Development of resistance to insects in Australian cotton varieties.

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Introduction

The reliance of the Australian cotton industry on insecticides for the control of insect pests represents a major economic and environmental concern. In the face of pesticide resistance in *H. armigera* and increasing environmental problems associated with pesticide use, the development of cotton varieties with enhanced resistance to pests, particularly *Heliothis* and mites, is a major objective of CSIRO to help lessen dependence of the industry on chemical pesticides. Both conventional breeding and genetic engineering are being used to produce pest resistant cottons. Here we discuss work to compare the levels of pest resistance in a variety of cotton cultivars which bear either morphological, or biochemical factors for resistance and to identify characteristics which may be valuable for incorporation in the highly successful breeding program at Narrabri.

Host plant resistance has long been a focus of the Narrabri breeding program. From 1984 to 1989 we evaluated several potential pest resistance characteristics by focussing on a series of morphological characters (okra leaf, glabrousness, nectariless and frego bract) which individually or in combination impart resistance to *Heliothis* and/or mites (Thomson and Lee 1980, Thomson, Reid and Fitt 1984, Fitt 1986, 1987, 1991, Wilson and Fitt 1987, Thomson 1987). All combinations of these 4 traits were studied over four seasons in small plot experiments to identify the combinations having most impact on pest abundance and damage. The okra leaf and glabrous traits were shown to reduce populations of both *Heliothis* and mites, both in these small scale experiments and in subsequent commercial scale experiments. These, and other, characteristics are now being incorporated into high yielding commercial cultivars of cotton, some of which have already been released (Siokra's). Other earlier releases included Sicot 3, a glabrous/frego bract variety and other combinations of traits (okra/glabrous, okra/nectariless) will soon be available in commercial varieties.

In addition to these morphological characters, there are a range of biochemical (allelochemical) factors which offer possible sources of pest resistance in cotton (Hedin et al 1983, Cullam and Hedin 1982, Zummo et al 1984). Most notable among these are increased levels of tannins and terpenoids, particularly gossypol. However, preliminary research at NARS in the past have shown these factors to be associated with unacceptable yield penalties and breeders have not been willing to allocate resources to them. Most research with biochemical resistance has been done by US breeders, who have sought significant pest resistance in association with high yield. Some lines have now been developed which boast these qualities, having high levels of resistance to *Heliothis* in particular.

The aims of the study described here were:

(i) to evaluate the pest resistance of cotton cultivars bearing different combinations of morphological and biochemical resistance factors in field experiments so as to identify any biochemical resistance factors which might usefully be incorporated in the main CSIRO breeding program to complement the morphological factors already in use to enhance pest resistance and

(ii) to investigate the mechanisms of any pest resistance (principally to *Heliothis* or mites) identified in the field using biological and chemical assays to better focus future breeding efforts and identify mechanisms suitable for genetic engineering of cotton.

Genotypes Studied.

We assembled a collection of genotypes with cover a broad range of pest resistance mechanisms (Table 1). Four genotypes from the Narrabri program were included (Siokra

1-4 (okra leaf, delta smooth), Sicala 33 (normal leaf, delta smooth), N74 (okra leaf, glabrous) and OGF (okra leaf, glabrous, frego bract)), plus groups from Mississippi with Multiple Hostplant Resistance (the MHR lines); from Texas with high levels of tannins (HT's and CS8310) and a group from Louisiana selected for high levels of gossypol (HG lines). In addition we have added a glandless DP16 which lacks terpenoid glands altogether and is generally regarded as being highly susceptible to insect attack. This is used as our susceptible control.

Table 1. Cotton genotypes evaluated for agronomic performance and pest resistance in the 1990/91 and 1991/29 seasons.
* = included only in 1990/91 **= included only in 1991/92.

	Cultivar	Characteristics
Multiple Hostplant Resistance (from Mississippi).	MHR10	normal leaf/ unknown heliothis resistance
	MHR11	normal leaf/ unknown heliothis resistance
	MHR14 *	normal leaf/ sucking pest resistance
	MHR15 *	normal leaf/ sucking pest resistance
	MHR17	normal leaf/ unknown heliothis resistance
High Gossypol Cultivars (from Lousiana)	HG660	normal leaf/ high gossypol
	HG065	normal leaf/ high gossypol
	HG063	normal leaf/ high gossypol
High Tannin Cultivars (from Texas)	HT-35-5-1 Smooth	normal leaf/ high tannin/ glabrous
	HT-35-5-1 Hirsute	high tannin/ hairy
	HT-35-14-3 **	high tannin/ normal leaf, hairy
	CS8310 **	normal leaf/ mite resistance
Commercial or Australian-bred cultivars	DP 90	commercial normal leaf
	Siokra 1-4	commercial okra leaf
	Sicala 3-3	commercial normal leaf
	N74-720-199B	okra/ glabrous
	OGF Line 8	okra/ glabrous/ frego bract
Standard Cultivar	DP16 Glandless **	normal leaf, lacks gossypol glands

Methods

Agronomic performance and pest resistance of all the genotypes have been studied over two seasons in small, replicated plots grown under both fully sprayed and unspryaed conditions at NARS. The field and laboratory experiments examined:

(i) the abundance of *Heliothis*, mites and other pest and beneficial insects on unsprayed field plots of each cultivar throughout the season

(ii) fruiting pattern, final yield and lint quality in sprayed and unsprayed plots

(iii) laboratory studies of growth rates and survival of *Heliothis* larvae on leaves and squares of each cultivar at different stages of plant development through the season

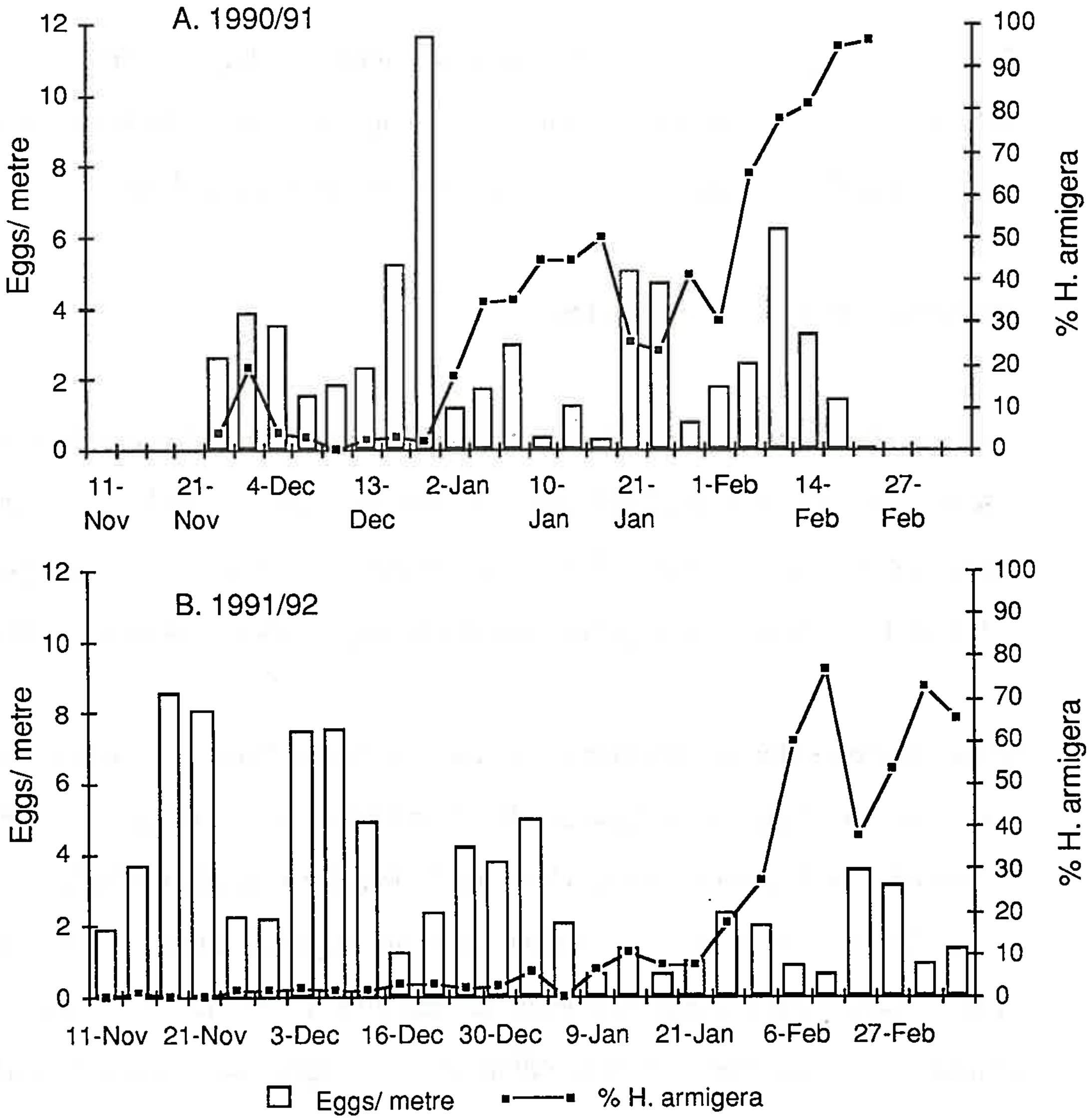
(iv) biochemical studies of the levels of gossypol, terpenoids, condensed tannins, nitrogen and sugars in each cultivar at different times throughout the season.

Field Evaluation of cultivars: abundance of *Heliothis*.

A total of 15 genotypes were evaluated in the 1990/91 season and 16 the following season (Table 1). *Heliothis spp.* were abundant early in both seasons, peaking at an average of 8-10 eggs/ metre in late December (Figure 1ab), and was generally more abundant late in the season in 1990/91 than in 1991/92. As usual there was a progressive increase in the proportion of *H. armigera* during each season (Figure 1ab), though this occurred somewhat later in 1991/92 than in 1990/91.

Measurements of insect densities showed consistent and significant differences between genotypes in the numbers of *Heliothis* eggs and larvae (Figure 2) and in susceptibility to mites. Egg and subsequent larval densities have been consistently lowest on glabrous genotypes, particularly the Okra leaf/ glabrous/ frego bract combination (OGF), whereas the MHR and high gossypol lines showed no evidence of non-preference resistance to

Figure 1. Seasonal abundance of eggs on unsprayed cotton plots and proportion of eggs which were *H. armigera* (averaged over all genotypes).



resistance to *Heliothis* (no reduction in numbers of eggs). MHRII consistently had the highest density of eggs.

Survival from egg to large larval stage was also highly variable between genotypes and in both seasons was lowest on the high gossypol and high tannin lines and the okra leaf genotypes in the field. Survival was highest on three of the MHR lines.

Resistance to mites and thrips.

Mites were considerably less abundant in 91/92 than in 90/91 (Figure 3), when severe infestations in the unsprayed field originated from an adjacent sorghum crop and caused defoliation of some genotypes. Mites were also difficult to manage in the sprayed plots in that year. In 91/92 mites were present but caused only minimal damage late in the season.

Some genotypes showed significant resistance to mites (Figure 3). The most resistant in both years were the okra glabrous (N74) and okra/ glabrous/ frego combinations, followed by the high tannin smooth lines and Siokra. Interestingly the high tannin hirsute (hairy) line was not particularly resistant, perhaps suggesting that it is the smooth leaf characteristic of the high tannin smooth line that gives it resistance rather than the levels of tannin. Most susceptible were the MHR lines, the high gossypol lines and DP90.

Thrips populations were sampled once at early squaring in 1990/91 and once at the 4 true leaf stage in 1991/92 (Fig 4). The early squaring samples showed considerable variation in thrips abundance between genotypes with okra leaf genotypes and the high tannin smooth most resistant. The sample in 1991/92 was taken earlier, before the leaf shapes had become distinguishable, and is probably a better indication of potential resistance to thrips. Some genotypes, such as high tannin smooth and HG660, appeared to have fewer

Figure 2. Genotypes ranked by the total number of eggs recorded on them during twice weekly checks over the season.

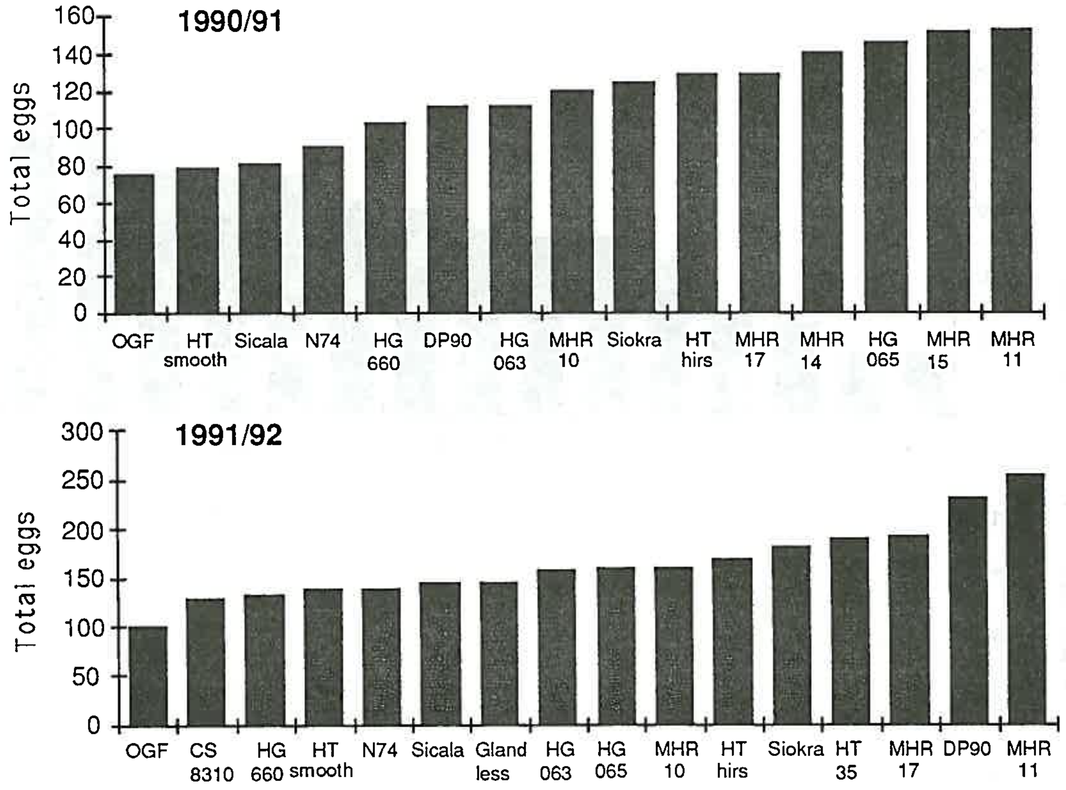


Figure 3. Total adult mites per leaf recorded on each genotype in weekly checks over the entire season.

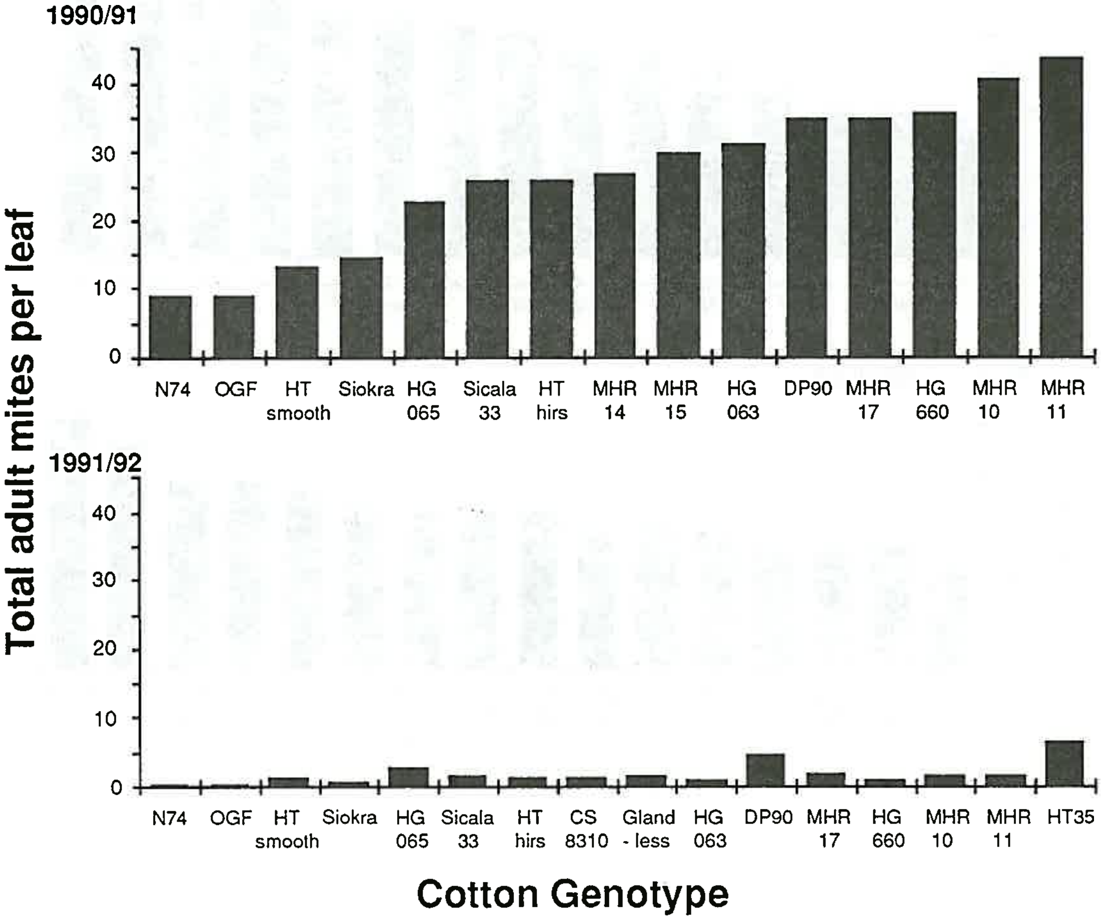
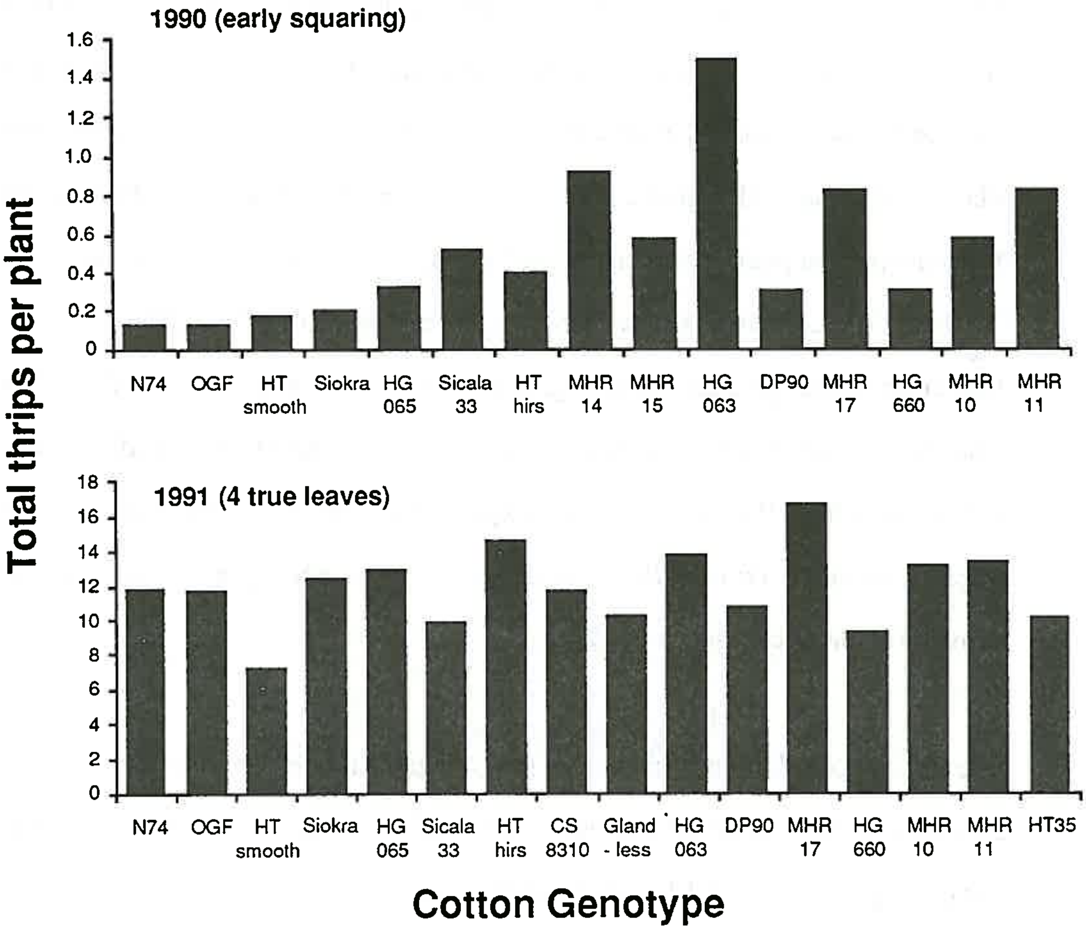


Figure 4. Total thrips (adults + larvae) per plant for samples at early squaring in 1990 and at 4 true leaves in 1991.



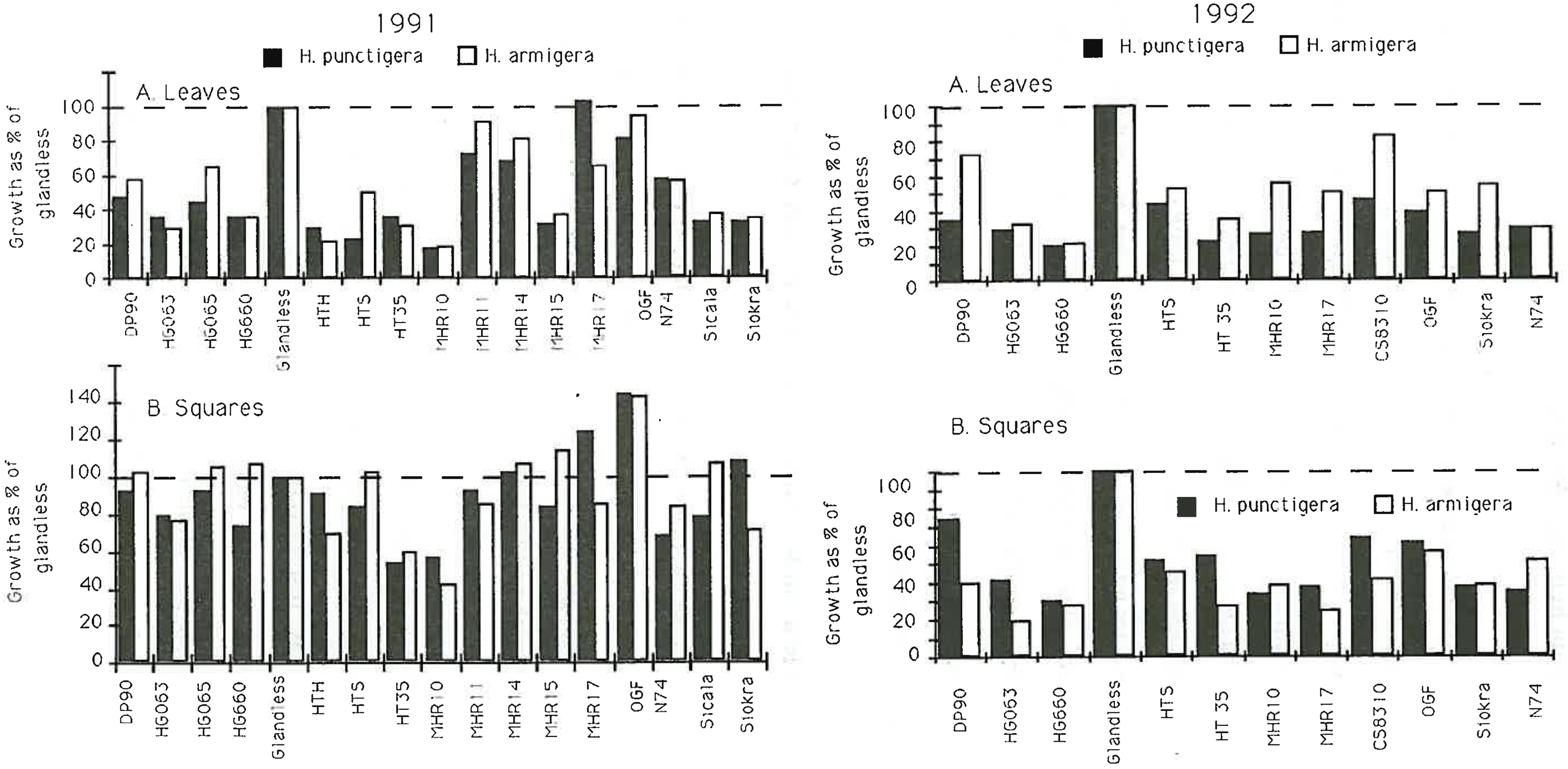
thrips in both seasons, but others were quite variable between years, especially the okra leaf genotypes. Resistance to thrips clearly warrants further study.

Mechanisms of resistance.

Observations of larval numbers and survival in the field reflect the actions of several interacting factors. To determine the importance of plant chemistry alone we conducted laboratory bioassays of larval survival and growth on leaves (node 3 from the top of the plant) and squares (1/3 grown) of most genotypes in each season. Material was collected from unsprayed plants growing in the field on 3 occasions in 1990/91 and 4 occasions in 1991/92. The collection times corresponded roughly to early squaring (December), early flowering (January), peak flowering (early February) and boll setting (late February/early March). Tissues from the glandless DP16 line were used as a standard. For simplicity, only results from the early February experiments are given here. Figure 5 shows growth rates of larvae of both *Heliothis* species on leaves and squares over 9 days relative to growth on glandless (set at 100%).

There was considerable variation in relative growth rates on leaves in both years (Figure 5a). Apart from OGF and some of the MHR lines, growth was markedly reduced. Surprisingly Siokra and Sicala limited growth as much as some of the high gossypol lines. Growth on leaves of the three high tannin lines was also severely depressed. Growth on squares was less variable in 90/91, but was generally reduced (relative to glandless) on most genotypes in 91/92. These results indicate considerable antibiotic resistance in leaves of most genotypes, probably due largely to terpenoids, and in squares of some lines.

Figure 5. Growth of *Heliothis* larvae reared on leaves or squares of various cotton genotypes relative to growth on the glandless genotype for tissues collected from plants during February 1991 and 1992.



Biochemical Assays.

In conjunction with the laboratory bioassays we collected leaf and square material for biochemical assays of total terpenoids, gossypol, condensed and hydrolysed tannins, sugars and nitrogen. Analyses of sugars and nitrogen indicate that all genotypes had adequate levels of these primary nutrients for larval growth, though there were seasonal and genotypic differences which may explain some of the variability in growth rates. In general levels of nitrogen declined in leaves and squares during the season, while soluble sugars remained steady or increased. Assays for gossypol and other terpenes are not yet complete but preliminary analyses indicate major variation in the diversity and concentration of terpenoids, particularly the so-called 'heliocides' which together with gossypol are toxic to *Heliothis* larvae. This variability warrants much greater investigation.

Yield under Sprayed and Unsprayed Conditions.

The bottom line in any assessment of pest resistance is of course final yield. Potential and relative yields for each genotype are summarised in Figure 6. Yields are all based on actual ginouts for each genotype. Ginout varies considerably, ranging from 31% for HT smooth to 42% for Siokra 1-4.

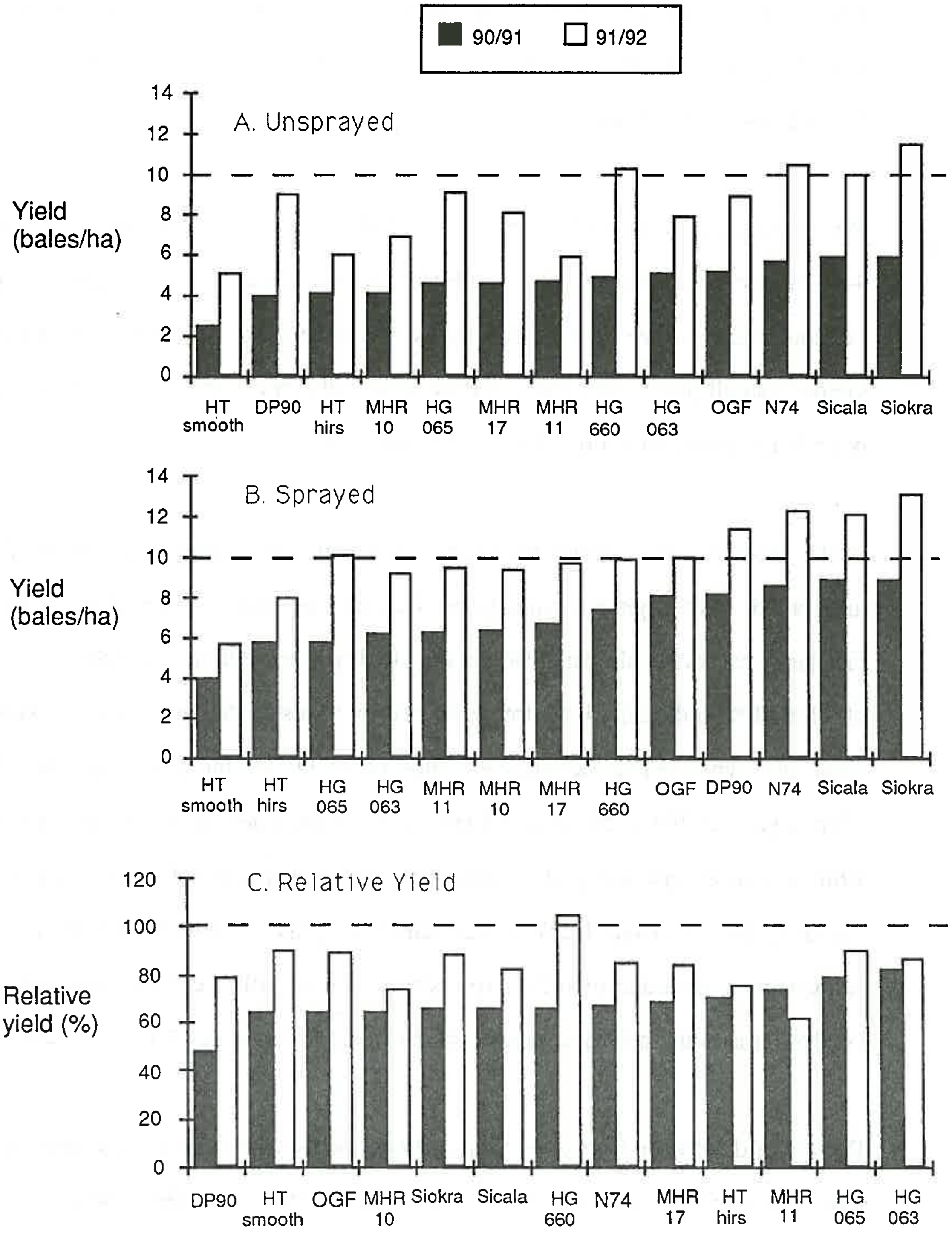
Potential yield is the yield when fully sprayed. The sprayed plots received a total of 11 sprays in 1990/91 and 9 sprays in 1991/92, while the "unsprayed" received 1 and 0 sprays respectively. Relative yield is the yield when unsprayed expressed as a percentage of the potential yield. Relative yield is thus a direct expression of the pest resistance of a genotype, independent of yield level, but is specific for the particular season and the pest pressure experienced. In both seasons the commercial cultivars (Siokra, Sicala, DP90) had high yield potential at 8-11 bales/ hectare, while the MHR and high gossypol lines had potential yields in the 6-9 bales/hectare range. One of the high tannin lines, HT smooth, performed poorly in both years despite a profuse fruiting behaviour. This line has extremely small bolls.

Yields were considerably higher in 1991/92 than in the previous year, in fact an average 44% higher in the sprayed plots and 80% higher in the unsprayed. Three genotypes had average yields above 10 bales/hectare when unsprayed. While we acknowledge that yields in small plots may be elevated by about 10% by a variety of factors, the important point in relation to insect resistance is relative yield.

In the 1990/91 season most most genotypes achieved relative yields of 65-70% when unsprayed. Two high gossypol lines, HG063 and H065, showed considerable pest resistance achieving almost 80% relative yield, while DP90 had the lowest relative yield of all at 48%, indicating its extreme sensitivity to insect damage. In the 1991/92 season most genotypes achieved relative yields of 75-90%. One genotype, HG660, had a relative yield of 101%, that is it yielded slightly more when unsprayed than when sprayed 9 times. This exceptional performance in the small unsprayed plots were also reflected in the bulk area (Siokra L22) of the same unsprayed field which averaged 10.08 bales/hectare (average of 6 four row strips picked with a commercial picker). In the 1990/91 season the bulk unsprayed cotton (Siokra 1-4) averaged 5.11 bales/hectare.

The major difference in relative yield between seasons and the changed performance of some genotypes is indicative of the differing conditions prevailing. Mites were a major problem in 1990/91, but were present only at low levels late in the season in 1991/92. This alone could explain the changed performance of DP90 (relative yields of 48% in 90/91 and 78% in 91/92), which is highly susceptible to mite and other insect damage.

Figure 6. Yield of genotypes of cotton in two seasons when (A) unsprayed or (B) sprayed, plus (C) relative yield (unsprayed/sprayed as a %). In each case genotypes are ranked according to 1990/91 results.



Similarly as Figure 1 shows there were fewer larvae during February (boll setting/ maturation) in 91/92 and very few of these were *H. armigera*. We have shown (Mares and Fitt unpub) that *H. punctigera* larvae are much less damaging to bolls than are *H. armigera*. These factors could explain the seasonal differences.

There are however, a multitude of other factors to consider, particularly when comparing genotypes. For example, there are major differences in blight tolerance among the genotypes being tested. All the CSIRO material is, of course, fully resistant to bacterial blight, whereas some of the US bred material is susceptible, some highly so. Assessments of blight infection and damage were made on all genotypes in the unsprayed plots in 91/92. The MHR genotypes and some HG genotypes were highly susceptible, having up to 45% infected leaves in January and 10-40% infected bolls in late February, which would undoubtedly have influenced their yield performance.

Conclusion

Our results illustrate quite clearly the value of various pest resistance characteristics and highlight differences in the way pest resistance can be achieved. The okra/glabrous genotypes (OGF & N74) achieve resistance through being relatively less attractive and hence receiving less eggs, while the high gossypol lines lead to reduced survival rates of larvae. The major point to note is that in both seasons the combination of high yield potential and some pest resistance in the commercial Australian bred lines gave them the highest yield when unsprayed. Of the lines with biochemical resistance factors the best were consistently the high gossypol lines HG660, HG065 and HG063. These lines may well have resistance characters worth incorporating in a high yielding Siokra background.

In the future we plan to extend our assessments of genotypic performance when unsprayed to several isolated locations to avoid the potential impact of drift and the difficulty of co-ordinating studies in areas surrounded by sprayed crops.

Acknowledgements

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