THE EFFECT OF COTTON CONDENSED TANNIN ON THE EFFICACY OF THE CRY1AC δ-ENDOTOXIN OF *BACILLUS THURINGIENSIS.*

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Introduction

The commercial release in Australia, in 1996, of transgenic cotton containing the cry1Ac gene of *Bacillus thuringiensis* Berliner var. *Kurstaki* (*Bt*), was a significant step towards reducing the use of conventional pesticides on cotton crops, for the control of *Helicoverpa armigera*. It is possible, however, that *H. armigera* could develop resistance to the *Bt* toxin, as has occurred with the use of other insecticides. The seasonal variations in the efficacy of *Bt* transgenic cotton, reported from field trials (Fitt *et al.*, 1994), could exacerbate the development of resistance (Daly, 1994), so there is a need for these variations to be better understood.

Since the level of tannins present in cotton plants tend to increase as the plant develops (Zummo *et al.*, 1984), they could be a factor in the variations in efficacy of *Bt* cotton. There are several publications on the effects of plant phenolic compounds on the efficacy of *Bt* against Noctuid larvae, reporting an enhancement (Ludlum *et al.*, 1990; Sivamani *et al.*, 1991; Gibson *et al.*, 1995; Morris *et al.*, 1995) or antagonism (Navon *et al.*, 1993). The activity of condensed tannins differs significantly, according to its plant origins and the insect species involved (Ayres *et al.*, 1997). We therefore selected four cotton cultivars, including two commercial lines, to test their effect specifically on the efficacy of Cry1Ac toxin against *H. armigera,* using bioassays. Polyethylene glycol (PEG) was used in a duplicate set of bioassays. Condensed tannins bind to PEG in preference to plant proteins (Jones and Mangan, 1977) and therefore negate the effect of the tannins. These bioassays could indicate if the tannins were actively involved in the changes in the efficacy of the *Bt* toxin.

Materials and Methods

The four cotton cultivars used were Siokra V15, Sicala V2, HT 35-14-3 (high tannin) and Imperial Red (high anthocyanin). The plants were planted outside in pots, in full sun, during

November and leaves harvested when the plants were at the first flower (mature) stage. Twenty to 24 plants per cultivar were bulk sampled. The leaves used were the first fully expanded leaf and the two nodes below that leaf. Leaves were collected onto liquid Nitrogen, ground, and stored at -60°C. A second set of plants was grown at the same time, as a control, in a growth room at 18 to 34°C and 16/8 hours day/night cycle. These were harvested before the pinhead square stage (immature), when tannin production would be low, and also at the same stage (first flower) as the plants outside. Four to eight plants per cultivar were bulk sampled. Sub-samples of all leaf material collected were kept at -60°C for tannin extraction and quantification. Field material tested was from the Sicala V2 Ingard cultivar (V2i), grown at the Plant Breeding Institute, Narrabri, during the 97/98 season. The first fully expanded leaves were collected onto liquid Nitrogen and freeze dried before storage at -12°C.

The bioassay method was based on the use of 32 celled rearing trays (Oliver Products Company, USA) containing an agar /sorbic acid layer on which the material to be tested was placed, separated by a self-adhesive paper dot label (Avery Dennison, Australia). Two variations of the method were used:

1. Cry1Ac toxin was added to ground leaf material. The toxin used was as a formulated product, MVP®, (Mycogen Corporation, USA). MVP contains Cry1Ac δ-endotoxin of *Bacillus thuringiensis* Berliner var. *Kurstaki*, encapsulated within killed and stabilised *Pseudomonas flourescens* cells (Soares *et al.*, 1994). Serial dilutions of MPV were prepared with autoclaved, filtered water. Aliquots of the doses were added in the ratio of 1:9 to weighed portions of the ground leaves. These were mixed and portioned out into the testing trays, one tray per dose. There were three or four doses per bioassay.

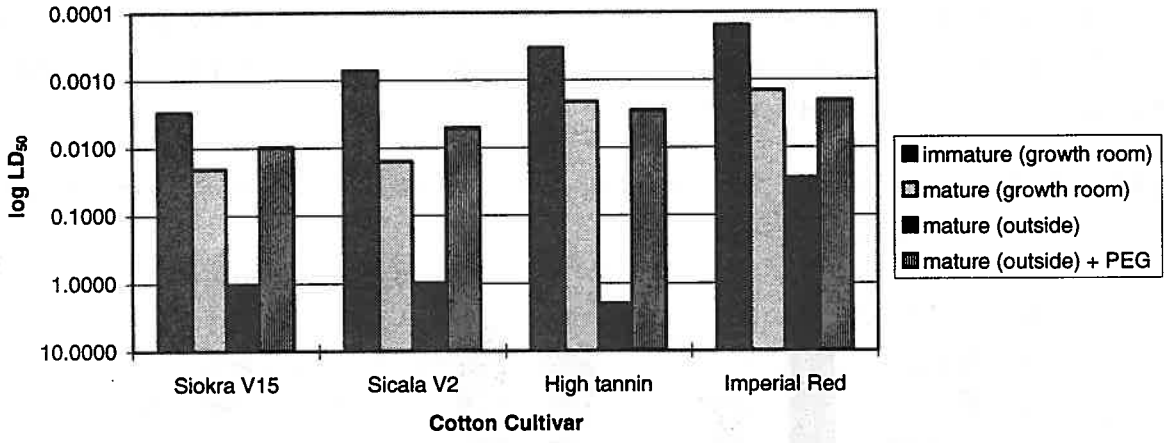
2. Freeze-dried *Bt* field material was incorporation into modified rearing diet. The diet consisted of 5g chickpea flour, 3g stabilised wheatgerm and 6.5g agar in 50ml of autoclaved, filtered water. Dried *Bt* leaf powder was weighed out and added to a proportion of the prepared diet to make up the highest dose. Serial dilutions were made with the diet as dilutant. The doses were portioned out into testing trays, with four doses per bioassay.

The *H. armigera* neonate larvae used were of a *Bt* susceptible, laboratory reared strain. They were reared on a diet modified from Teakle and Jensen (1985). Polyethylene glycol (mol. wt 8000), when used, was incorporated into the ground leaf or diet as 3% by weight. This is sufficient to bind the condensed tannin present in cotton leaves. Condensed tannin was extracted and quantified by the method of Li *et al.* (1996).

Results and Discussion

There was a drop in efficacy of 7 to 23 fold, for the Cry1Ac toxin (MVP) when mixed with ground leaves of immature plants compared to mature plants, of all cultivars grown in the growth room (Figure 1). The decrease in efficacy was larger when comparing LD_{50}s of MVP added to leaves of mature plants which were grown inside and mature plants grown outside, from 20 fold for Imperial Red, and up to 948 fold, for HT 35-14-3, the high tannin cultivar. Since plants were at the same growth stage, secondary compounds (eg tannins) stimulated by the higher light levels outside, could be contributing to the decreased efficacy of MVP. There was a large increase in the efficacy when PEG was added to the ground leaf of plants grown outside, ranging from 14 fold (Imperial Red) to 710 fold (high tannin). This indicated that tannins may be implicated in the low efficacy of Cry1Ac in leaves of mature plants, grown outside.

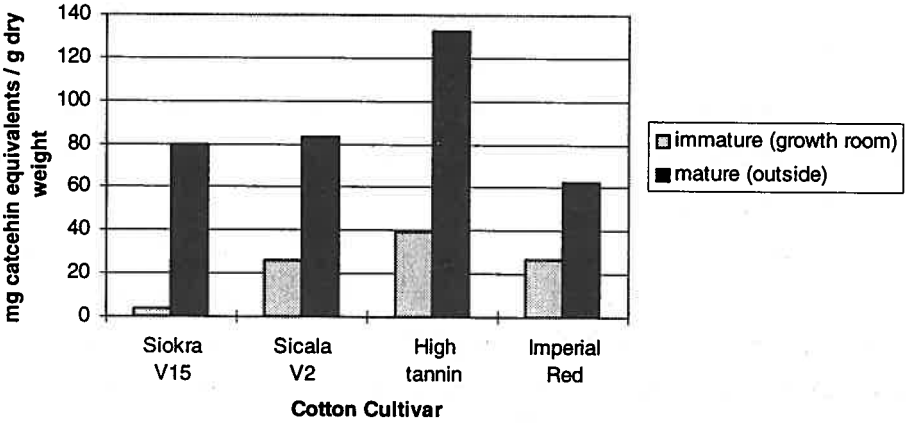
Figure 1. Effect of cultivar, plant growth stage, position and PEG on the LD_{50} of Cry1Ac toxin (mg/ml) for *armigera* neonate larvae.



Correlating the results in Figure 1 with the levels of condensed tannins measured in the leaf material used (Figure 2), showed that MVP added to leaves with low tannin levels (immature), was more efficacious than MVP added to leaves with higher levels (mature, grown outside). Of the leaf samples from the four cultivars grown outside, MVP was most efficacious in leaves of Imperial Red, which had the lowest tannin level, and least efficacious in leaves of the high tannin cultivar, which had the highest tannin level. This correlation also suggests that tannins may be actively reducing the efficacy of Cry1Ac toxins.

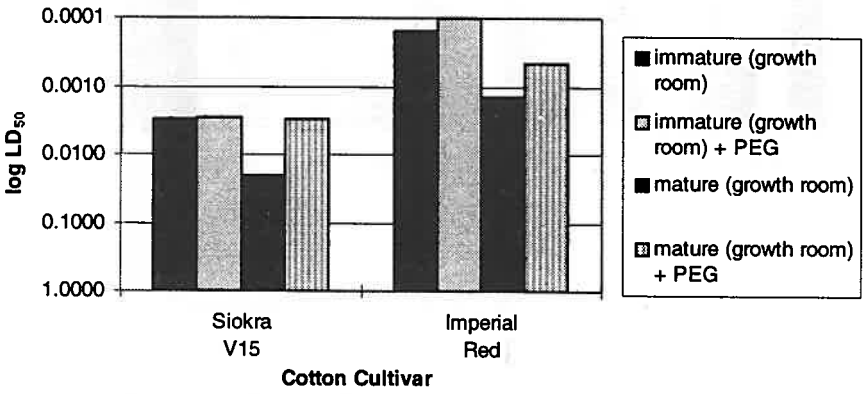
As a control, PEG was also added to bioassays with ground leaves from Siokra V15 and Imperial Red, grown in the growth room, to test the effect of PEG in leaves with relatively

Figure 2. Levels of condensed tannins measured in leaves of immature plants, grown in the growth room, and mature plants grown outside, for four cultivars.



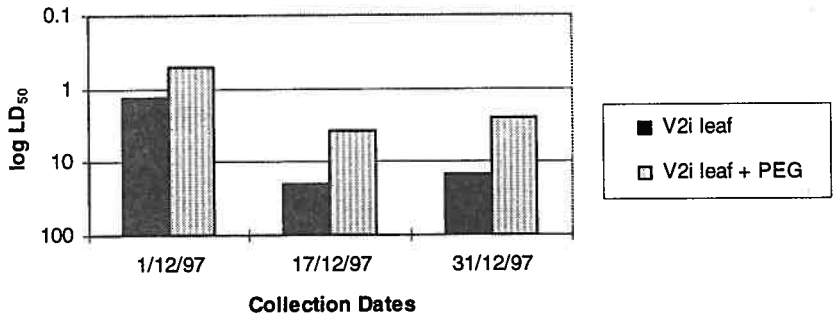
low tannin levels. There was less than a 2 fold difference in LD_{50}s for bioassays using immature plant leaves and 3 to 7 fold for mature plants (Figure 3). In controls set up with leaf or diet, with and without PEG, but no MVP, mortality remained low: 1% of 172 neonates without PEG and 4% of 145 neonates with PEG. This indicates that where tannin levels were low (as in the immature plants, Figure 2) or absent, PEG alone did not substantially increase larval mortality.

Figure 3. Effect of PEG on the LD_{50} of Cry1Ac toxin in leaves of two cultivars from the growth room.



When PEG was added to the diet incorporation bioassays of V2i field material, for three collections made in December 1997, a 2.6 to 5.8 fold increase in efficacy was seen (Figure 4). For the collection made on 16/1/98, the efficacy of the *Bt* leaves dropped below the sensitivity of the bioassay (LD_{50} of above 25% leaf in diet). Adding PEG to this bioassay did not increase the efficacy above this level. These results suggest that tannin may play a part in the seasonal decline in efficacy of *Bt* cotton in the field, but is possibly not the only factor involved.

Figure 4. Effect of PEG on the LD_{50} (% leaf in diet) of field collected *Bt* leaves



Overall our results suggest that condensed tannin may have an antagonistic effect on the efficacy of Cry1Ac toxin against *H. armigera*. However, they are not entirely consistent with the similar finding of Navon *et al.* (1993), who concluded that tannin, acting mainly as an antifeedant, was inhibiting the efficacy of the *Bt* toxin against *H. virescence*. We did not observe a reduction in feeding of neonate larvae on leaves with the highest tannin levels, Sicala V2 and HT 35-14-3, grown outside. Larvae on controls of these samples (no *Bt*, no PEG) grew faster than those on leaves of immature plants of the same cultivars, 77% reaching 3rd instar over the experimental period as opposed to 17%. It is possible that the condensed tannin in our experiments had a more direct effect on the Cry1Ac toxin as also suggested by Lüthy, et al. (1985) in experiments with *Peris brassicae*.

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