CHAPTER 14

Stewardship – Reducing The Risk Of Resistance

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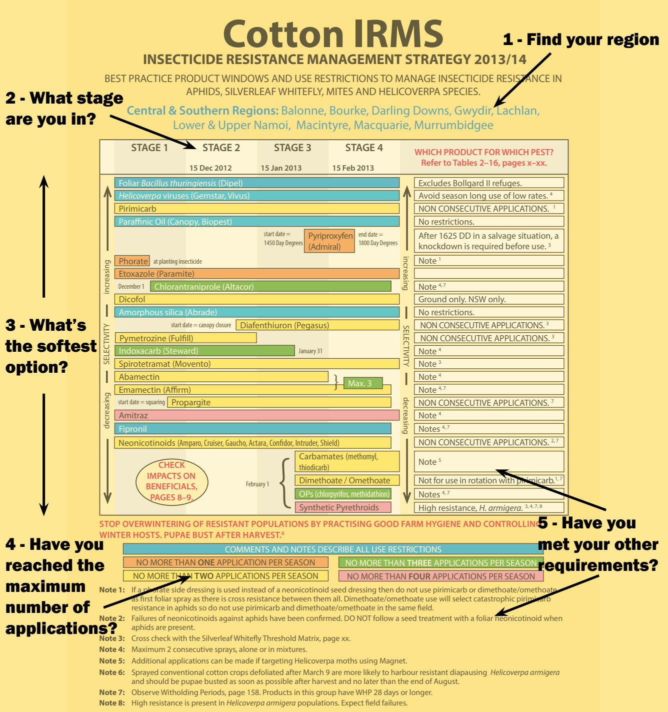
Insecticide stewardship refers to protecting the efficacy and longevity of biotechnology traits and insecticides used to control pests in the Australian cotton industry.

Resistance is an outcome of exposing pest populations to a strong selection pressure, such as an insecticide. Genes for resistance usually naturally occur at very low frequencies in insect populations. They remain rare until they are selected for with exposure to a toxin, either from an applied pesticide or from a biotechnology trait, such as the Bt toxins within Bollgard II. Once a selection pressure is applied, resistance genes can increase in frequency because the insects that carry them are more likely to survive and produce offspring. If selection continues, the proportion of resistant insects relative to susceptible insects may continue to increase until reduced effectiveness of the toxin is observed in the field.

Cotton IRMS: The FIRST place to go when selecting a spray



Insecticide stewardship

The use of pesticides can select for resistance in pest populations. The cotton industry has implemented an Insecticide Resistance Management Strategy (IRMS) to manage the risk of resistance in aphids, mites and *Helicoverpa* spp. both in conventional and Bollgard II cotton.

The evolution of the IRMS is driven by the Transgenic and Insect Management Strategies (TIMS) Committee. TIMS is an industry committee facilitated by Cotton Australia. The results from the insecticide and miticide resistance monitoring programs, carried out each season, are used to inform the committee of any field scale changes in resistance levels. TIMS consults extensively with cotton growers and consultants in all cotton regions as part of finalising the IRMS each season. The IRMS is updated annually and printed in the Cotton Pest Management Guide.

The IRMS aims to minimise selection across consecutive generations of the pest. The way in which this can be practically achieved, depends on the lifecycle of the pest species, as the life cycles of *Helicoverpa* spp. and the sucking pests are very different, the IRMS recommends different use patterns for different chemistries.

The life cycle of the *Helicoverpa* spp. is 42 days (average). To minimise selection pressure across consecutive generations most chemicals that target *Helicoverpa* spp. are restricted to windows of between one and two generations. The life cycle of mites and aphids is much shorter than that of *Helicoverpa* spp. The resistance strategy for these sucking pests depends on rotation and non-consecutive use of chemistries. There are also restrictions on the maximum number of uses for individual products and chemical groups to further encourage rotation of chemistries. Non-consecutive use of chemistries is particularly important for aphids because they reproduce asexually, which means that all offspring from a resistant aphid will also be resistant.

The IRMS is split into two regions: Northern and Central/Southern. This delineation reflects the different growing seasons from central Queensland through to southern NSW. Since *Helicoverpa* spp. and mirids are capable of travelling long distances, the delineation is also designed to reduce the chance that pests moving between regions would be reselected repeatedly by the same insecticide group by limiting the time period in which most insecticides are available.

Pupae busting is an important tool in the IRMS. It is an effective, non-chemical method of preventing resistance carryover from one season to the next. The guidelines for performing pupae busting in sprayed conventional cotton are based on the likelihood that larvae will enter diapause before a certain date. This means that in specific field situations pupae busting is not necessary. The estimated commencement date of diapause is based on the model which drives the *Helicoverpa* Diapause and Emergence Tool on the Cotassist website. The web tool uses long term average weather data to predict the timing of diapause.

Adherence to the IRMS is an important tool in pest management, particularly in Bollgard II cotton. Infestations of aphids, mites, mirids and silverleaf whitefly often require intervention with foliar insecticides to protect cotton yield and quality and as such there is a risk of resistance developing in these populations. The IRMS chart seeks to directly manage the risk of resistance in pests as well as reduce risk of inadvertent selection of pests that are not the primary target of the insecticide.

Large areas of Bollgard II will not change the frequencies of resistant genes being carried by *H. armigera* moths. The same proportion of resistant and susceptible moths will continue to lay eggs in cotton – be it conventional or Bollgard II. Hence the likelihood of resistance developing to foliar and soil applied insecticide remains the same, even if the overall size of the *H. armigera* population is reduced. Continuing to follow the IRMS will ensure that the industry retains the ability to control *H. armigera* effectively with the insecticides on cotton both now and in the future. The IRMS should always be consulted when making a spray decision, even in Bollgard II cotton.

Stewardship of Bollgard II cotton

The introduction and adoption of biotechnology traits has delivered significant benefits for Australian cotton growers and cotton production systems. Following the introduction of the first biotechnology trait (INGARD® cotton in 1996), biotechnology has today become a very important feature of cotton production and a key component in cotton breeding programs. In 2012, approximately 98% of the cotton planted in Australia contained at least one biotech trait which underlines the importance of the technology to Australian cotton growers.

Biotechnology has delivered real benefits to cotton growers and has significantly reduced some of the production risks associated with the crop. This has directly enabled growers to focus on key management strategies to drive yield and fibre quality outcomes. The technology has also allowed growers in non-traditional growing areas to explore cotton as a mainstream cropping option and adapt their farming system (irrigated or dryland) to benefit from cotton as part of their rotation.

Currently there are two broad classes of cotton biotechnology traits which are approved and available in Australian cotton varieties providing either insect protection, herbicide tolerance or in varieties which are ‘stacked’ with a combination of both traits.

The herbicide tolerant varieties are discussed in more detail in chapter 15. For more information on obtaining access to these biotechnologies please refer to chapter 3 of the Production Manual. This chapter will focus on the stewardship of biotechnologies that provide insect protection.

Bollgard II

Bollgard II cotton was introduced to the Australian market in 2005. It contains two genes derived from the common soil bacterium *Bacillus thuringiensis* (Bt). These bacteria produce a large array of crystalline proteins, two of which are produced in Bollgard II cotton, Cry1Ac and Cry2Ab. Cry 1Ac is specific to Lepidoptera (moths, including our major pests, *Helicoverpa* spp.) and Cry2Ab to Diptera (flies) and Lepidoptera, giving inbuilt protection against the larvae of *Helicoverpa* spp. (source: Monsanto).

The introduction of insecticidal transgenic varieties into the Australian cotton market has allowed the industry to reduce its pesticide use by more than 90% and is arguably the most important technology the industry uses. However, resistance is a great threat to the continued availability and efficacy of Bollgard II cotton in Australia. Even though the Bt proteins are derived from plant tissues, they still select for survival of resistant individuals. The Resistance Management Plan (RMP) for Bollgard II was established by regulatory authorities to mitigate the risks of resistance developing to either of the proteins contained in Bollgard II cotton.

When the RMP for Bollgard II was developed the frequency of resistance to both of the toxins that it expresses (Cry1Ac and Cry2Ab) was expected to be low. Screening for resistance in *H. armigera* and *H. punctigera* began around the time that Bollgard II was commercially released, revealing that baseline frequencies were much higher than anticipated.

While in both *H. armigera* and *H. punctigera* the first isolations of alleles conferring resistance to Cry1Ac were recently detected, these alleles remain rare (< 1 in 1,000). But, since developing the RMP for Bollgard II, CSIRO's monitoring has shown that in both of the main target species resistance to Cry2Ab is present, is higher than expected, and is probably increasing.

This is the case not only for *H. armigera* which has a track record of developing resistance to conventional insecticides, but also for *H. punctigera* which has shown limited ability of evolving resistance to conventional insecticide sprays. The continued efficacy of Bollgard II cotton is therefore dependent on the effective implementation of the RMP.

The Bollgard II RMP

The RMP is based around 5 key elements that impose limitations and requirements for management on farms that grow Bollgard II. These are mandatory growing of refuges; control of volunteer and ratoon plants; a defined planting window; restrictions on the use of foliar Bt; and pupae destruction. In theory the interaction of all these elements should effectively slow the evolution of resistance.

Planting windows

The purpose of planting windows is to confine crop development and maturity to limit the number of generations of *Helicoverpa* spp. exposed to Bollgard II cotton each season. This measure effectively restricts the selection pressure on key pests to develop resistance to Bollgard II.

The planting window concept was originally part of the voluntary Insecticide Resistance Management Strategy (IRMS) and was based on a scientific understanding of the ecology of *Helicoverpa* spp. The start date of the planting window is based on the date that moths are likely to emerge in a region using long term temperature data and the window length is one lifecycle of the pest, based on daily temperatures around the start date, which is about 42 days. Imposing a start date is especially important in warmer regions where pupae do not necessarily enter a diapause over the winter and where there is no climatically driven restriction on when planting can begin.

There are usually 3-4 generations of *Helicoverpa* spp. in a cotton growing season, depending on temperatures for that year, so the risk strategies around the RMP have been developed based on these numbers. In warmer cotton gorwing regions there is not always a climatic limit on how long crops can be grown, so the RMP includes an end date for crops in Central Queensland, and all Bollgard II and associated trap crops must be destroyed by July 31.

Mandatory refuges

The aim of a refuge crop is to generate significant numbers of susceptible moths that have not been exposed to the Bt proteins in Bollgard II. Moths produced in the refuge will disperse to form part of the local mating population where they may mate with any resistant moths emerging from Bollgard II crops, delaying the development of resistance. This strategy works because resistance to the Bt proteins has so far been found to be recessive, so if a resistant moth (rr) from the Bollgard II crop mates with a susceptible moth (ss) from the refuge, the offspring they produce (rs) are also killed by the Bt toxins.

The current RMP options for irrigated Bollgard II refuges are 100% sprayed cotton, 10% unsprayed cotton or 5% pigeon pea (relative to the area of Bollgard II cotton grown) with almost 70% of refuges grown being pigeon pea. No matter which refuge is grown, it is critical that they are managed to be most attractive to *Helicoverpa* moths when Bt cotton is also most attractive.

It is a requirement of the RMP for growers to ensure that their refuge crops receive adequate nutrition, irrigation (for irrigated refuges), and weed and pest management (excluding *Helicoverpa* sprays) so that they remain attractive while Bollgard II is grown. An important characteristic of mandatory refuges is their synchronicity with the corresponding Bollgard II crop. The timing of refuge planting is dependent on the timing of Bollgard II cotton planting so that the refuge is flowering (both pigeon pea and cotton refuges) at the same time as the Bollgard II. Ideally, refuges should be as or more attractive to *Helicoverpa* than the corresponding Bollgard II crop to attract females to lay eggs in the refuge.

Role of non-mandatory refuges

Helicoverpa are polyphagous which means that they feed on a wide range of host crops and vegetation, including cotton. Bt cotton dominates the total area of cotton grown in Australia but at a landscape scale it often forms part of a mosaic of other crops and vegetation. Non-cotton crops and natural vegetation are known to be important for Bt resistance management by providing alternative sources of Bt susceptible moths apart from those produced by the mandatory refuges. But we cannot confidently rely on these unstructured refuges to produce moths because their effectiveness and distribution is highly variable between seasons and regions.

Control of volunteer and ratoon plants

The presence of volunteers within a refuge diminishes the value of a refuge, as some of the moths emerging from that refuge have had some exposure to the Bollgard II proteins. Larvae that carry the gene for resistance, Heterozygous (RS) individuals, may emerge and develop on the refuge (conventional cotton or pigeon peas) crop before moving onto a Bollgard II volunteer within the refuge. In this way, the RS larvae become exposed to the Bt proteins at a later growth stage when they can survive to produce offspring. This will lead to an increase in the frequency of resistant individuals in the population.

The same risk to resistance from increasing exposure to the Bollgard II technology applies not only to Bollgard II volunteers within refuge areas but also in fallow fields and non-cropping areas. The good farm hygiene practice of removing all volunteers in and around cropping areas is not only important in removing disease and pest carryover hosts but also in reducing the resistance risk to Bollgard II technologies.

Restrictions on use of foliar Bt sprays

Sprayed cotton refuges are grown for commercial cotton yields, requiring active control of *Helicoverpa* with foliar insecticides. To ensure that no selection for Bt resistance can take place in this type of refuge, the use of foliar Bt insecticide is excluded. Sprayed cotton refuges are much larger than unsprayed refuge types because of the lower rates of *Helicoverpa* survival.

In unsprayed cotton and pigeon pea refuges, 'unsprayed' is in reference to insecticides which control *Helicoverpa* species. In these refuges, all foliar applied insecticides with activity against *Helicoverpa* species are excluded. These refuges are able to produce high numbers of *Helicoverpa* moths from much smaller areas.

Pupae destruction

Helicoverpa larvae enter a diapause phase in the soil as temperatures begin to cool and daylength decreases in early autumn. This dormancy strategy allows the pest to survive the winter months in temperate regions when host plants are scarce and temperatures are generally too low to allow successful development.

Cultivation of the soil between seasons, during the dormancy phase, is an effective way of preventing any moths that developed resistance in the previous year from contributing to the population in the following year.

In Central Queensland, due to the warmer temperatures and smaller changes in daylength, *Helicoverpa* pupae produced late in the season do not remain in the soil but emerge within 15 days of pupating, making pupae busting ineffective. Late season trap crops are used as an alternative. Trap crops of pigeon peas are timed to be at their most attractive after the cotton has cut out. Moths emerging from the Bollgard II fields late in the season should be attracted to the pigeon peas to lay their eggs. Once the cotton has been harvested the trap crops are destroyed and cultivated to kill the larvae and pupae.

Pupae destruction is an important part of the RMP. (Photo: Trudy Staines)

Evaluating the effectiveness of the RMP

To evaluate the effectiveness of the RMP the CRDC funds a program that monitors field populations of moths for resistance to Cry1Ac and Cry2Ab. Monsanto Australia operates a separate but complimentary monitoring program. The data provides an early warning to the industry of the onset of resistance to Bollgard II. The results are used to make decisions about the need to modify the RMP from one season to the next to ensure its ongoing effectiveness at managing resistance. Any changes to the RMP are put forward by Monsanto to the Australian Pesticides and Veterinary Medicines Authority. Industry is given an opportunity to have input into these changes via the TIMS Committee.

The full details of the RMP are published annually in the Cotton Pest Management Guide along with the latest annual results from the resistance monitoring program.

The future – third generation Bt technology

The industry's third generation Bt technology is being developed. It is based on the same platform as Bollgard II but with a new protein (Vip3A) added.

CSIRO has begun performing screens against the new protein in Bollgard III (Vip3A) and found that in *H. armigera* the frequency of genes conferring resistance is around 1 in 20 moths. Not only is this higher than expected, it is much greater than the starting frequencies for Cry2Ab. Vip3A resistance genes have also been detected in *H. punctigera* at a frequency that is higher than expected and higher than the starting frequencies for Cry2Ab.

Work is underway to characterize this Vip3A resistance. This information, along with data on the efficacy of Bollgard III against *Helicoverpa* (also underway), will be used with information on the frequencies of Cry1Ac, Cry2Ab and Vip3A to determine the RMP for Bollgard III. At this stage it is almost certain that we will not be developing a RMP with a clean resistance slate.

Managing resistance to Bollgard II is critical in the lead up to the introduction of Bollgard III to ensure the efficacy of this technology is maintained, both now and into the future.

For more information the following resources and tools are available at https://www.mybmp.com.au/auth_user/grower_tools_and_resources.aspx

- **Cotton Pest Management Guide**
- **Biotechnology Module**