IPM with two-gene cotton

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Introduction

The title of my paper reflects the over-riding precedence of Integrated Pest Management (IPM) in the future of pest management for Australian cotton. Bt cotton, whether with one gene or two, will only ever be a component of sustainable IPM systems, not an answer in itself. IPM seeks to utilise a diverse array of pest control tactics to achieve pest management without excessive reliance on pesticides. Many of the components of IPM systems are used in Australian cotton production (sampling, thresholds, soft pesticides, cultivation of crop residues, planting windows, pest tolerant varieties, beneficial insects). Based on research over the last decade we are now seeing greater efforts to maximise biological sources of mortality for pests (the cotton plant itself, beneficial insects, weather) and an increasing willingness on the part of growers to co-operate in areawide management systems for key pests. These developments, supported by production of the Australian Cotton CRC's IPM Guidelines for Australian Cotton (Mensah and Wilson 1999), suggest that IPM will progressively become the norm for Australia. Pesticides are, and will remain, a part of IPM, but with greater reliance on less disruptive compounds and more objective decision making associated with their use.

Where does Bt cotton fit into this development? Bt cottons further increase biological mortality of *Helicoverpa* by modifying the suitability of the cotton plant, but do not directly disrupt other parts of the crop environment. They thus offer a sound foundation for sustainable IPM.

The Australian industry now has four years experience of single gene INGARD cottons. Over that period the area grown has increased to 25% of total cotton area and most growers would have had some experience with the technology. Despite the hype which often surrounds them, transgenic crops should not be perceived as a "silver bullet" solution to pest problems. Experience in Australia has shown that the efficacy of varieties expressing the CryIAc Bt protein is not consistent through the growing season and can be highly variable (Fitt *et al* 1994, Fitt *et al* 1998). Efficacy against *Helicoverpa* spp. typically declines through the boll maturation period, to the point where survival of larvae is little different to that on non-transgenic cotton (Fitt *et al.* 1994, Fitt *et al* 1998), although growth rates of survivors on the INGARD crops are still dramatically reduced (Fitt unpublished). Clearly this pattern is not consistent with a high dose strategy and the changing efficacy of Bt cotton imposes additional risks for resistance management. The decline in efficacy begins during flowering and supplementary *Helicoverpa* control has been necessary on INGARD crops, particularly in the last third of the growing season. Despite this INGARD crops have reduced the need for pesticide sprays by at least 40-60% in the first few years of commercial use (Pyke and Fitt 1998) - a spectacular achievement for any IPM technology.

Two-gene Bt cottons have been under development in Australia for some time. The initial two-gene cottons expressed the CryIAc protein (as in INGARD) and a second Bt protein (CryIIA). This second protein is also active against *Helicoverpa* and a similar suite of Lepidopteran pests, but has no activity against sucking pests or beneficials. While commercial development of these initial two-gene cottons has unfortunately ceased, to be replaced by the combination of CryIAc and CryX, we can use the information available for the CryIAc/CryIIA combination to infer how two-gene Bt cottons may fit into IPM systems. Given regulatory approvals, INGARD/CryX combinations should be commercially available in a range of varieties for the 2003 planting season. Here I will discuss some of the issues associated with future use of two-gene cotton and its role in sustainable pest management. These include efficacy, resistance management, secondary pests and the integration of two-gene Bt cotton with other components of IPM.

Efficacy of Two-gene Bt Cotton

While efficacy of INGARD cotton can be variable and subject to numerous environmental factors (Fitt 1998, Daly and Fitt 1998), the addition of a second gene increases efficacy overall and provides more consistent efficacy late season. Figure 1 compares efficacy of current INGARD cottons with two-gene lines (and controls) averaged over a season. While INGARD lines showed the typical seasonal decline in efficacy with survival peaking at 70% in late February, the two-gene lines never exceeded 15% survival and averaged <10% survival. No larvae survived to third instar and those larvae that did survive were half the size of those on INGARD. These results suggest that in the field very few larvae will survive on two-gene cotton and this was the case for the large scale trials completed to date. All the evidence available from laboratory and small scale field work with INGARD/CryX lines suggest its performance will be no less. So we will have two-gene Bt varieties capable of providing highly effective control of *Helicoverpa* and substantially reducing the need for pesticides.

Resistance Risk and Management.

Resistance is the achilles heel for Bt cotton. With INGARD we have instituted a comprehensive management plan to guard against the risk of resistance. The current strategy is based on certain assumptions about the nature and initial frequency of possible Bt resistance mechanisms in natural *Helicoverpa* populations. All the elements of the strategy (refuge crops, planting window, overwintering cultivation, restricted area) are important to ensure we do not have detectable resistance to the CryIAc protein before the two-gene cottons are released. Simulation models

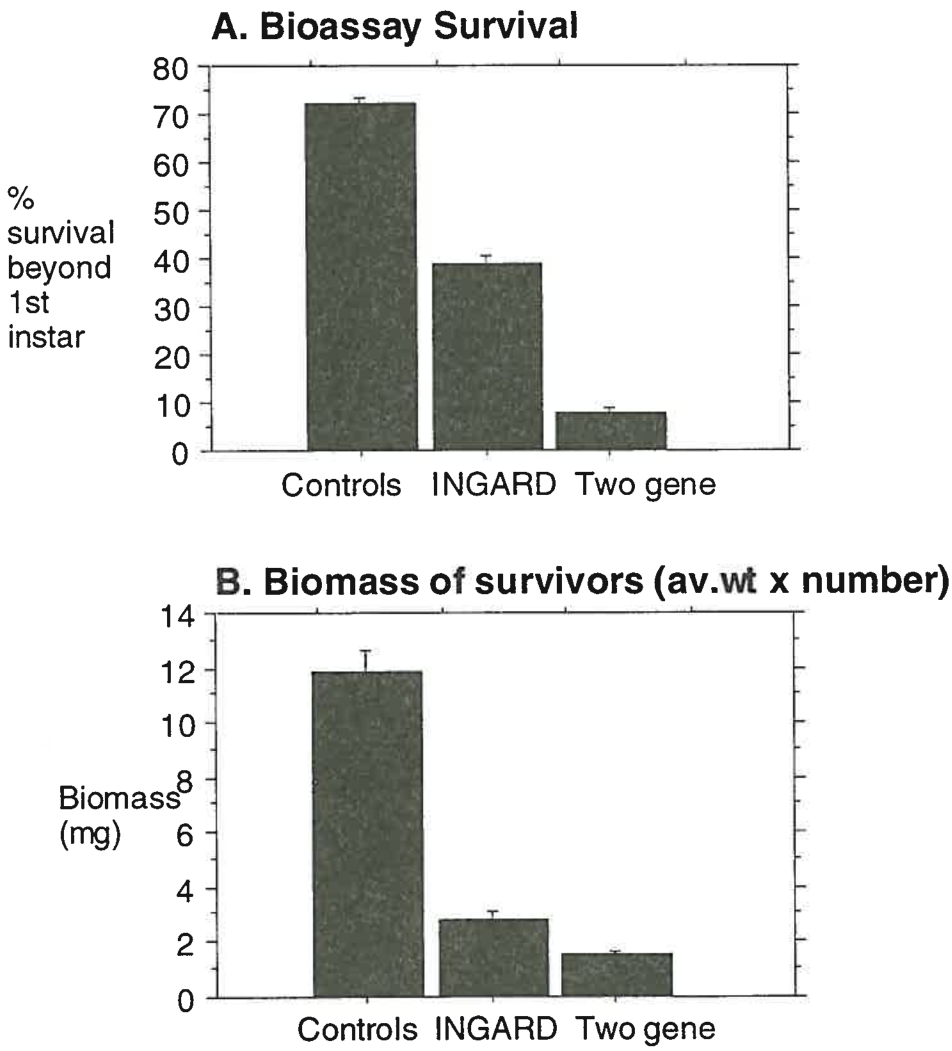

Figure 1. Survival and growth of Helicoverpa larvae in a laboratory bioassay with leaf tissues from conventional (control) cotton lines, INGARD lines and two gene lines. Survival and growth recorded after five days of expsoure. Histograms show results averaged over 8 sampling dates from late November 1997 to early March 1998.

(Roush et al 1998) suggest a huge payoff in terms of reduced risk of Bt resistance if we can achieve that. *Helicoverpa* will find it much more difficult to evolve resistance to two independently acting Bt proteins than to INGARD alone. Nonetheless it will be necessary to maintain a resistance management strategy which will include the same components as for INGARD, although some of the details will change. Provided effective refuges are maintained, and auditing can demonstrate this, the advent of two-gene cotton will allow much larger areas of Bt cotton to be grown. If growers are prepared to utilise effective unsprayed non-cotton refuge options then theoretically a high proportion of all cotton grown could be two-gene Bt cotton. This prospect immediately opens up great possibilities for wider adoption of IPM and increased collaboration in area-wide management groups. However, there are some caveats. Firstly, we need to rigorously adopt conservative resistance management plans, closely monitor *Helicoverpa* populations for Bt resistance and be prepared to react quickly with more restrictive strategies if needed. Secondly to achieve sustainable IPM and maintain minimal requirements for disruptive pesticides we need to research IPM compatible options for managing pests other than *Helicoverpa*, particularly the sucking pests. This will involve predators and parasitoids, trap crops, conventional host plant resistance and biotechnology options through discovery of genes for insecticidal proteins to target sucking pests.

The threat of secondary pests

Early in the development of INGARD cotton the possibility that sucking pests may become a more significant problem was highlighted. Reduced pesticide sprays for *Helicoverpa* may allow mirids, aphids and late season sucking bugs to survive and become more damaging. To date there has been little evidence of such changes in Australia, although aphids occur commonly in unsprayed INGARD fields and often reach high levels earlier than normal. However, some of the aphid outbreaks are probably related to drift onto INGARD crops, from nearby commercial cotton, which reduces beneficial populations. In other instances, where drift has not been a problem, aphids have usually been controlled by beneficial insects, particularly parasites (Wilson pers.obs). With heightened concern about the possible involvement of aphids in bunchy top and with increasing levels of pesticide resistance in aphid populations (Herron et al in press) the need for timely, non-disruptive options for aphid management is great.

The green mirid, *Creontiades dilutus*, appears no more abundant in Bt cotton than in conventional cotton in Australia. By contrast in Mississippi, *Lygus* bugs have required more control on BOLLGARD crops than on conventional crops (Layton et al 1998). Here also we need to maintain effort to utilise trap crops for mirid management where possible, to fully utilise plant compensation for early season fruit loss, to develop selective chemical or biological spray options for mirids, and if possible to enhance the tolerance of cotton varieties to mirids through conventional or transgenic traits. All these areas of research are being pursued.

Late season sucking bugs present another challenge. The risk that bugs such as the green vegetable bug, *Nezara viridula*, or the harlequin bugs may become significant late season problems was highlighted some years ago (Mares and Fitt 1997). In some production regions of the eastern US late season "stink bugs" have emerged as a serious problem in BOLLGARD cotton. Populations of *Nezara viridula* have resulted in up to 38% yield loss in unprotected Bt cotton (Turnipseed and Greene 1996). The same pattern is emerging in Australia in unsprayed INGARD crops. In commercial INGARD crops, declining late season efficacy and consequent sprays for *Helicoverpa* means that in most seasons "stink bugs" have not been a problem. Even so there have been instances in which *Helicoverpa* was rare and sprays were not needed (Darling Downs 1999/2000), where green vegetable bugs have caused significant damage. This risk of late season stink bugs may become a greater issue with two-gene cotton. At present we have few soft options for managing these pests. If we are not to lose the advantage of reduced pesticide requirement on two-gene cotton then IPM compatible means of managing GVB and aphids will be needed. Parasitoids and trap crops are two options which should be researched.

Two-gene Bt cotton as a foundation for IPM

Rather than silver bullets, Bt cotton varieties should be viewed as a foundation on which to build IPM systems which incorporate a broad range of biological and cultural tactics (Fitt in press, Fitt and Wilson in press, Figure 2). I will discuss some of these elements in detail.

Beneficial insects.

Research has shown little effect of INGARD cotton on non-target species, including non-lepidopterous pests, beneficial insects, and other canopy dwelling and soil dwelling species (Fitt *et al.* 1994; Wilson, Fitt and Forrester, unpublished data). Densities of predators and parasitoids have been shown to be two to three times higher than in conventionally sprayed crops (Wilson and Fitt unpublished). Reduced use of disruptive pesticides will allow more emphasis on the management and manipulation of beneficial species, using nursery crops and food sprays (Mensah 1997, 1998; Mensah and Khan 1998), or other means of conservation and augmentation (Schellhorn et al, this proceedings). Furthermore, since many of the beneficial insects in cotton are generalists (Hearn and Fitt 1992; Wilson *et al.* 1998), their increased abundance can minimise the risk of outbreaks of a

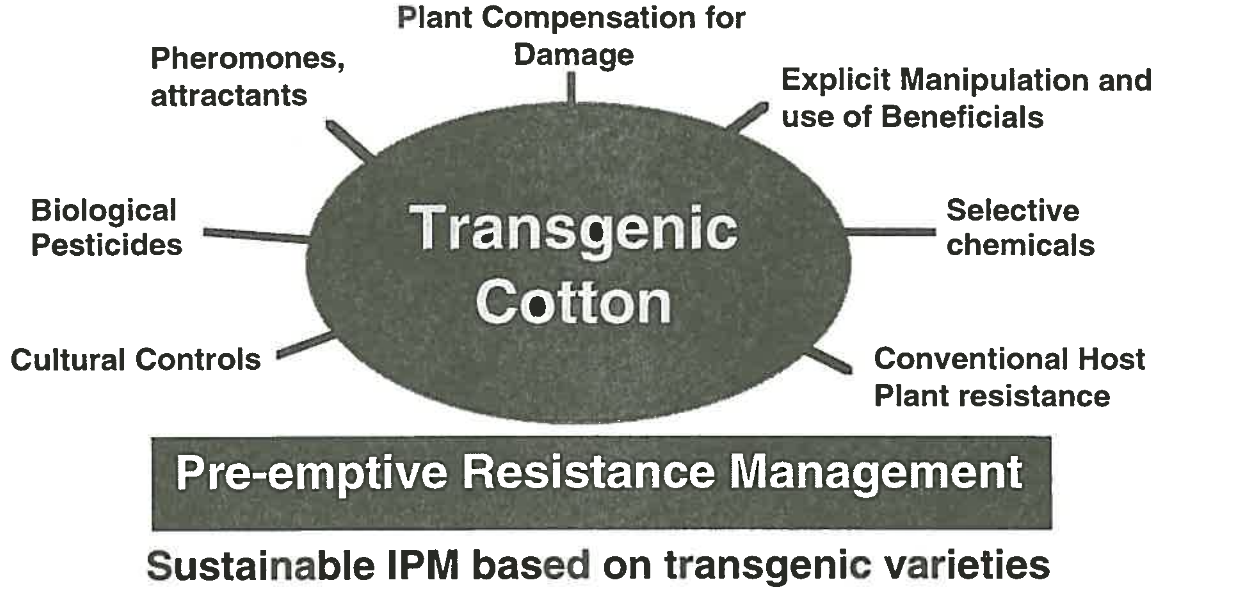

Figure 2. IPM components which can be built on a foundation of effective Bt cottons

range of secondary pests. Australian researchers are seeking to add diversity to cotton production systems to assist in IPM. For example, an IPM system based on in-field lucerne strips, a predator food spray (Envirofeast™) (Mensah, 1997, 1998; Mensah and Khan, 1997) and biological pesticides such as Bt and nuclear polyhedrosis virus can be effective. When combined with two-gene Bt cotton such a system could be even more effective. In this case lucerne strips have a dual function in providing a nursery for generalist beneficials and a trap crop for green mirids. The combination of lucerne and food spray provides more stability to predator populations. Ongoing research with nursery crops for parasitoids and trap crops for *Helicoverpa* all provide options for enhanced biological control.

Selective Pesticides

Selective chemicals used only when essential will be an important component for IPM systems based on two-gene Bt cotton. These options are discussed fully in Holloway and Forrester (1998) and Wilson *et al* (1998a). Research by Jonathon Holloway (pers. comm.) shows that the efficacy of many selective pesticides is enhanced on INGARD cottons, probably because feeding larvae are already suffering physiological stress from Bt intoxication even if they have not been killed by the plants. While with more efficacious two-gene cottons we would hope that larvae are quickly killed and additional pesticide needs would be minimal these important results nonetheless confirm the compatibility of Bt cottons with soft options chemistry for IPM. Highly selective biological insecticides will also have a role in pest management at the cropping system level. Formulations of

Nuclear Polyhedrosis Virus (eg. GEMSTAR), will provide alternative control options for *Helicoverpa* which may survive on transgenic crops or on other crops (eg. sorghum and legumes) growing in the same agro-ecosystems where cotton is grown. By utilising alternative control tactics in different crops where *Helicoverpa* develops (eg. transgenic Bt in cotton, virus sprays in sorghum) we can minimise exposure of the whole population to the same selective agents and so minimise selection for resistance.

Conventional Host Plant Resistance (HPR) and Compensation

A combination of Bt genes with other HPR characters through classical plant breeding may also enhance the stability of IPM systems. Bt genes have been incorporated in Australian okra leaf varieties to provide enhanced resistance to both *Helicoverpa* and mites. A range of insecticidal secondary compounds are also found in *Gossypium hirsutum*. For instance the terpenoid aldehydes such as gossypol or the related 'heliocides' reduce survival and growth rates of *Helicoverpa* spp. (Fitt, Mares and Benson unpublished). Synergism between Cry IAc protein and high gossypol levels has been shown and CSIRO breeders are seeking to combine these traits in commercial cultivars.

Cotton varieties have a considerable capacity to compensate, even overcompensate, for insect feeding damage (Sadras 1995). With two-gene Bt cottons early season fruit loss from *Helicoverpa* should be minimal, but mirid damage to pinhead squares will remain an issue. In regions where compensation for early fruit loss is reliable, much greater use could be made of this capacity through the application of appropriate thresholds which take into account both pest density and plant growth. On Bt cotton crops thresholds for *Helicoverpa* must allow time for larvae to feed sufficiently to ingest a lethal dose of the insecticidal protein, yet still allow intervention while larvae are of a size where they can be controlled effectively with insecticides (generally less than 6 mm) and before economic loss occurs. Thresholds for other pests remain largely unchanged. Cotton genotypes vary in their ability to compensate for pest damage (Sadras and Fitt 1997). Selection for genotypes with higher compensatory ability in combination with Bt genes could allow the use of higher thresholds for all pests with less risk, therefore reducing the need to intervene with disruptive insecticides.

Cultural Techniques for Management

Cultural techniques integrate easily with Bt cottons and include the mandatory requirement to cultivate crop residues to destroy any surviving *H. armigera* pupae in the soil through winter (Fitt and Daly 1990). The use of trap crops to concentrate *Helicoverpa* populations as part of area-wide approaches to population management or as a means for on-farm management of other pests such as mirids is becoming increasing feasible. On going research with trap crops for mirids (lucerne) and for *Helicoverpa* (pigeon pea etc) is providing further options to minimise pesticide requirements, maximise beneficial abundance and allow greater co-ordination among growers in areawide approaches (see Dillon this proceedings). Using trap crops to concentrate and destroy *Helicoverpa* will still be highly beneficial with two-gene Bt cottons since they will reduce the population size exposed to Bt plants and so act to reduce selection for Bt resistance.

Behavioural Manipulation

Management of pests through behavioural manipulation with attractants, repellants or mating disruptants may also be feasible with two-gene cottons. Behavioural controls could be useful for pests other than *Helicoverpa*, but they may also play a role in conjunction with refuge crops for resistance management to ensure random mating among moths from refuge and Bt crops.

Other transgenes

Finally, the current reliance on Bt genes in transgenic cotton varieties represents only the first wave of insecticidal proteins for pest management. While Bt genes are the most advanced commercially much research effort is focussed on alternative transgenes with activity against the major Lepidopteran and Hemipteran pests of cotton (Llewellyn and Higgins 1998). These offer possibilities for pyramiding with Bt genes to provide more sustainable resistance management (Roush et al 1998) or control of minor pests.

Conclusions

IPM systems for future cotton production will, of necessity, be more complex than the pesticide based systems currently in place, and will require greater effort on the part of crop managers whether they be professional consultants or farmers themselves. Transgenic cottons expressing two Bt proteins with activity against one or more key pests offer great scope to further reduce pesticide requirements for cotton production. Provided they are supported with conservative resistance management strategies two-gene cottons should provide a foundation for sustainable IPM systems. The real challenge for researchers is to achieve an integration of approaches that manages secondary pests with biological and system wide options that can rival the predictability of conventional insecticides. A significant challenge for researchers and funding agencies alike is to recognise that work on a range of IPM components must continue alongside the increasing focus on biotechnology. Transgenic insecticidal cottons will not be sustainable technologies alone; they must be supported with other approaches which will require continued research.

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