Assessment of Pest Resistance in Wild Australian *Gossypium* species using a *Heliothis* Cell Culture Assay

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Introduction

The wild Australian *Gossypium* species are subdivided into three sections, *Sturtia*, *Hibiscoidea* and *Grandicalyx*. The first two sections together contain five species, and include the widespread and locally abundant *G. sturtianum* and *G. australe*, respectively. Section *Grandicalyx* contains a further twelve species restricted to the Kimberley region of northwestern Australia and the Cobourg Peninsula in the Northern Territory. Until recently, the *Grandicalyx* taxa were poorly known, and phytochemical data have not previously been reported. Indeed, the phytochemistry of species belonging to sections *Sturtia* and *Hibiscoidea* is only now being investigated closely.

The genus *Gossypium* is characterised by its ability to synthesise gossypol and a series of related terpenoid aldehydes that are known to retard growth of several larval pests of cotton (Stipanovic *et al.*, 1977, 1990; Chan *et al.*, 1978; Elliger *et al.*, 1978; Hedin *et al*, 1983). That certain of the more accessible wild Australian species display high levels of resistance to *Helicoverpa* larvae has been known for some time (Benson *et. al.*, 1994), and we are currently investigating the mechanisms of this resistance using larval feeding assays. The CSIRO *Gossypium* germplasm collection now contains representatives of all Australian species currently known. However, the small number of *Grandicalyx* specimens grown for seed increase precludes the use of these species for larval bioassays, which require relatively large quantities of plant material. Given the potential of the wild Australian *Gossypium* species for integration into the breeding program, we are developing phytochemical screens and bioassays which can be conducted concurrently using a minimum of plant material. The use of tissue-cultured cells in place of intact *Helicoverpa* larvae enables such screening. Cells (in

this case obtained from ovaries of *Heliothis virescens* pupae) can be induced to grow and divide indefinitely under suitable conditions. After an appropriate period of incubation with a toxic substance added to their growth medium, the number of cells surviving can be correlated with the concentration of that substance. Besides enabling bioassays to be conducted where material would otherwise be insufficient, the method is precise, faster and less labour intensive than larval-feeding studies (Stipanovic *et al.*, 1990). A further advantage is that it enables the separation of chemical resistance factors from potentially confounding morphological factors, such as the presence of leaf hairs. This article details some preliminary results of our research utilising this technology.

Plant Materials

Seedling plants of the five *Gossypium* species belonging to sections *Sturtia* and *Hibiscoidea* were obtained from CSIRO Division of Plant Industry, Canberra and grown in the field at Myall Vale alongside the *G. hirsutum* and *G. barbadense* genotypes. Due to the rarity of *Grandicalyx* material, the twelve species in this section were sampled from glasshouse-grown specimens at the Division of Plant Industry facility at Black Mountain.

Freshly expanded leaves (usually node three from the top of the plant) were collected. Where available, squares (approximately 1/3 grown) and boll coats (from 1/3 to 1/2 grown bolls) were also sampled. Paucity of *Grandicalyx* material allowed for limited within-accession sampling, and in some cases data are based on leaves from one plant only. For *G. hirsutum*, *G. barbadense* and species within sections *Sturtia* and *Hibiscoidea* pooled samples from ten or more specimens were analysed.

Cell Culture Bioassay

Heliothis virescens cells were incubated with the same acetonitrile extracts used for chemical analysis (see below), at 10% in cell culture medium. Controls were incubated with the acetonitrile solution alone at 10% in culture medium. For details of the general method from which this bioassay was adapted, see Stipanovic *et al.*, 1990.

To date, cell-culture bioassay results have been obtained for four section *Grandicalyx* species (*G. londonderriense*, *G. populifolium*, *G. rotundifolium* and *G.*

species novum) leaf extracts (Figure 1). For comparative purposes, they were bioassayed alongside *G. nelsonii* and *G. hirsutum* (DP16 glandless, HG063 and Siokra 1-4) leaf extracts, and *G. barbadense* (Pima S-7) boll coat extract.

Cells incubated with extract from DP16 glandless leaf showed the least growth inhibition (approximately 10%) relative to controls (Figure 1). Since glandless cotton is free of terpenoid aldehydes this may indicate a minor source of resistance independent of terpenoid content (eg. tannins or flavonoids).

There is a clear trend of declining cell growth with increasing total terpenoid aldehydes. A major exception to this trend is *G. nelsonii*, which suppresses cell growth to a greater extent than would be expected on the basis of terpenoid content alone. This species was also extremely effective against *Helicoverpa* larvae in feeding trials (Benson *et al.*, 1994). We are currently investigating other sources of resistance in this species.

The cell-culture bioassay tests the ability of compounds to kill cells *in vitro*. This is quite different to the possible modes of action of the same compounds when ingested by larvae. Good agreement has however been shown between the cellular and whole-larval bioassays with the tobacco budworm, *Heliothis virescens*, from which these cells were derived (Stipanovic *et al.*, 1990). Our own preliminary data indicate similar correlations between the cellular assay and feeding studies using our Australian pest species *Helicoverpa armigera*. Further work is needed to fully correlate the two bioassays.

Chemical Analysis

The seventeen known wild Australian *Gossypium* species were analysed for terpenoid aldehydes. *G. hirsutum* (glandless DP16, Siokra 1-4 and HG063) and *G. barbadense* (Pima S-7) lines were also analysed. In each case 50 mg samples of freeze-dried, finely-ground material were extracted into 5 mL of 80% acetonitrile solution and analysed directly by High Performance Liquid Chromatography. The method was modified from Brubaker *et al.*, 1996.

Results of the chemical analysis are presented in Table 1. For clarity, data are presented for one species in each of sections *Sturtia* and *Hibiscoidea* and four *Grandicalyx* species (selected to indicate the range and qualitative diversity found in

this section). *G. hirsutum* and *G. barbadense* data are included for comparative purposes.

No terpenoid aldehydes were detected in glandless *G. hirsutum* (DP16). In healthy green tissues the glands are thought to be the site of both synthesis and storage of these compounds.

The analyses of Australian wild species were from single accessions, which may or may not be representative of their respective species. In addition, the very limited within-accession sampling from species in section *Grandicalyx*, has not yet enabled us to make generalisations about the constancy or otherwise of chemical composition within accessions. The data presented in Table 1 do however reveal considerable variation in the quantity of total terpenoids present across the taxa, and a high degree of qualitative diversity. *G.* species novum, an as yet undescribed species discovered in 1993, is unique in that it contains 38000 ppm or 3.8% terpenoids by dry weight, of which 82% is the single compound methoxyhemigossypolone. Further sampling is ongoing as material becomes available, and will determine whether these unusual patterns represent aberrant chemical composition or are truly representative of species/accessions.

Conclusion

There is a general trend of declining cell growth with increasing total levels of terpenoid aldehydes, irrespective of the large qualitative differences detailed in Table 1.

The data suggest that total levels are more important than differences in the concentrations of individual components, even where these differences are very marked. We must now determine whether this also holds true for larval-feeding assays. If so it may have major implications for the cotton breeding program.

Figure 1. **Relationship between cell growth and total terpenoid aldehydes extracted from nine cotton species or lines**

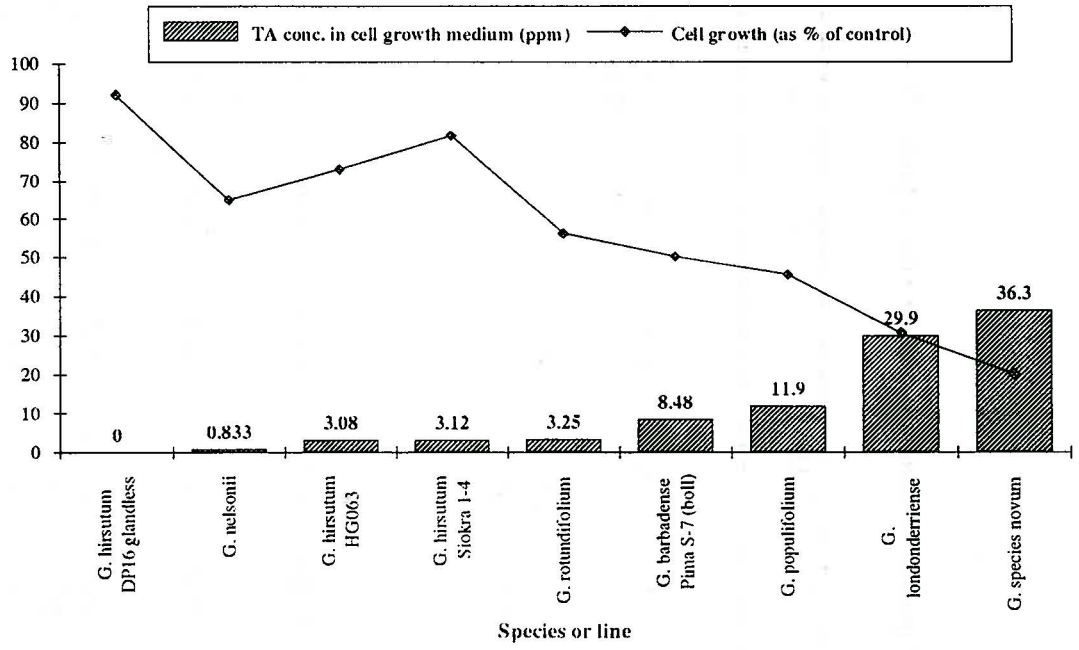

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Table 1. **Relative percentages and total concentrations (ppm dry weight) of terpenoid aldehydes in selected commercially-grown and wild Australian *Gossypium* species**

	HGQ	MHGQ	G	H4	H1	H3	H2	B4	B1	B2/B3	TOTAL PPM
Commercially-grown species											
G. barbadense Pima S-7 leaf	31.2	34.3	3.36	5.41	9.85	trace	1.33	5.76	8.78	trace	**6580**
G. barbadense Pima S-7 boll coat	11.1	23.1	1.28	9.42	18.2	0.97	1.27	8.12	25.4	1.05	**8500**
G. hirsutum DP16 (glandless) leaf	-	-	-	-	-	-	-	-	-	-	**-**
G. hirsutum HG063 leaf	25.8	-	6.88	8.17	20.5	8.84	29.9	-	-	-	**3110**
G. hirsutum Siokra 1-4 leaf	36.5	-	9.87	12.7	20.5	4.39	16.1	-	-	-	**3120**
Australian wild species											
Section *Sturtia*											
G. sturtianum leaf	30.3	63.4	trace	0.288	0.430	0.263	0.920	0.836	2.16	2.01	**11200**
Section *Hibiscoidea*											
G. nelsonii leaf	23.7	trace	5.59	23.3	41.2	trace	6.09	trace	trace	-	**801**
Section *Grandicalyx*											
G. londonderriense leaf	2.49	27.3	1.33	8.16	15.5	0.819	2.38	0.625	13.7	27.7	**29300**
G. populifolium leaf	2.16	-	1.97	26.9	45.2	6.34	17.0	trace	0.412	trace	**11900**
G. rotundifolium leaf	4.48	2.64	8.87	31.9	52.1	trace	trace	-	-	-	**3260**
*G.*species novum leaf	2.71	81.9	-	0.159	0.314	0.0596	0.183	4.18	7.53	3.09	**37600**

HGQ = hemigossypolone, MHGQ = methoxyhemigossypolone, G = gossypol, H1 - H4 = heliocides, B1 - B4 = methoxyheliocides

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