation by aphids which suck rather than chew. In an article in the previous edition of The Australian Cottongrower, Gary Fitt points out that conventional cotton plants increase their production of defensive chemicals when attacked by herbivores.

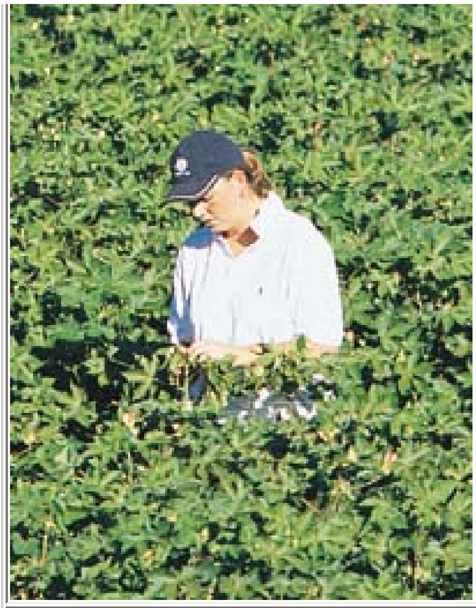
Unlike the stress-induced response, the major change in efficacy known to occur with the maturation of the plants was found to be due to a reduction in the amount of Bt toxin. Earlier work had shown that Bt concentrations decline markedly after squaring, and molecular analyses pinpointed the change to a decline in the production of mRNA (the template for the production of the Bt toxin).

It became clear that the expression of the Cry1Ac transgene fell away at this stage of plant growth. Expression of the gene is under the control of a “promoter”, and it was shown that expression of another gene influenced by the same promoter also declined at squaring.

One significant outcome of this work is the understanding the scientists now have of the importance of the role of the promoter in the production of Bt toxin in post-squaring cotton. This has triggered research into possible new promoters that will induce a more consistent production of Bt throughout the life of the cotton plant.

A second important outcome relates to the value of ELISA tests (Enzyme Linked ImmunoSorbent Assay) to measure the levels of Bt toxin and thereby predict efficacy against H. armigera. In general, the test can predict efficacy quite well as it detects the major change in the amount of Bt associated with plant growth stage. But of course it failed to detect the quite marked changes in efficacy associated with the plant response to environmental stress as the amount of Bt toxin had not changed.

This research concentrated on evaluating the interaction of stress and the mortality induced while Bt levels are high (before squaring occurs) and it showed that quite mild ‘stresses’ on what are otherwise cosseted plants, grown in the controlled experimental environment, are capable of inducing a response from cotton that causes marked impacts on insect survival.



Larval mortality is higher in the field.

Su Young scoring a surface treatment bioassay. This involves scoring dead and live larvae to gain an indication of the effectiveness of a Bt toxin against a resistant strain of H. armigera.

It is known that field-grown cotton is generally less favourable for H. armigera survival than glasshouse-grown plants. Plants in the field are exposed to a far greater temperature range and environmental assaults (such as wind and hail damage, waterlogging, nutritional stresses, insect damage and root damage) and so are perhaps in a continual state of stress.

It is possible that, under some conditions of plant stress, Bt toxin and the plant-produced chemicals will have an additive effect on H armigera mortality.

It may be that this additive effect will prolong the period when insecticidal sprays are not required even when the toxin levels start to decline.

Whatever the duration of the additive effect of the two mechanisms, it is likely to influence insect survival in Bt cotton crops.

This has two consequences.

First, it will reduce the period during the season when insecticides are required and, second, it will reduce the number of generations individuals carrying resistant alleles (to Bt) might be advantaged and thus increase in frequency.

These results raise the interesting question of what impact these non-Bt responses of plants would have on Bt resistant strains of H. armigera. In the future it should be possible to improve existing resistance management strategies for Bt cottons by enhancing the existing defensive mechanisms of plants.

Definitions

Gene: The fundamental unit of heredity. Genes are located on chromosomes.

Transgenic/transgene: An organism which has a foreign gene (transgene) incorporated into its genome.

Cry1Ac: The transgene which produces the Bt protein in cotton.

Promoter: Controls when and in which cells a given gene will be expressed.

mRNA: The class of RNA (ribonucleic acid) which copies genetic information from DNA and enables the production of the particular protein.

ELISA: A highly sensitive technique for detecting and measuring antigens or antibodies.

Alleles: Alternative forms of a gene. A single allele is inherited from each parent.

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