Preamble to the Resistance Management Plan (RMP) for Bollgard II 2013–14

Sharon Downes and Lewis Wilson, CSIRO
Kristen Knight, Monsanto Australia Limited
Greg Kauter, Cotton Australia
Tracey Leven, Cotton Research & Development Corporation

Resistance is the greatest threat to the continued availability and efficacy of Bollgard II cotton in Australia. Even though the Bt proteins in Bollgard II are delivered in the plant tissues, there is still the selection for the survival of resistant individuals. The 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. As it is difficult to be precise about the probability of resistance developing in *Helicoverpa* spp. to the proteins contained in Bollgard II cotton the industry implemented a pre-emptive management plan that aims to prevent field level changes in resistance.

A key component of the RMP for INGARD was a limitation on the area of INGARD cotton that could be planted. This restriction limited selection for resistance to the Cry1Ac protein in INGARD. The industry has so far been able to preserve the efficacy of this gene. When Bollgard II replaced INGARD, the constraint on the area of transgenic cotton was removed. Bollgard II contains both Cry1Ac and Cry2Ab. Computer simulation models of resistance development indicate that it will be more difficult for a pest to develop resistance to both of the insecticidal proteins. However, it is not impossible for *Helicoverpa* spp. to adapt to this technology. Recent work has shown that for *H. armigera* and *H. punctigera* the assumed baseline frequency of Cry2Ab resistance genes in populations is substantially higher than previously thought. The continued efficacy of Bollgard II cotton is therefore even more dependent on the effective implementation of the RMP.

The total area of cotton planted in the 2013–14 season is predicted to remain at the large scale that it increased to in 2010–11 and the Bollgard II acreage will still represent over 90% of the total area planted to cotton in Australia. Given the selection pressure exerted by Bollgard II cotton, as well as the high baseline frequency of genes conferring resistance to Cry2Ab in *Helicoverpa* spp. it is critical to abide by the obligations under the RMP.

Future transgenic cottons may also rely on either of the two existing insecticidal genes within Bollgard II. In particular, Monsanto's third generation Bt-cotton, Bollgard III, will build on the existing Bollgard II cotton platform. Protecting Bollgard II cotton therefore also represents an investment in the protection of future transgenic technology for the Australian cotton industry. If field resistance to Bollgard II cotton were to eventuate it may make it more difficult to market new transgenic products in cotton, and the perceptions of other industries, growers and the public could be unduly affected. Modelling undertaken by CSIRO also suggests that Cry2Ab resistance levels in *Helicoverpa* spp. at the time of introducing Bollgard III will directly impact on the requirements for the RMP for that technology. Therefore, it is critical that the industry complies fully and effectively with the RMP for Bollgard II.

The 5 Elements of the Bollgard II RMP

The five elements of the RMP 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 mandatory cultivation of crop residues. In theory the interaction of all of these elements should effectively slow the evolution of resistance.

Your questions answered

How do we test whether the RMP is effective?

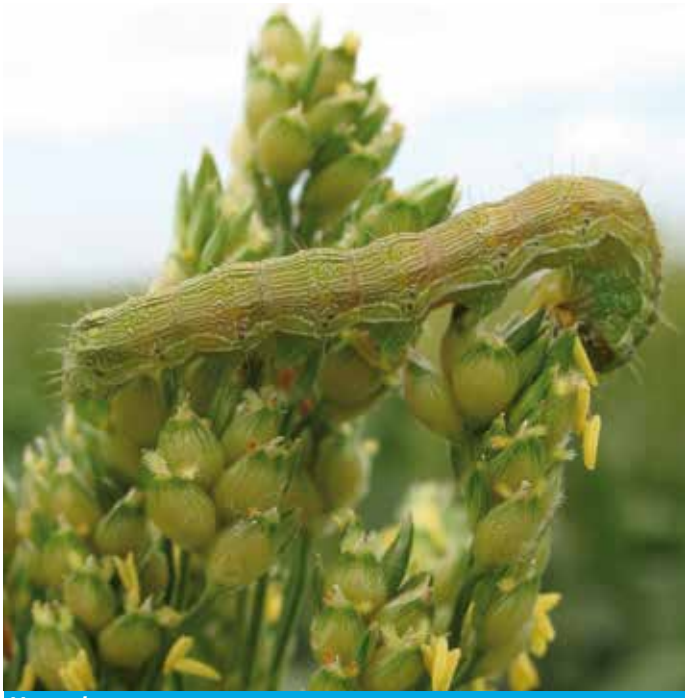
To evaluate the effectiveness of the RMP the CRDC funds a program that monitors field populations of moths for resistance to Cry1Ac and Cry2Ab. Work has also commenced on monitoring field populations of moths for resistance to the new vip3A gene contained in Bollgard III technology. Monsanto Australia operates a separate but complimentary monitoring program. The data provides an early warning to the industry of the onset of resistance to Bollgard II and the potential risk of resistance developing to Bollgard III. 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.

Two sorts of tests are conducted. F2 screens involve testing the grandchildren of pairs of moths raised from eggs collected from field populations, and therefore take about 10 weeks to run. To increase the number of insects that could be processed during the season, CSIRO developed protocols for testing the frequency of the Cry2Ab resistance gene detected with F2 screens using a shorter method called an F1 test. F1 screens involve testing the offspring of single-pair matings between moths from resistant strains maintained in the laboratory and moths raised from eggs collected from field populations. They take around 5 weeks to conduct.

What is the current situation for Bt resistance in *H. armigera* in Australia?

A gene is present in field populations of *H. armigera* that has the potential to confer high-level resistance to Cry1Ac. CSIRO and Monsanto data suggests that this gene occurs at a low frequency which is probably less than 5 in 10,000 (<0.0005or 0.05%). This gene does not confer cross-resistance to Cry2Ab and in certain environments is largely recessive. It also has a high fitness cost (i.e. resistant individuals develop slowly and are more likely to die) but this disadvantage is not likely to greatly impact on the development of resistance. In addition, Dr Robin Gunning (NSW DPI) suggests that other resistance mechanisms may be present in *H. armigera*.

A gene that confers high level resistance to Cry2Ab is present in field populations of *H. armigera*. This gene does not confer cross-resistance to Cry1Ac. The most extensively studied colony of insects with this resistance (called SP15) appears to be as fit as susceptible insects. The resistance in such colonies is recessive. The mechanism conferring resistance to Cry2Ab in *H. armigera* has been shown to be an alteration of a binding site in the gut of the insect. F2 tests indicated that the frequency



H. armigera. (Melina Miles, Qld DAFF)

of the gene for resistance to Cry2Ab in 2012–13 was 1 in 100 (0.01, 1%) or less.

In 2004 CSIRO developed protocols for testing the frequency of resistance using a modified and shorter version of the F2 method called an F1 test. This method assumes that the various isolates of Cry2Ab detected so far are of the same kind. These protocols were immediately adopted by Monsanto. During the following two years CSIRO performed experiments which verified that the same mechanism appears to confer resistance in all of the isolates of Cry2Ab detected to date. In 2007–08 CSIRO began F1 tests in *H. armigera* in earnest. Results with *H. armigera* show that the estimate of Cry2Ab resistance frequency for F1 screens.

At the end of the 2012–13 season is approximately 2 in 100 (0.02, 2%). Currently, we believe that the frequencies obtained from the F1 screens are likely to most accurately reflect the situation in the field.

What is the current situation for Bt resistance in *H. punctigera* in Australia?

Before 2008–09 more than 4000 genes from *H. punctigera* had been screened and none had scored positive for resistance to Cry1Ac. However, since 2008–09 at least a five individuals which carry a gene that confers resistance to Cry1Ac have been isolated from field populations of *H. punctigera*. F2 tests indicate that the frequency of this gene is still quite rare ats less than 1 in 1000 (0.001, 0.1%). It is not cross-resistant to Cry2Ab.

A gene that confers high level resistance to Cry2Ab is present in field populations of *H. punctigera*. This gene does not confer cross-resistance to Cry1Ac. The most extensively studied colony of resistant insects (called Hp4–13) demonstrates the same broad characteristics as the SP15 strain of Cry2Ab resistant *H. armigera.* The resistance is recessive, occurs at a high level, and is due to an alteration of a binding site in the gut of the insect. F2 tests indicated that the frequency of this gene in 2012–13 was 1 in 100.

In 2007–08 and 2009–10 CSIRO and Monsanto respectively began F1 tests in *H. punctigera*. As with *H. armigera*, the Cry2Ab resistance frequency in *H. punctigera* for F1 screens is higher than that determined with the F2 tests. At the end of the 2012–13 season, the frequency of Cry2Ab genes in *H. punctigera* was approximately 12 in 1000 (0.012, 1.2%).

Why is there a high baseline frequency of Cry2Ab genes in field populations?

The high frequency of individuals carrying the Cry2Ab resistance gene in field populations is unexpected because, until the widespread adoption of Bollgard II, there has presumably been little exposure of *Helicoverpa* spp. to this toxin and therefore little selection for resistance. Although the Cry2Ab toxin from Bt is present in some Australian soils, it is not common. In contrast, the Cry1Ac toxin is far more common in Australian soils, yet resistance to this toxin in *Helicoverpa* spp. is rare. Mutations that confer resistance to Cry2Ab may occur in field populations of *Helicoverpa* spp. at a very high rate.

Collection of *H. punctigera* moths from inland regions were made in winter 2009 to see if these populations, which would have little exposure to Bollgard II, carry resistance to Cry2Ab. F1 screens conducted by CSIRO on these populations show they carry the same Cry2Ab resistance gene present in the cropping areas but at a much lower frequency of 5 in 1000 (0.005, 0.5%) compared to a sample from cropping populations collected at the same time (5 in 100, 0.05, 5%). We do not have an F1 resistance frequency for Cry2Ab in *H. punctigera* prior to the widespread adoption of Bollgard II.

Is the frequency of Cry2Ab genes increasing in field populations of *H. armigera*?

CSIRO F2 data for *H. armigera* suggest a gradual increase in frequency of Cry2Ab resistance genes in recent years. The frequency obtained for 2010–11 was significantly greater than for previous years, but since then has not continued to increase. Monsanto began collecting F2 screen data for *H. armigera* in 2003–04 and since then there has been no significant change in frequency of Cry2Ab resistance genes over time with an average of 9 in 1000 (0.009 or 0.9%).

Since 2004–05 Monsanto has used the F1 protocol developed by CSIRO to screen for resistance to Cry2Ab. CSIRO also has F1 screen data for *H. armigera* since 2007–08. Both data sets analysed independently show that there is no significant difference in the frequencies of Cry2Ab resistance alleles over the longer term; although the frequencies in 2010–11 were higher than in previous years they have since declined. Irrespective of changes through time the frequencies of Cry2Ab in *H. armigera* are higher than expected and this finding is a concern (see above).

Is the frequency of Cry2Ab genes increasing in field populations of *H. punctigera*?

At the end of 2008–09 the F2 and F1 data sets from CSIRO demonstrated significant increases in the frequency of Cry2Ab resistance genes in field populations of *H. punctigera*. CSIRO began collecting F2 screen data for *H. punctigera* in 2002–03 and afterwards there was a gradual increase in resistance frequencies over time which became statistically significant in 2007–08 and remained highly significant in 2008–09. After declining in 2009–10, resistance frequency increased again in 2011–12 to the highest recorded level (2 in 100, 0.02 or 2%) before declining to 1 in 100 (0.01, 1%) in 2012–13. The complete data set continues to demonstrate a significant gradual increase in frequency over time.

Monsanto began F2 screens with *H. punctigera* in 2007–08 and in 2010–11 detected a Cry2Ab resistance frequency that was significantly higher than in previous years. However, this may have been an overestimate in frequency as all positives were from one larval collection. In 2012–13 the Cry2Ab resistance frequency is at a similar level to that recorded in 2008–09 (7 in 1000, 0.007 or 0.7%). If the probable overestimation in frequency last season in 2010–11 is taken into account there has been no significant change in the Cry2Ab resistance frequency over time.

The 2008–09 CSIRO F1 data set for *H. punctigera* demonstrated a 5 fold increase in frequency compared to 2007–08 (from 1 in 100 to 5 in 100 or 0.01 to 0.05). The frequencies obtained from 2009–10 until 2012–13 are lower than those detected in 2008–09. From 2009–10 until 2011–12 there was a gradual increase in frequency from 1 in 100 (0.01 or 1%) to 4 in 100 (0.04 or 4%) but in 2012–13 the frequency declined to 15 in 1000 (0.015 or 1.5%). The shifts in F1 screen data from 2007–08 to 2012–13 mirror those of the F2 screen data, however since the data set is restricted to the last four years only, it is not possible to look for longer term shifts over time. Monsanto began F1 screens for *H. punctigera* in 2009–10 and have recorded no change in frequency of Cry2Ab resistance genes over time with an average of 1 in 1000 (0.012 or 1.2%).

Why has *H. punctigera* shown signs of developing resistance to Cry2Ab when it has no history of resistance to insecticide sprays?

H. punctigera has the capacity to develop resistance to insecticide sprays but it has been presumed that any resistance selection in cotton regions was kept in check by dilution from susceptible immigrants from central Australia each spring. There may be some recent changes to the ecology of *H. punctigera* that could impact on their ability to develop resistance including a greater tendency to overwinter in cotton regions and less immigration of inland individuals than in the past due to low rainfall inland. The decline in Cry2Ab resistance frequencies in *H. punctigera* in 2009–10 may reflect some dilution due to immigration of inland individuals but this hypothesis is difficult to test.

***H. punctigera*.** (Melina Miles, Qld DAFF)

What is known about resistance to Vip3A protein in *H. armigera* and *H. punctigera*?

Monitoring for resistance to the Vip3A protein has revealed that genes allowing survival against this toxin already exist in *H. punctigera* and *H. armigera*. Data obtained by CSIRO suggest that the frequency of vip3A resistance genes in *H. punctigera* is around 1 in 100 (0.01, 1%). This estimate is based on both F2 screens and F1 screens; unlike the situation for Cry2Ab, there is no significant difference among the frequencies obtained with both methods and therefore the frequency reported is from the pooled data. The frequencies of Vip3A resistance alleles in *H. armigera* obtained from F2 screens are higher than those for *H. punctigera*, at 3 in 100 (0.03, 3%). Therefore, as with Cry2Ab, the early data indicate that there is an unexpectedly high frequency of individuals in field populations that carry a gene conferring resistance to Vip3A protein. In 2010–11 Monsanto began screens for Vip3A resistance genes in both *Helicoverpa* spp. and estimate from a small sample a frequency for *H. armigera* based on F1 and F2 screens of 1 in 100 (0.01 or 1%). The estimate of Vip3A resistance frequency for *H. punctigera* based on F1 and F2 screens is also 1 in 100 (0.01 or 1.0%).

Is the current RMP adequate for controlling further increases in resistance frequencies?

There have been no reported field failures of Bollgard II due to resistance. However the finding of a higher baseline frequency of Cry2Ab genes using F1 tests than previously detected using F2 screens is a major concern. It is imperative that all users of Bollgard II steward the technology responsibly. In particular, it is critical that closer attention is paid to managing Bollgard II cotton associated refuges and that effective pupae busting occurs in a timely fashion.

In addition, Monsanto and the TIMS Bt Technical Panel will continue to work together to assess annually new information on resistance frequencies in *Helicoverpa* spp. and knowledge of tactics for Bt resistance management to provide background information and recommendations for the Cotton Australia convened TIMS Committee. Additional measures could be taken in response to significant increases in resistance frequencies to the Cry2Ab toxin in Bollgard II cotton by *Helicoverpa* spp. to mitigate the risk of levels being attained that would lead to field failures. Note that the RMP will continue to be the document that informs growers of their responsibilities in managing Bollgard II cotton while the contingency plan will contain other mitigation strategies that may be introduced into the RMP.

1. Refuges

What is the purpose of refuges?

The aim of refuge crops is to generate significant numbers of susceptible moths (SS) that have not been exposed to selection pressure from the Bt proteins. Moths produced in the refuge crops will disperse to form part of the local mating population where they may mate with any potentially resistant moths (RR) emerging from Bollgard II crops. This reduces the chance that resistant moths will meet and mate. The offspring from matings between one resistant and one susceptible moth will carry one gene from each parent (RS) and are referred to as heterozygotes. In the cases of Bt resistance that have so far been identified, heterozygotes are still controlled by Bollgard II cotton. Therefore, the critical function of the refuge is to dilute the frequency of RR individuals within the population. It is

crucial that the timing of the production of moths from refuges matches that of Bollgard II crops. While the use of planting windows and use of two Bt genes in Bollgard II cotton are aimed at reducing selection pressure for Bt resistance, the use of refuge crops is to try to balance or counter the selection that will still occur.

How were the current requirements for refuge crops determined?

The relative sizes of refuge crops required in the RMP are based on models and knowledge of Helicoverpa moth emergence for different crop types. The likely moth productivity of the different refuge options has been determined through large-scale field experiments conducted by researchers within the Cotton CRC over several seasons. Only refuge options that have been assessed in this way are currently approved by the APVMA. In these experiments, a refuge of 10% unsprayed cotton was considered as the reference point. On average pigeon pea produced twice as many moths as the same area of unsprayed cotton, hence a 5% refuge, half that of an unsprayed cotton refuge, is required for this crop. Initially, sorghum and corn were included as refuge options in the RMP because they were effective at producing *H. armigera* moths. However, since they are not a preferred host for *H. punctigera,* from 2010–11 sorghum and corn were removed from the RMP as refuge options.

Is there a minimum size to a refuge crop?

Where sprayed conventional cotton is grown on the farm unit, each refuge crop must be at least 48 metres wide and a minimum of 2 hectares. This is to minimise the risk of spray drift onto the refuge, as this would decrease the effectiveness of the refuge in producing moths.

If no sprayed conventional cotton is grown on the farm, the minimum size of a refuge must be 24 metres wide and 24 metres long. Sprayed and unsprayed refuges must be planted separately.

Can mixtures of the refuge crop options be used to meet the refuge requirements?

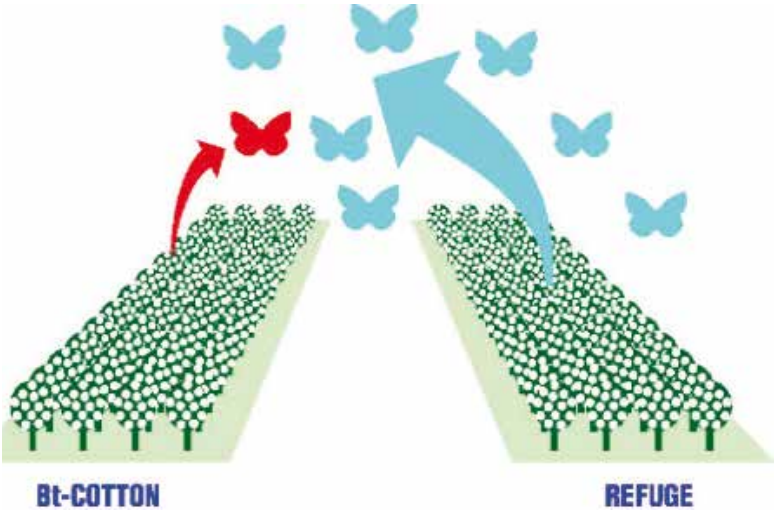
It is possible to combine more than one type of refuge, provided that the total requirements for area equivalence are met. For example, 1 hectare of pigeon pea can be grown alongside 1 hectare of unsprayed cotton, rather than 2 hectares of either. Each type of refuge must be managed so that it is productive and other restrictions on minimum dimensions, number of plantings and location also need to be met. However, sprayed and unsprayed refuge options cannot be mixed in the same field. For example, it would not be acceptable to use 1 hectare of pigeon pea grown alongside 30 hectares of sprayed cotton as a substitute for 2 hectares of pigeon pea.

Why can't a conventional crop from a neighbouring property act as a refuge?

In some cases, a conventional crop grown on a neighbouring property may satisfy the requirements of a refuge for Bollgard II. However, the crop may not be managed in a way that complies with the RMP. Since growers cannot control the management of a neighbour's crop, it is not sensible to rely on these areas as refuges for Bollgard II.

Why do the refuge options differ for dryland Bollgard II and irrigated Bollgard II?

For dryland Bollgard II crops the only available dryland refuge options are sprayed or unsprayed cotton.The reason for this



Moths produced from refuges dilute resistance genes in the population.

is that the other refuge option available in irrigated Bollgard II (pigeon pea) tends to be planted after the cotton and it's a requirement that dryland refuges must be planted within the 2 week period prior to the first day of planting Bollgard II cotton. However CSIRO and Monsanto have conducted work on the suitability of pigeon pea as a dryland conventional cotton treated and not treated by slashing as a potential refuge option. There are also irrigated refuge options for dryland Bollgard II cotton. These options are sprayed or unsprayed irrigated cotton and unsprayed irrigated pigeon pea, and were chosen because to date they have been the most widely adopted refuges for irrigated Bollgard II.

How can the 'effectiveness' of an individual refuge be evaluated?

The productivity of refuges will vary considerably across regions and seasons. It is not possible to place a value on the effectiveness of each refuge. Looking after refuges, including nutrition, weed control, timely irrigation and all factors that make the refuge 'attractive' to female moths laying eggs, is the key to ensuring that they are effective. Managing resistance is a population level activity, and every refuge makes an important contribution to the overall RMP for the valley, and because *Helicoverpa* spp. disperse widely, on a larger scale for the whole industry. It is imperative that all refuges produce their quota of susceptible (SS) moths. Monsanto audits the quality of refuges on every farm that grows Bollgard II to ensure that they are well maintained and effective.

Why is the location of refuge crops important?

For the refuge principle to be successful, refuge crop areas must be in close proximity to the Bollgard II crop(s) to ensure that it is highly likely that moths emerging from the Bollgard II will mate with susceptible moths from the refuge crop. Helicoverpa moths are capable of migrating long distances, but during the summer cropping season a significant part of the population may remain localised and move only a few kilometres within a region. The level of movement will depend on the mix of crops and their attractiveness at the time of moth emergence. For this reason the best location for a refuge crop is close as possible to the Bollgard II crop, within 2 km.

Is there an alternative to growing refuges for resistance management?

No, though alternatives are being investigated. It is important to recognise that the costs associated with refuge crops are an investment in the longer term value of transgenic technology for the industry. The costs associated with growing an attractive refuge should be considered as an integral part of growing Bollgard II.

2. Volunteers

Why is it important to control conventional cotton volunteers or ratoon plants in Bollgard II?

In terms of the RMP, it is important to prevent the establishment of conventional cotton in Bollgard II fields because larger larvae that have grown on conventional cotton plants are moderately tolerant to Bt. If large larvae migrate to neighbouring Bt plants, those that are heterozygotes (RS) may survive and contribute to increasing the frequency of resistance genes in the *Helicoverpa* spp. population. In the cases of Bt resistance that have so far been identified, heterozygotes are controlled by Bollgard II cotton. By removing conventional volunteers from Bollgard II fields, heterozygotes will have no opportunity to grow large enough to be able to tolerate Bt plants and therefore contribute their resistance genes to the next generation of moths.

Why is it important to control Bollgard II volunteers or ratoon plants in conventional cotton and all refuges?

The same logic applies as in the previous question. The presence of Bollgard II volunteer plants in a conventional crop or refuge exerts a selection pressure for Bt resistance. Heterozygous (RS) larvae that emerge from eggs laid on conventional cotton may grow and during their development move onto Bollgard II volunteers. In this way RS larvae become exposed to Bt at later growth stages when they can survive to produce offspring. This will lead to an increase in the frequency of resistant individuals (both RS and RR) in the population. If the field is designated as a refuge crop, the presence of the Bollgard II volunteers will diminish the value of the refuge.

3. Planting windows

Why do we need a Bollgard II planting window?

The purpose of restricting the planting window is to limit the number of generations of *H. armigera* that will be exposed to Bollgard II in any one season. This measure effectively restricts the selection pressure on *H. armigera* to develop resistance to Bollgard II.

Is it possible to vary the Bollgard II planting window?

Where exceptional circumstances exist, requests for a variation to the planting window will be considered. In the past Monsanto approached the APVMA on behalf of a grower or Cotton Grower's Association to consider requests. From 2006–07 onwards, the TIMS Committee will consider requests. Requests must satisfy a number of criteria as outlined in the 'Request for variation to the Bollgard II planting window' document, found on page 74. If a request is approved, the variation only affects the planting window component of the RMP for the requestee/s for the current season. All other components of the RMP remain the same.

4. No Bt sprays

Why is it important that foliar Bt sprays are not used on refuges?

By preventing the use of foliar Bt on all refuges (sprayed and unsprayed), the likelihood of producing moths that are susceptible (SS) rather than resistant (RR) to Bt is maximised. This is an important part of the RMP because susceptible refuge moths are presumed to mate with any resistant moths in the population to produce heterozygotes (RS) that are killed by Bollgard II.

With regard to refuge crops, what does the term 'unsprayed' mean?

The term 'unsprayed' encompasses all management activities which are likely to reduce the survival of Helicoverpa in these crops. Insecticides with activity against Helicoverpa cannot be used in unsprayed refuges. Food sprays cannot be used in unsprayed refuges as these aim to reduce Helicoverpa survival through increased predation and parasitism. Similarly, Trichogramma and other biolocial control agents cannot be released in unsprayed refuges as they too aim to reduce Helicoverpa survival.

5. Pupae destruction

Given that few larvae survive in Bollgard II, why is it important to pupae bust?

Cultivating between seasons prevents any moths that developed resistance in the previous year from contributing to the population in the following year. Although we expect few larvae to survive in Bollgard II, those that do are most likely resistant and these are precisely the ones that must be killed so that the next generation of moths (emerging the following spring) are not enriched with resistant individuals. This is especially the case in a drought year because of the increased opportunity for 'resistance genes' to increase in frequency.

Am I required to pupae bust in my refuges?

Refuges must produce moths during the cotton season when Bollgard II is grown but unsprayed refuges can continue to provide benefits for resistance management by being left in place until the following spring. By doing this any pupae produced in the autumn may be carried over the spring and provide additional genetic dilution of resistant survivors. Once Bollgard II crops begin flowering and are highly attractive to Helicoverpa moths, the corresponding refuge should not be cultivated (e.g. for weed control, row formation etc).

Why are there requirements for trap cropping in central Queensland?

In central Queensland *Helicoverpa* spp. pupae produced late in the cotton season do not remain in the soil, but emerge within 15 days of pupating. Pupae busting is not an effective resistance management tool in these warmer areas and trap crops are required as an alternative. Trap crops of pigeon peas are planted after the cotton and are timed to be at their most attractive after the cotton has cut-out. Thus moths emerging from Bollgard II cotton fields at the end of the season will be attracted to the trap crops and are likely to lay their eggs in the trap crop. The egg and larval stages can last 30+ days. Once the cotton has been harvested, the trap crop should be destroyed, removing the food source from the larvae (which will then die) and the soil then cultivated to destroy any pupae. It is critical to time the destruction so that it corresponds with the period of most effective kill of the range of life stages of Helicoverpa. See the 2010–11 RMP for more details.

Guidelines for Helicoverpa management in Bollgard II cotton

Since 2005–06 there have been occasional reports of larvae surviving for several weeks at threshold levels in Bollgard II fields. All affected fields were at mid-flowering to late-flowering and the survivors included *H. armigera* and *H. punctigera*.

Work conducted by CSIRO and Monsanto demonstrated that these larvae did not survive on Bollgard II due to Bt resistance or because of the absence of Bt genes in the cotton. Recent work suggests that larvae exhibit strong behavioural responses to the Bt proteins in Bollgard II plants. Detection and avoidance of the Bt toxins results in frequent movement of larvae, potentially within and between plants, resulting in an apparent feeding preference for flowers. These behaviours, coupled with the sometimes temporal and spatial variability of Bt toxin expression in Bollgard II cotton, can result in a proportion of larvae becoming established.

For resistance management reasons, it is recommended that if larvae reach thresholds in Bollgard II fields they should be controlled by spraying. However work conducted by Monsanto suggests that it is unlikely that there will be a yield penalty associated with larvae survival in Bollgard II fields. This is supported by a recent study that used the distribution of larval damage in fields that carried larvae at the current thresholds as the basis for an artificial damage experiment. The work showed that Bollgard II plants could tolerate up to 100% square loss at early flowering, up to 100% square removal alone or in combination with 30% boll damage at peak flowering, and 30% boll damage at late flowering, without impacting yield or quality. Therefore Bollgard II cotton seems to compensate well for damage caused by larvae and the current threshold can be used in most situations without causing significant yield reduction.

With the increased risk of resistance to Cry2Ab in Helicoverpa it is critical that we monitor the distribution and proportions of fields that are affected by surviving larvae, and the number of fields that are sprayed to control Helicoverpa. Part of the end of season general survey of CCA members includes questions about control of Helicoverpa in Bollgard II fields.

If you experience above threshold levels of Helicoverpa in your Bollgard II fields please immediately contact:

- **Sharon Downes: 02 6799 1576–0427 480 967; or,**
- **Kristen Knight 07 4634 8400–0429 666 086.**

Insecticide selection for Bollgard II crops

When controlling Helicoverpa within Bollgard II crops, insecticide selection should comply with the cotton industry's Insecticide Resistance Management Strategy (pages 59–67). The predator/pest ratio (described on page 11) should also be given careful consideration when the application of an insecticide is being considered. If an insecticide is required, try to choose the most effective product that is the least disruptive to the beneficial complex. Refer to pages 8–9. While foliar Bt can be used on Bollgard II crops, it is a requirement of the Bollgard II Resistance Management Plan that foliar Bt not be used on any refuge crops.

Helicoverpa thresholds

Do not include any larvae <3 mm long in spray threshold counts. For economic management of Helicoverpa, larval populations should be controlled with an insecticide if a threshold of:

- 2 larvae /m >3 mm long are found over 2 consecutive checks; or,
- 1 larvae /m >8 mm long is found in any check.

Application of these thresholds requires careful and accurate assessment. Checks should be made over the whole plant including the terminals, squares and especially flowers and small bolls. Be sure to objectively assess larval size. A complete description of the sampling protocols for Helicoverpa can be found on page 10.

GUIDELINES FOR AMENDING BOLLGARD II PLANTING WINDOWS 2013–14

Planting Windows in the Bollgard II® RMP are the key element in the strategy for restricting the number of generations of Helicoverpa spp. exposed to Bollgard II® in a region. This is necessary to limit the rate of evolution of resistance to Bt toxins. These guidelines allow a degree of flexibility to accommodate unforseen circumstances without jeopardising this objective.

The TIMS Committee will only consider requests for a variation to the planting window in situations in which exceptional circumstances exist.

If the request is accepted and agreed to by the TIMS committee then a "Bollgard II® Planting Window Variation Notice" will be issued by Monsanto. This variation only affects the planting window component of the Resistance Management Plan (RMP). All other components of the RMP remain the same.

Process

Monsanto is responsible for the issuing of a "Bollgard II® Planting Window Variation Notice" under the APVMA Notice of Variation of Registration of Agricultural Product – Bollgard II® cotton (March 23, 2006).

Cotton Growers who wish to request a variation to Bollgard II® planting window dates for them or their region will need to make a formal request to the TIMS Committee who will make a written recommendation to Monsanto. The request must be in writing from their local CGA and received, where possible, by the end of August.

It is essential that there has been wide consultation regarding the proposal including; CGA members, local consultants, Industry Development & Delivery Team and researchers and the local Monsanto Regional Business Manager. Requests that are supported by TIMS will be approved by Monsanto. The Variation Notice will be communicated to relevant organisations and individuals by TIMS and Monsanto.

Criteria for assessing the application to change a planting window

1. The Cotton Growers Association (CGA) must request and approve the change with a majority vote (an absolute majority is more than 50% of all CGA members eligible to vote).
2. The majority decision affects TUA compliance for all licensed growers. This means that all growers, even the minority that voted against the change, must abide by the majority decision. The CGA must advise all growers of the outcome of the vote, and their obligation to abide the decision. Evidence in writing of this process will be required from the CGA, together with the information requested below.
3. The region (or individual grower) requesting the variation is more than 100 kms from any other significant Bollgard II® planting.
4. Planting of Bollgard II® in the region requesting the variation has not exceeded 10% of the anticipated Bollgard II® cotton area.
5. No Bollgard II® cotton has been planted in excess of 21 days prior to the opening of the new window.
6. There are no known threats to the efficacy of refuges in the region (e.g., plague locust pressure).
7. The requested planting window variation must be a 42 day window that falls entirely within the period September 1 to December 31.

Essential information to be submitted with a request for a Bollgard II® planting window variation

1. Detailed description of the reasons for the request.
2. Proposed new window start and finish dates.
3. Map or description of the region concerned.
4. Distance of the relevant region to nearest neighbouring cotton.
5. Time of first Bollgard II® cotton planted in the region.
6. Area of Bollgard II® already planted in the region.
7. Projected total area to be planted to Bollgard II® in the region.
8. Statement confirming that all cotton growers in the region, even those that voted against the change, will abide by the requested changes to the window.
9. Statement confirming that all cotton growers in the region acknowledge that they must meet the pupae busting requirements in the RMP even when a later planting window is requested.

TIMS Committee, C/- Greg Kauter, Cotton Australia Ltd. Suite 4.01, 247 Coward St., Mascot NSW 2020.
Ph: 02 9669 5222 M0b: 0429 700 711 E: gregk@cotton.org.au