

Enhancing host plant resistance of Australian cotton varieties

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We are often told that cotton is highly susceptible to insect pests. In fact cotton is not the preferred host plant for the main pest, *Helicoverpa* spp. and the plant has a wide variety of defences against insects. For example, cotton is rich in terpenoid compounds like gossypol and the even more potent 'heliocides' which are toxic to *Helicoverpa*.

The terpenoids are stored in the small black glands that are easily visible over most parts of the plant. Cotton also contains significant levels of tannins that disrupt feeding and growth of chewing insects like *Helicoverpa* and other similar species.

There are also morphological characteristics such as leaf shape (okra vs normal), hairiness (glabrous vs hairy), bract shape (frego bract vs normal bract) and the presence or absence of nectar-producing glands on leaves or flowers. These morphological traits all change the plant environment for insect pests in ways that make the plant less attractive or expose the pest to additional mortality factors (predators, parasites, extremes of temperature), which reduce their survival or growth.

For some years CSIRO entomologists and plant breeders have collaborated to exploit these natural defences by researching and implementing natural host plant resistance (HPR) in cotton. This work has been supported in part by CRDC and has involved field trials at Narrabri, Dalby, Biloela and in the current year at Kingaroy.

In all cases our work has involved a broad range of cotton varieties and breeding lines (usually 40–60 different types each year) in which we compare pest abundance, insect damage and final yield in both fully sprayed and unsprayed treatments. The unsprayed plots fully test the capacity of most genotypes to tolerate insect pests. In most years we have also had a



HPR trials are sprayed with groundrigs under strict wind conditions to minimise disruption to the unsprayed plots.



treatment that was sprayed regularly with Bt to remove most *Helicoverpa*.

By using all three treatments we can estimate the effects of *Helicoverpa* or sucking pests on yield and so identify plant types with enhanced tolerance to specific groups of pests.

TRANSGENICS VERSUS CONVENTIONAL HPR

Much emphasis is now rightly placed on transgenic forms of resistance to pests, initially through the Bt genes (Ingard and Bollgard II) which provide dramatic improvements in tolerance for *Helicoverpa*. Bt genes represent a quantum leap forward in pest tolerance and many other new transgenes for insect control are in the pipeline.

Even so there is still considerable value in conventional HPR traits for other pests, and also for *Helicoverpa*. Conventional breeding for pest resistance makes small incremental improvements in the tolerance of new varieties to insect feeding or damage.

Any change to the plant that makes it less attractive to pests or more tolerant to damage will only enhance the value of genetically-engineered traits by providing a stronger, more stable basis on which to manage those genes.

Likewise, current efforts to understand and utilise the physiological capacity of cotton to compensate for damage (work of Dr Tom Lei and Dr Lewis Wilson of CSIRO Plant Industry Narrabri) is also important to the robustness of IPM systems. Compensation is a powerful attribute of cotton plants that allows them to recover from damage and is one of the main reasons why one to two *Helicoverpa* larvae per metre of row have been shown time and again not to do economic damage.

HPR is of course only one of many priorities for cotton breeders. They must maintain a focus on yield and fibre quality, seek to develop tolerance to challenging new diseases, consider regional adaptation, incorporate a range of transgenic traits and attempt to improve conventional tolerance to insects.

TOLERANCE TO HELICOVERPA

Norm Thomson's pioneering work with okra leaf, frego bract and glabrous traits firmly established the importance of these for *Helicoverpa* tolerance. That early work clearly showed very

Setting up a glasshouse experiment to measure cotton responses to insect damage.

FIGURE 1: Relative abundance of whiteflies on unsprayed plots of 36 cotton genotypes grown at Narrabri, 1995–96 season

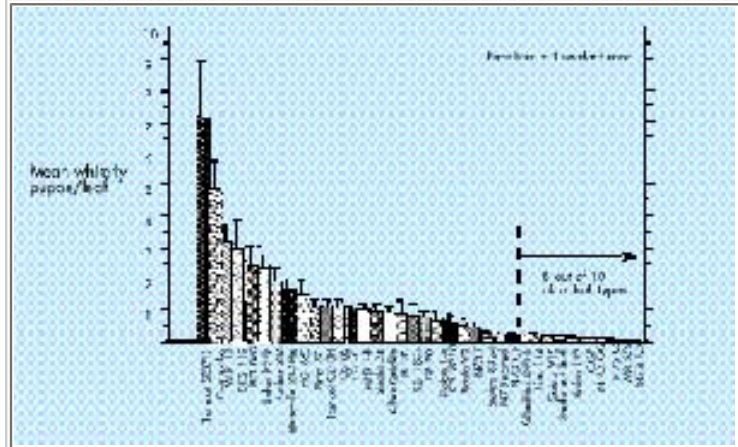
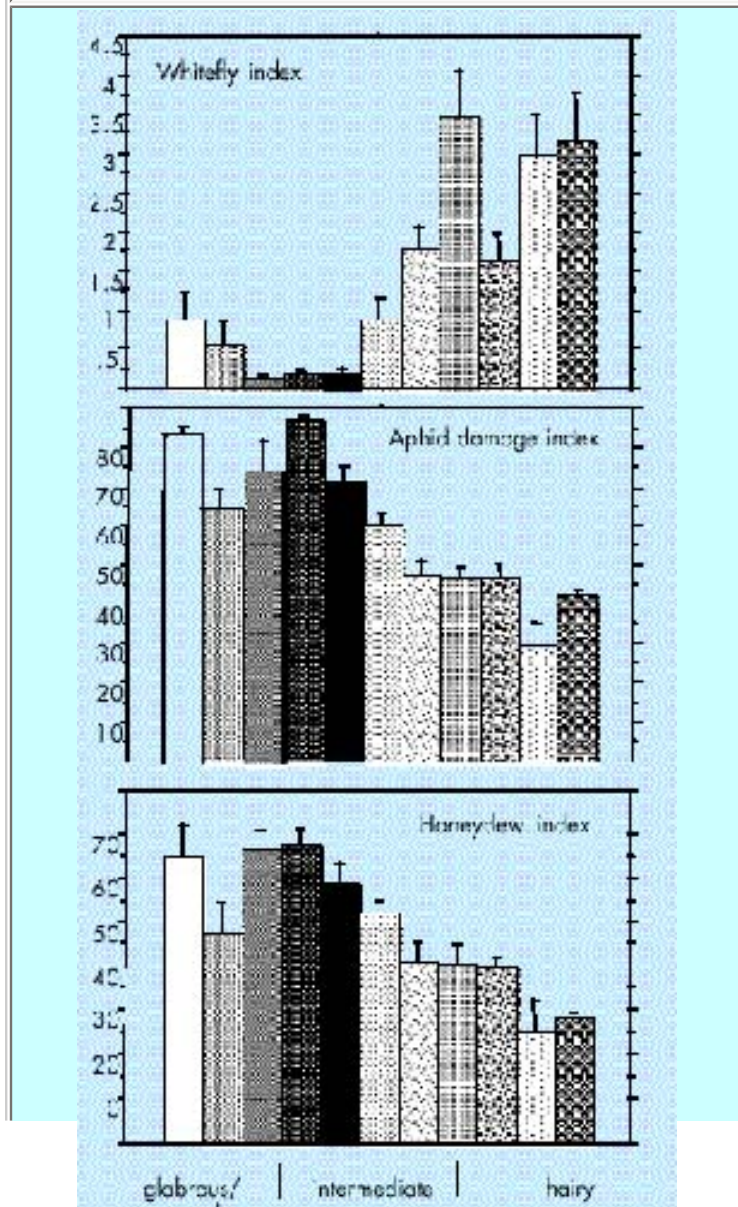


FIGURE 2: Effect of leaf hairiness on abundance of whiteflies, aphid damage aphid honeydew index (Data are means \pm standard error)



big benefits for okra under high insect pressure. In addition it showed agronomic penalties for frego and glabrous types. Our work has also identified high gossypol and high tannin varieties with enhanced tolerance to *Helicoverpa*. These traits have now been introduced into commercial varieties and also combined with Bt genes where there may be synergies that give additional control of *Helicoverpa*.

One complication here is that high gossypol and high tannin are often associated with a yield penalty, probably because of the metabolic costs of producing and maintaining these chemicals. Another complication is that some plant chemicals such as tannins may interfere with the activity of the Bt protein (work by Karen Olsen and Joanne Daly, CSIRO Entomology, Canberra already suggests this in Ingard varieties). Tannins can also be important for disease tolerance.

Clearly to get value from these biochemical HPR traits without compromising yield or Bt efficacy requires considerable skill in breeding and a lot of field evaluation. These new combinations are now undergoing field trials and may appear as varieties some time in the future.

TOLERANCE TO WHITEFLY AND APHIDS

Both aphids and whitefly may emerge as significant issues for the cotton industry. Aphids have developed resistance to a number of pesticides, while the whitefly (B type *Bemisia tabaci*) is a 'sleeper' for the industry and is now present in some fields in Emerald. We have shown that some morphological HPR characteristics can provide significant tolerance to these pests.

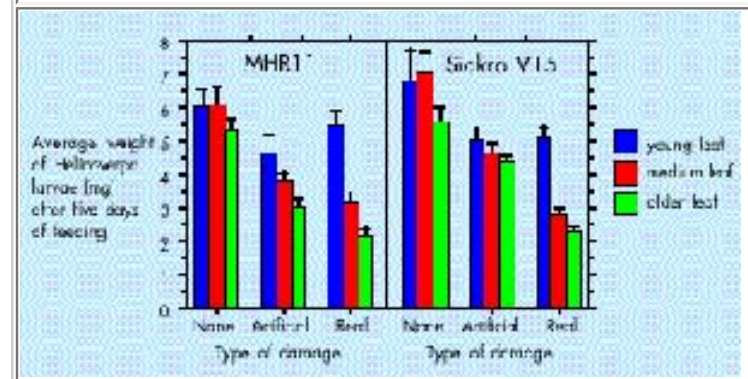
Figure 1 shows an index of whitefly numbers on 36 different cotton genotypes. The large variation in numbers between genotypes was related to differences in leaf shape and leaf smoothness. Eight out of 10 of the lines with the lowest whitefly index had okra leaves. On average these had just 12 per cent as many whiteflies as those with normal leaves.

Whitefly numbers were also affected by leaf hairiness. Much higher numbers of whiteflies occurred on hairy (hirsute) varieties with hair ratings of seven or above (Figure 2). Current commercial varieties have a hairiness rating of six (Deltasmooth), but the more glabrous types (very smooth, with ratings of two or less) clearly



Partitioning cotton squares into their many parts to determine where Bt protein and other chemicals are present.

FIGURE 3: Response of two cotton varieties to artificial or real damage to leaves



had fewer whitefly. Collaborative research with Dr Eric Natwick of the University of California has also shown much higher whitefly HPR with CSIRO okra leaf types (especially glabrous) when compared with normal leaf US lines.

One complication is that aphids show the opposite trend. Aphids were more abundant, did more leaf and stem damage and produced more honeydew on the more glabrous types.

We also know from our earlier work that glabrous types may be more susceptible to mirid damage. Obviously it is difficult to get one plant characteristic that will give resistance against all these pests!

MIRID TOLERANCE

Elsewhere in the world mirid resistance has been associated with exceptionally hirsute (hairy) cottons, with increased levels of terpenoids and with the nectariless trait. Because hairy cottons are not commercially acceptable in Australia (because of poor grade and susceptibility to *Helicoverpa*), we have focused on the nectariless trait for mirid tolerance.

Some nectariless/Bt combinations from the breeding program are undergoing their first field mirid evaluations this season. Yields of these types have been promising both locally and in the Ord. The mirid evaluation involves collaboration with Dr Moazzem Khan (DPI, Kingaroy) and Dr Paul Grundy (DPI, Biloela) to monitor mirid numbers in a range of lines.

'INDUCED' RESPONSES TO INSECT DAMAGE

Cotton is not a passive player in the game against pests. Like many plants, cotton is known to respond to damage from herbivores like *Helicoverpa* or infection by pathogens (*Verticillium* for example) by showing an increase in levels of defensive chemicals at the site of damage and elsewhere in the plant. This 'induced' response is a natural mechanism that allows plants to avoid some of the metabolic costs of having high levels of defence all the time (so-called constitutive defences), by rapidly inducing the defensive compounds only when they are challenged by a disease or herbivore.

The phenomenon of induced defences is well known in the resistance of cotton to fungal and bacterial pathogens and to mites. When attacked, the damaged tissues produce a



Okra leaf and frego bract: Two important HPR traits.

chemical signal that switches on the defence systems throughout the plant. Some of the biochemicals and signals have been identified, but there is much more to learn about this process.

RECOVERY CAPACITY

Some years ago Dr Victor Sadras showed significant variation in the capacity of a range of cotton genotypes to recover their growth after terminal damage. The 'growth recovery index' was found to correlate closely with our estimates of pest resistance of those same genotypes in the field.

These results suggested that genetic variation in recovery capacity could be an important component in variation in resistance to insect pests. This relationship could have potential for developing new robust varieties for future IPM systems where plants will be required to suffer and tolerate increased levels of damage from some pests.

Varieties that respond more rapidly or more strongly to damage by producing natural defensive compounds are likely to be more robust in IPM systems where compensation and tolerance play a key role. Likewise the interaction of induced biochemical responses with expression of Bt genes needs to be understood for future management of Bollgard II cottons.

So we began some work to determine whether some varieties respond to damage by producing more chemical defences more rapidly than others. Being able to select genotypes that show rapid and strong defensive responses will be an advantage.

The first step was to develop techniques to damage plants in repeatable ways; techniques to detect changes in the suitability of those damaged plants for subsequent insect feeding; and suitable biochemical techniques to measure changes in the chemistry of damaged plants.

So far we have made progress on the first two of these. Using laboratory bioassays similar to those used in our work with Bt cottons, we have shown that leaves of selected varieties, when damaged by feeding of *Helicoverpa* larvae or by artificial means, show dramatic reductions in suitability for subsequent larvae.

Growth rates of neonate larvae placed on

damaged leaves were only 40–60 per cent of those for larvae on undamaged leaves of Siokra V-15 and MHR11 — a variety highly suitable for *Helicoverpa* feeding (Figure 3). In both varieties the older leaves showed stronger responses to damage than the first fully unfurled leaf at the top of the plant. In Siokra V-15 the response to real damage was greater than to artificial damage (48 per cent reduction in *Helicoverpa* growth rate compared to 28 per cent), while in MHR11 the responses were similar (35 per cent and 38 per cent reduction in growth rates).

The next step is to screen a broader range of varieties, including most of the current commercial ones, to measure variation in the rate or intensity of response to damage. This work also involves Bt varieties where we are interested to see what happens to Bt levels in damaged plants. We will use ELISA techniques to measure changes in Bt, and also measure changes in tannins and a range of oxidative compounds previously identified in damaged cotton in US work.

FUTURE DEVELOPMENTS

Several of the characteristics identified in this work have been introduced into new CSIRO breeding lines that are undergoing field evaluation. Some of them may end up in commercial varieties in the future — many will not. Conventional HPR traits are unlikely to give high levels of tolerance to pests. They can nonetheless help develop new robust varieties for future IPM systems and in combination with Bt genes or other transgenes may show synergies that magnify the impact of both.

Although we have outlined some work to identify HPR traits for sucking pests, this has really been a sideline to work on *Helicoverpa*. With a range of sucking pests likely to have increased pest status as we move into a future of increasing use of Bt cotton, we believe there is a need for more focused work on sucking pests to identify conventional or transgenic HPR traits to assist in future management. The research summarised here gives us clear directions for new work to identify a broad range of HPR traits needed for a range of pests.

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