Changes in the efficacy of Bt cotton against *Helicoverpa armigera* (Hübner): Interpretation of assays

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ABSTRACT

The insecticidal efficacy of cotton varieties that produce Cry1Ac protein toxin declines over the growing season. Additional changes of shorter duration have also been recorded. Some laboratory assays may not measure the actual efficacy presented to lepidopteran insect pests by Bt plants. ELISAs for Bt toxin and protein and mRNA assays, indicate that the levels of these products in the plants are correlated with the decline in efficacy over the growing season, as seen in bioassays. However, proanthocyanins and other plant compounds affect the efficacy of the Bt toxin. Therefore, leaf or whole plant bioassays would be more accurate monitors of season long efficacy than Bt ELISAs or mRNA assays. Interpretation of assays becomes more complex when plants are subjected to environmental factors. Bt ELISA and mRNA assays were unable to detect changes induced by environmental factors. Similarly, diet incorporation assays using ground leaf material were unable to detect these changes as preparation of the leaf material altered insecticidal activity. Conventional cotton varieties (non-Bt) responded to environmental factors, implying that physiological changes within the plant affect efficacy. Whole-plant assays are therefore the most accurate measure of bio-efficacy of Bt varieties while other bioassays are useful in certain circumstances. Bt ELISA and mRNA assays are a reliable measure of these products only.

Introduction

Varieties of cotton that produce Cry1Ac protein toxin become increasingly less toxic to *H. armigera* larvae as the growing season progresses (Fitt *et al.*, 1994). Additional efficacy fluctuations of shorter duration have also been recorded within seasons, possibly as a result of environmental stress (Pyke and Fitt, 1998). In earlier work, we have found that higher temperatures and insect damage significantly increase efficacy, whereas lower temperatures decrease efficacy (Mahon *et al.*, 2002).

While investigating both short- and long-term changes in insecticidal efficacy, we found that although commonly used laboratory assays provide measures of efficacy, they can be an imperfect indication of the actual efficacy presented to lepidopteran pests by intact, growing Bt plants. Here we assess the techniques used to assay Bt cotton efficacy, which include biochemical assays, bioassays using ground leaf material, whole-leaf or leaf-discs and bioassays on whole plants.

Season-long Changes in Efficacy

Assaying mortality and protein and Bt titres throughout the season, revealed a general reduction in efficacy that is correlated with declining levels of protein in the leaves and also a decline in the amount of Bt toxin (Figure 1). However, some shifts in efficacy within the season were not reflected in changes in the titre of Bt as determined by ELISA. RNA assays indicated that Bt mRNA synthesis declines during the growing season (Finnegan *et al.*, 1998).

The concentration of proanthocyanins in leaves increases as the season progresses (Zummo *et al.*, 1984). Bioassays involving the addition of polyethylene glycol (PEG), which binds to proanthocyanins, indicate that pro-anthocyanins interact antagonistically with Bt toxin (Olsen *et al.*, 1998). This effect was most pronounced mid-season when proanthocyanins levels have increased and the level of toxin has not fallen substantially (Figure 2).

Olsen and Daly (2000) also demonstrated a plant-toxin effect related to plant growth stage. Experiments where Bt (either as ground leaf from Bt-expressing plants or the commercial product MPV®) was added to ground leaf from conventional cotton plants, indicated that the outcome of the bioassay is dependent on growth stage of cotton plants (Figure 3). The efficacy of the MVP® was 28 fold greater and that of Bt leaves was 57 fold greater when mixed with the leaves of pre-square plants compared to those from plants with bolls.

This is further evidence that the physiological background of the plant plays a major role in the efficacy of transgenic plants either by enhancing or sequestering the Bt toxin. Therefore leaf, or whole plant bioassays would seem to be more appropriate than Bt ELISAs or mRNA assays in monitoring season-long efficacy.

Intra-season Changes in Efficacy

Interpretation of assays becomes more complex when plants are subjected to environmental factors such as temperature change or insect damage, which would be the norm in the field and possibly the reason for the smaller shifts in efficacy seen during the season. Experiments investigating the effect of insect damage (from *H. armigera* larvae) on efficacy using leaf-disc bioassays, showed a clear effect of insect damage (a five-fold difference between treatments) (Figure 4). In contrast, mRNA assays (unpublished data) and Bt ELISA were unable to detect these changes. Surprisingly, diet incorporation assays using ground leaf material, were also unable to detect these changes, and the differences between treatments was only 0.8-fold (Figure 4).

Diet incorporation assays were also unable detect efficacy changes induced by modifying temperatures (unpublished data).

Further tests revealed that freezing or grinding Bt leaf material, (as in diet incorporation assays) altered its insecticidal activity in a manner that masked insect damage induced changes (Figure 5). While the effect of insect damage remained detectable on whole plants, the difference between treatments was markedly reduced in comparison with leaf-disc assays, presumably as a result of defences triggered within the intact plants by the insect infestation. Conventional cotton varieties (non-Bt) also responded to insect damage. Figure 6 shows the results of an experiment where the freezing and grinding of leaves altered them in a manner that impacted on larval growth.

Clearly, shifts in the efficacy of Bt plants may not always be the result of changes in the amount of Bt present but can be due to unrelated physiological changes within the plant or plant material during assay preparation. Bioassays using ground leaf material are therefore inappropriate for monitoring changes in efficacy, especially those brought about by environmental factors.

Conclusions

Whole plant assays provide the most complete measure of efficacy of Bt varieties as they incorporate the full array of insecticidal components as well as plant responses to the insect infestation. Whole-leaf or leaf-disc assays provide a convenient snapshot of the efficacy of a plant at a particular time, but because they exclude the chemicals produced by plant's defence mechanisms during infestation, they are likely to overestimate insect survival. Bioassays using ground leaf material measure changes in toxin levels and some interactions with plant secondary chemicals, but other interactions can be masked. Bt ELISA and mRNA assays measure only those products and so cannot be used to accurately monitor overall efficacy. Conversely, leaf or plant bioassays cannot be used reliably to predict Bt toxin levels in the plants.

Acknowledgments

We thank Gary Fitt and Greg Constable for supplying the cotton seed, Janelle Scown, Anna Grabowski and Suzanne Young for their excellent technical assistance. This work was funded by the Australian Cotton Research and Development Corporation and the Australian Cotton Co-operative Research Centre.

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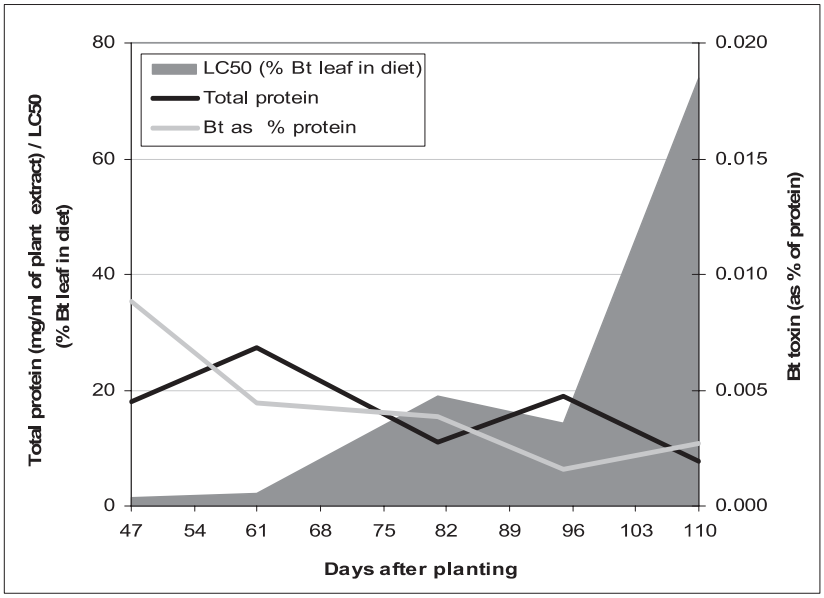

Figure 1.
Protein levels, Bt toxin and efficacy of two Bt (Cry1Ac) cotton varieties sampled over two seasons.

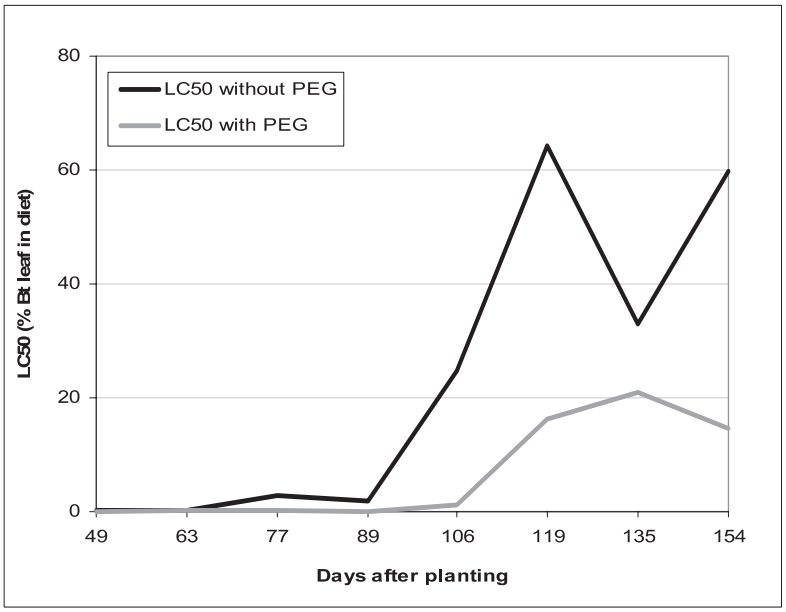

Figure 2.
Efficacy of Bt leaves collected over the season, determined by diet incorporation assays, with and without the addition of polyethylene glycol (PEG), which binds proanthocyanidin in the leaves.

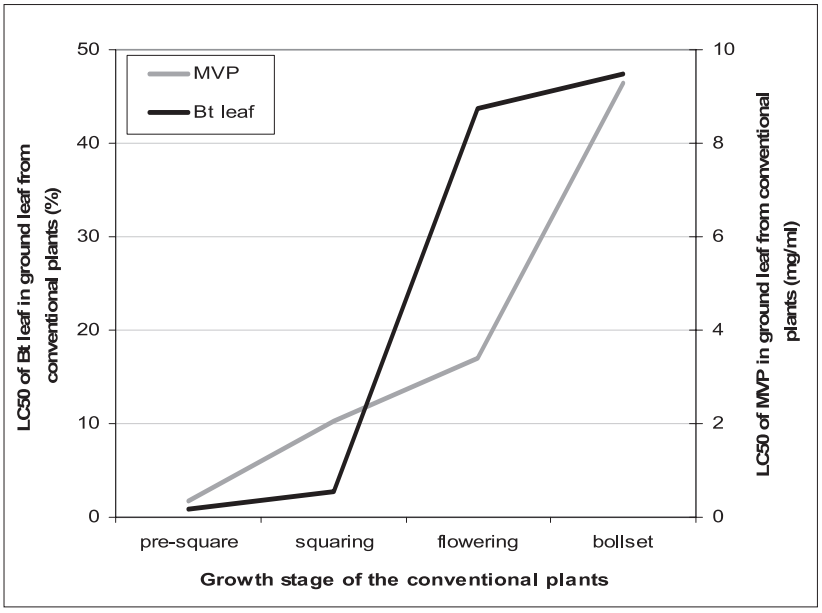

Figure 3.
Two sources of Bt toxin (Bt leaf and MVP) assayed in ground leaf material from conventional cotton plants at four different growth stages.

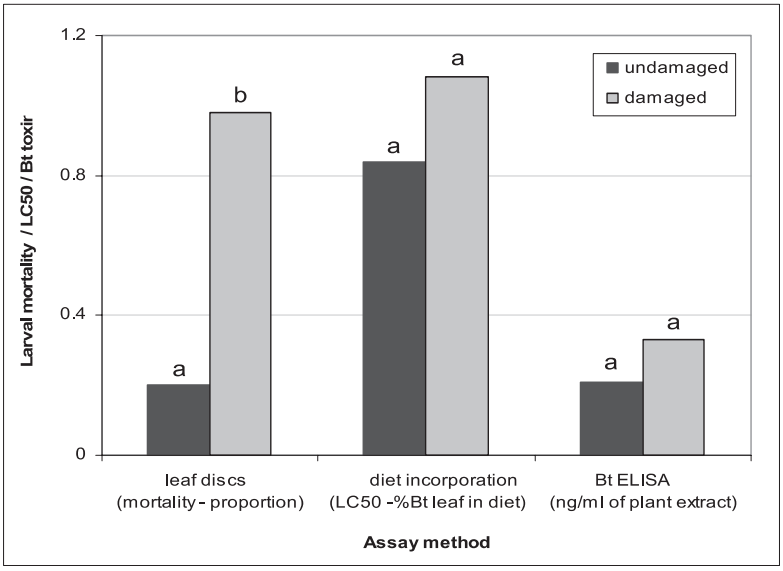

Figure 4. *Differences in the efficacy and level of Bt toxin in leaves from insect damaged and undamaged Bt cotton plants using different assay methods. Different letters above bars, within the same assay method, indicate significant differences (F-test, $P<0.05$).*

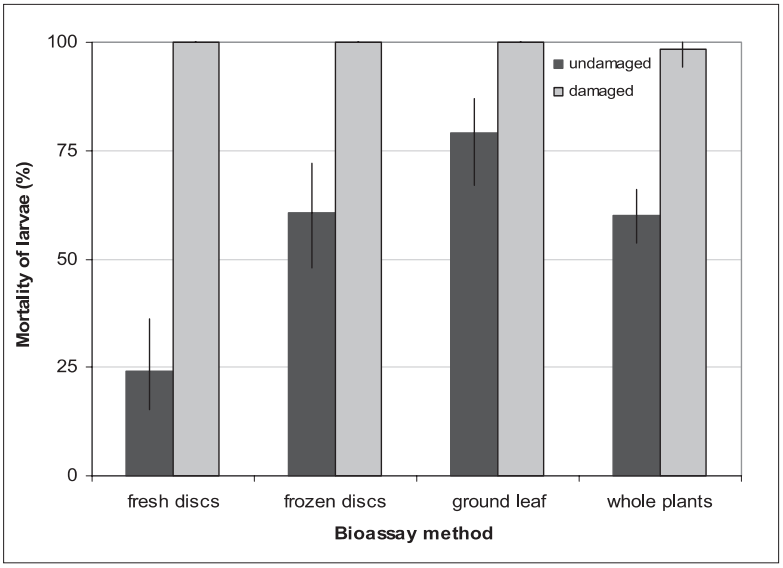

Figure 5. *Differences in the efficacy of leaves from insect damaged and undamaged Bt cotton plants with different handling of leaf material. Methods compared together within one experiment. Error bars represent 95% confidence intervals.*

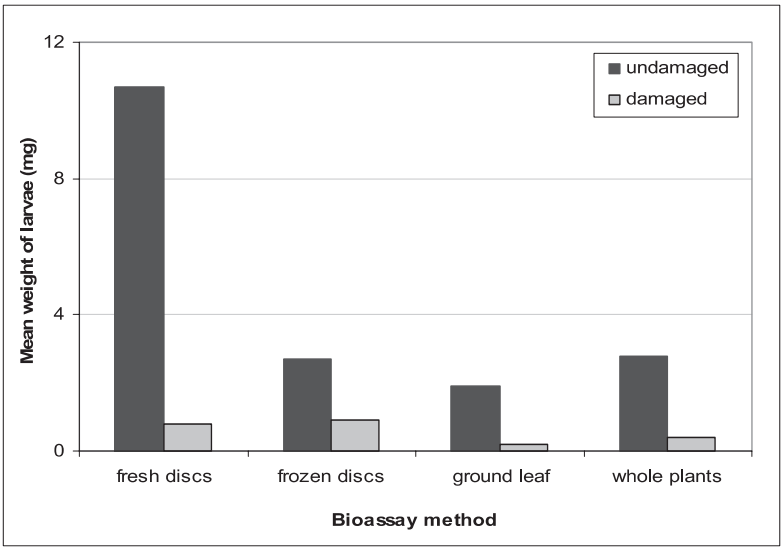

Figure 6. *Differences in the weight of larvae fed leaves from insect damaged and undamaged conventional cotton plants with different handling of leaf material from one experiment (individual weights of larvae not recorded).*