

Preamble to the Resistance Management Plan (RMP) for Bollgard II 2014–15

Sharon Downes and Lewis Wilson, CSIRO
Kristen Knight, Monsanto Australia Limited
Greg Kauter, formerly 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.

Bollgard II acreage will represent over 90% of the total area of cotton planted in the 2014–15 season. 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 3, 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 3 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 3 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 3. 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 have been 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. This method was incorporated into the monitoring program by CSIRO in 2002 and Monsanto in 2003 and detects all previously detected and potentially new types of resistances but is very labour intensive.

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. 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. 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 each of the isolates of Cry2Ab detected until then was the same type of resistance, and subsequently adopted F1 tests.

From 2002 to 2012 CSIRO continued to perform tests which showed that all newly isolated resistances to Cry2Ab were the same type that was initially identified. Similarly, work on Vip3A from 2009 to 2012 showed that all newly isolated resistances were the same type that was initially identified. In 2013 CSIRO shifted to performing only F1 screens to focus on the frequencies of the known resistances. In addition to screening F1 families against the toxin of interest (e.g., Cry2Ab), they introduced screens against all classes of Bt toxins (e.g., Cry1Ac and Vip3A) in an effort to detect any novel forms of resistance that carry dominance. Every 4 or 5 years CSIRO will incorporate F2 screens into the program to check for any new recessive forms of resistance. In 2013 Monsanto continued to perform both F2 screens and F1 screens.

This chapter uses the 2013 data from the program run by Monsanto for F2 screens and from the CSIRO and Monsanto programs for the F1 screens.

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



H. armigera. (Melina Miles, DAFF Qld)

less than 5 in 10,000 (<0.0005 or 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. Monsanto did not isolate any positive alleles in *H. armigera* from 100 tested using F2 screens in 2013–14. Results with *H. armigera* show that the estimate of Cry2Ab resistance frequency for F1 screens at the end of the 2013–14 season is approximately 2 in 100 (0.02, 2%) or less, which is higher than for F2 screens. 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 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 at less than 1 in 1000 (0.001, 0.1%). It is not cross-resistant to Cry2Ab. In

GET MORE THAN WATER SAVINGS

www.valleyirrigation.com/au

Get even more than water and labor savings with your new pivot or lateral. For a limited time, when you buy a new machine you will receive a FREE Valley® Jacket.



www.valley-au.com

Ph: 07-3457-8830

Email: vaus@valmont.com



H. punctigera. (Melina Miles, DAFF Qld)

2013/14, CSIRO used F1 tests against *H. punctigera* for Cry1Ac resistance and detected a frequency of 4 in 1000 (0.004, 0.4%). This is the first year that these F1 screens were performed.

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 performed by Monsanto indicated that the frequency of this gene in 2013–14 was 1 in 500 (0.002, 0.2%).

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 2013–14 season, the frequency of Cry2Ab genes in *H. punctigera* was approximately 1 in 100 (0.01, 1.0%).

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* from 2002 to 2012 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 1 in 250 (0.004 or 0.4%).

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 demonstrates a 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 2013–14 the Cry2Ab resistance frequency is at a similar level to that recorded in 2008–09 (1 in 185, 0.005 or 0.5%). If the probable overestimation in frequency 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 2013–14 are lower than those detected in 2008–09 and most recently have declined to the levels first detected in 2007–08. 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 62 (0.016 or 1.6%).

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.

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

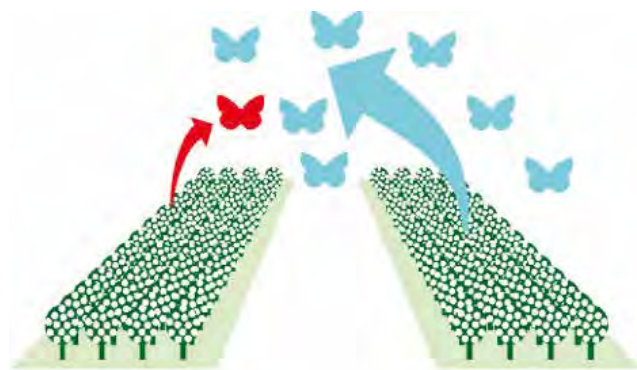
Monitoring for resistance to the Vip3A protein has revealed that genes allowing survival against this toxin already exist in *H. punctigera* and *H. armigera*. Data obtained by CSIRO suggest that the frequency of vip3A resistance genes in *H. punctigera* is around 1 in 100 (0.01, 1%). This estimate is based on F2 screens (2009–2012). 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 160 (0.006 or 0.6%).

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



Moths produced from refuges dilute resistance genes in the population.



InsectTech™

HELICOVEX®

High Potency & Highly selective Heliothis NPV Insecticide



- Contributes to resistance management of traditional insecticides
- High quality formulation
- Excellent shelf life and spray tank stability.

INTRODUCTORY OFFER

2 FREE Heliothis moth monitoring traps supplied with every 1L sold.



20% off MyTraps pest monitoring software packages with every purchase.
Use coupon code OCP.



NSW/WA	James	0408 025 139
SA	Plamen	0488 583 333
VIC/TAS	Scott	0488 717 515
QLD	Andrew	0448 016 551

www.ocp.com.au



RMP tactics

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 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 which is especially important in warmer growing regions. 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?

A permit (PER14804) was issued to vary Bollgard II planting windows by the APVMA on the 25th August 2014. Refer to summary on page 76.

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 biological 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 do not need to be pupae busted. 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 2014–15 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 Crop Consultants Association (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 66–69). The predator/pest ratio (described on page 10) 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.



SUMMARY OF BOLLGARD II PLANTING WINDOW PERMIT

A permit (PER14804) was issued to vary Bollgard II planting windows by the Australian Pesticides and Veterinary Medicines Authority (APVMA) on 25th August 2014. Specifically, this permit is designed to study the effect of an extended planting window on end of season crop management.

Overview of Planting Window Permit

Region	Overview of Permit Window	Mitigation Strategies
NSW and Southern QLD (incl. Darling Downs)	All Bollgard II must be planted by November 30 (an additional 15 days after the RMP window close)	Bollgard II planted within the permit period (November 15–30) must be destroyed within 30 days of completion of harvest
Central QLD	An additional 2 weeks to be allocated adjacent to the current RMP window, at the discretion of the CGA (can be prior or following the current window or 1 week prior and 1 week after current window)	<ul style="list-style-type: none"> • Bollgard II planted within the permit period must be destroyed within 30 days of completion of harvest • Bollgard II planted later than the current RMP window will require an additional 1% refuge area

In order to take advantage of this permit, the RMP prescribed planting window cannot be varied. In the event that a variation notice is submitted and approved, this permit will no longer apply to the valley in question. The approval of this permit means that the previous planting window extension permit in Emerald is no longer valid and cannot be used.

Our last line of defence.



Pupae bust in winter to slow down resistance
Because we are in this together
www.mybmp.com.au

Kate Dhu Photography

Best Practice

SCHEDULE A – RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2014–15

Developed by Monsanto Australia Limited and the Transgenic and Insect Management Strategy (TIMS) Committee of Cotton Australia Ltd.

The resistance management plan is based on three basic principles: (1) minimising the exposure of *Helicoverpa* spp. to the *Bacillus thuringiensis* (Bt) proteins Cry1Ac and Cry2Ab; (2) providing a population of susceptible individuals that can mate with any resistant individuals, hence diluting any potential resistance; and (3) removing resistant individuals at the end of the cotton season. The three principles are supported through the implementation of 5 elements that are the key components of the Resistance Management Plan. These elements are:

1. Refuge crops;
2. Planting window;
3. Pupae busting/Trap crops;
4. Control of volunteers and ratoon cotton; and,
5. Spray limitations.

Growers of Bollgard II cotton are required to practice preventative resistance management as set out below.

Compliance with the Resistance Management Plan is required under the terms of the Bollgard II Technology User Agreement and under the conditions of registration (*Agricultural and Veterinary Chemicals Act 1994*).

Section 1 is applicable to all regions in New South Wales and Queensland that grow cotton while sections 2 and 3 detail specific requirements for New South Wales and Southern Queensland, and Central Queensland respectively.

New South Wales, Southern Queensland & Central Queensland

1. Refuges

Growers planting Bollgard II cotton will also be required to grow a refuge crop that is capable of producing large numbers of *Helicoverpa* spp. moths which have not been exposed to selection with Bt proteins Cry1Ac and Cry2Ab. These unselected moths are expected to dominate matings with any survivors from Bollgard II crops and thus help to maintain resistance to Bt proteins Cry1Ac and Cry2Ab at low levels.

All refuge options are based on the requirement of a 10% unsprayed cotton refuge or its equivalent, as determined by the relative production of *Helicoverpa* spp. from each of the refuge types as described in Tables A and B, for irrigated and dryland production scenarios respectively. Irrespective of the irrigation regime for the Bollgard II cotton, all pigeon pea refuges must be fully irrigated so that they are attractive to *Helicoverpa* spp. during the growing period of the Bollgard II cotton.

For each area of irrigated Bollgard II cotton planted, a grower is required to plant a minimum of one or a combination of the following:

TABLE A: Irrigated Bollgard II cotton refuge options

Crop	Conditions	% of Bollgard II
Cotton	Irrigated, sprayed conventional cotton	100
	Irrigated, unsprayed conventional cotton	10
Pigeon pea	Fully irrigated, unsprayed	5

TABLE B: Dryland Bollgard II cotton refuge options

Crop	Conditions	% of Bollgard II
Cotton	Dryland or irrigated, sprayed conventional cotton	100
	Dryland or irrigated, unsprayed conventional cotton	10
Pigeon pea	Fully irrigated, unsprayed	5

No other refuge options are approved for dryland Bollgard II.

Note: Unsprayed means not sprayed with any insecticide that targets any life stage of *Helicoverpa* spp.

Bt products must not be applied to any refuge (including sprayed cotton).

If the viability of an unsprayed refuge is at risk due to early or late season pressure by *Helicoverpa* spp., or any other caterpillar species, contact Monsanto immediately. With prior approval from the Monsanto Compliance and Stewardship Manager, a non-Bt heliocide can be applied.

An unsprayed refuge should not be planted in the same field as any crop sprayed with a rate of insecticide that is registered for *Helicoverpa* spp., with the exception of Bollgard II. Sprayed crops and unsprayed refuges that are planted in adjacent fields must be separated by sufficient distance to minimise the likelihood of insecticide drift onto the unsprayed refuge.

For the purposes of this Resistance Management Plan, conventional cotton includes any cotton varieties that do not have Bt proteins in the plant that control *Helicoverpa* spp. moths.

SCHEDULE A – RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2014–15

General conditions for all refuges:

- (a) Refuge crops are to be planted and managed so that they are attractive to *Helicoverpa* spp. during the growing period of the Bollgard II cotton varieties.
- Irrigated:** It is preferable that all refuge is planted within the 2 week period prior to planting Bollgard II. If this is not possible, refuge planting must be completed within 3 weeks of the first day of sowing of Bollgard II. At this time, sufficient refuge must have been planted to cover all of the Bollgard II cotton proposed to be planted for the season (including Bollgard II already planted and any that remains unplanted). Should additional Bollgard II planting be made after this date, which is not already covered by refuge, additional refuge must be planted as soon as possible and no more than 2 weeks after sowing of the additional Bollgard II.
- Dryland:** A dryland refuge must be planted within the 2 week period prior to the first day of planting Bollgard II cotton.
- (b) Pigeon pea refuges should not be planted until the soil temperature reaches 17°C, which is a requirement for germination, and should also be planted into moisture to ensure successful germination. If soil temperatures are not suitable to allow germination of pigeon peas in line with condition (a), an alternative refuge must be planted in its place within the prescribed period (under (a) above).
- (c) Once Bollgard II cotton begins to flower the corresponding refuge must not be cultivated.
- (d) Insecticide preparations containing Bt may be used on Bollgard II cotton throughout the season BUT NOT on any refuge crops.
- (e) All refuges are to be planted within the farm unit growing Bollgard II cotton. Subject to clause (f) below, all reasonable effort should be taken to plant the refuge either on one side of, or next to a Bollgard II cotton field and all Bollgard II fields must be no more than 2 km from the nearest associated Bollgard II refuge.
- (f) To minimise the possibility of refuge attractiveness being affected by herbicide drift, non-herbicide tolerant refuges should be separated from herbicide tolerant Bollgard II cotton crops by a sufficient distance to minimise such drift, but no more than 2km from the Bollgard II cotton.
- (g) To account for possible insecticide drift, the options for the width of refuge crops vary according to spray regime. If any sprayed conventional cotton is grown on the same farm unit, Bollgard II refuge crops must be at least 48 metres wide and each refuge area must be a minimum of 2 hectares. If no sprayed conventional cotton is grown on the same farm unit, Bollgard II refuge crops must be at least 24 metres wide and 24 metres long. Different unsprayed refuge options may be planted in the same field as a single unit; however a sprayed conventional cotton refuge must not be planted in a field that is also planted to an unsprayed refuge type.
- (h) In all regions, destruction of refuges should only be carried out after Bollgard II cotton lint removal has been completed.
- (i) Refuges for dryland Bollgard II cotton crops must be planted in the same row configuration as the Bollgard II crop unless the refuge is irrigated. If an irrigated option is utilised for a dryland Bollgard II crop, then that refuge may be planted in a solid configuration. Dryland cotton is measured as green hectares (calculated as defined in the Technology User Agreement).

2. Control of volunteer and ratoon cotton

Volunteer and ratoon cotton may impose additional selection pressure on *Helicoverpa* spp. to develop resistance to the Bt Cry1Ac and Cry2Ab proteins produced by Bollgard II cotton.

Growers must ensure that volunteer and ratoon plants are removed as soon as possible from all fields, including fallow areas, Bollgard II crops, conventional cotton crops and all refuges. **The presence of Bollgard II volunteers/ratoon cotton in any refuge will diminish the value of the refuge and must be removed as soon as possible.**

Note: The refuge should preferably be planted into fallow or rotation fields that have not been planted to cotton in the previous season.

3. Post-harvest crop destruction

As soon as practical after harvest, Bollgard II cotton crops must be destroyed by cultivation or herbicide so that they do not continue to act as hosts for *Helicoverpa* spp.

Section 2: New South Wales & Southern Queensland only

1. Planting windows

All Bollgard II crops are to be planted into moisture or watered-up by 15 November, unless otherwise advised by a Bollgard II Planting Window Variation Notice.

2. Pupae destruction

In Bollgard II cotton fields, each grower will be required to undertake *Helicoverpa* spp. pupae destruction after harvest according to the following key guidelines:

- Bollgard II crops should be slashed or mulched and fields cultivated for pupae control within 4 weeks of harvesting. All pupae busting must be completed by July 31.
- Ensure disturbance of the whole soil surface to a depth of 10 cm.
- All fields that are sown to any winter crop following a Bollgard II crop must be inspected by the Technology Service Provider before sowing commences in order to ensure that pupae busting has occurred.

SCHEDULE A – RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2014–15

In Refuge crops:

In New South Wales and Southern Queensland, to ensure maximum emergence of late pupae from associated refuges, soil disturbance of refuge crops should not be undertaken until after the pupae busting in Bollgard II cotton crops on the farm unit is complete. All unsprayed refuges, should preferably be left uncultivated until the following October.

3. Failed crops

Bollgard II crops that will not be grown through to harvest for various reasons and are declared to, and verified by, Monsanto as failed must be destroyed within two weeks after verification, in such a way that prevents regrowth. Crops abandoned before February 28 do not require pupae busting. Crops abandoned on February 28 or later must be pupae busted.

Section 3: Central Queensland only

1. Planting Windows

Central Highlands: All Bollgard II crops are to be planted into moisture or watered-up in the period between September 15 and October 26, unless advised otherwise by a Bollgard II Planting Window Variation Notice.

Dawson Callide Valleys: All Bollgard II crops are to be planted into moisture or watered-up in the period between September 15 and October 26, unless advised otherwise by a Bollgard II Planting Window Variation Notice.

Belyando/Clermont: All Bollgard II crops are to be planted into moisture or watered-up in the period between November 4 and December 15, unless advised otherwise by a Bollgard II Planting Window Variation Notice.

Mackenzie: All Bollgard II crops are to be planted into moisture or watered-up in the period between November 4 and December 15, unless advised otherwise by a Bollgard II Planting Window Variation Notice.

2. Refuges

Pigeon Pea refuge should preferably be planted into a fallow or rotation field that has not been planted to cotton in the previous season to avoid volunteer and ratoon cotton.

In Central Queensland soil disturbance of refuge crops can only occur 2 weeks after final defoliation of the Bollgard II cotton.

3. Late summer pigeon pea trap crop

A late summer trap crop (pigeon pea) must be planted for all Bollgard II cotton grown in Central Queensland. The planting configuration of the trap crop should be the same as that of the Bollgard II crop.

Irrigated Bollgard II must have an irrigated trap crop. Table 3 shows the requirements for the late summer pigeon pea trap crop. Dryland Bollgard II growers who do not have any irrigated cotton on their farm should contact their Monsanto Regional Business Manager for alternative options.

Refuge and late summer trap crops have different purposes and, if pigeon pea is selected for both, two separate plantings may be required. However, where a pigeon pea refuge is utilised as a trap crop the full 5% pigeon pea refuge area must be managed to become the late summer trap crop and must adhere to the requirements in Table 3 below.

TABLE 3: Late summer pigeon pea trap crop requirements in Central Queensland

Criterion	Trap crop*
Minimum area & dimension (Requirement)	A minimum trap crop of 1% of planted Bollgard II cotton crop is required. If sprayed conventional cotton is grown on that farm unit: the trap crop must be at least 48m x 48m. If no sprayed conventional cotton is grown on that farm unit: the trap crop must be at least 24m x 24m.
Planting time	The trap crop should preferably be planted between November 1 and November 30. Note: if growers choose to plant their trap crop to coincide with the planting of pigeon pea refuges they must manage the trap crop in such a way that it remains attractive to <i>Helicoverpa</i> spp. 2-4 weeks after final defoliation.
Planting rate **	35kg/ha (recommended establishment greater than 4 plants per metre)
Insect control	The trap crop can be sprayed with virus after flowering, while avoiding insecticide spray drift, except where a pigeon pea refuge is converted to a trap crop. In this case the full 5% pigeon pea refuge area managed to become the late summer trap crop can only be sprayed with virus after the first defoliation of Bollgard II cotton.
Irrigation	The trap crop must be planted into an area where it can receive the additional irrigation required to keep the trap crop attractive to <i>Helicoverpa</i> spp. until after the cotton is defoliated.
Weed control	The trap crop should be kept free of weeds and particularly volunteer Bollgard II cotton. When using the full 5% pigeon pea trap crop option, weed control must not be carried out by cultivation once flowering of the associated Bollgard II cotton crop has commenced.
Crop destruction	The trap crop must be destroyed 2-4 weeks (but not before 2 weeks) after final defoliation of the Bollgard II cotton crop, (slash and pupae bust – full soil disturbance to a depth of 10cm across the entire trap crop area). All Bollgard II and associated trap crops must be destroyed by July 31.

* A pigeon pea trap crop is to be planted so that it is attractive (flowering) to *Helicoverpa* spp. after the cotton crop has cut out, and as any survivors from the Bollgard II crop emerge. Planting pigeon pea too early (e.g. before November) or too late (e.g. mid December) is not adequate for cotton crops planted during September through to October.

** The planting rate is a recommendation based on a minimum of 85% seed germination.

NB: If any grower encounters problems in complying with the RMP, please contact your Monsanto Regional Business Manager. For further background information on the various components of this plan see the "Preamble to the Resistance Management Plan for Bollgard II" (see pages 70–76).

SCHEDULE A – RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2014–15

Ord River Irrigation & Burdekin Bowen Basin & Richmond areas

Developed by Monsanto Australia Limited and the Transgenic and Insect Management Strategy (TIMS) Committee of Cotton Australia Limited.

The resistance management plan is based on three basic principles: (1) minimising the exposure of *Helicoverpa* spp. to the *Bacillus thuringiensis* (Bt) proteins Cry1Ac and Cry2Ab; (2) providing a population of susceptible individuals that can mate with any resistant individuals, hence diluting any potential resistance; and (3) removing resistant individuals at the end of the cotton season. The three principles are supported through the implementation of 5 elements that are the key components of the Resistance Management Plan. These elements are:

1. Refuge crops;
2. Planting window;
3. Pupae busting/Trap crops;
4. Control of volunteers and ratoon cotton; and,
5. Spray limitations.

Growers of Bollgard II cotton are required to practice preventative resistance management as set out below.

Compliance with the Resistance Management Plan is required under the terms of the Bollgard II Technology User Agreement and under the conditions of registration (Agricultural and Veterinary Chemicals Act, 1994).

This RMP is for the following areas:

- Ord River Irrigation Area, Western Australia
- Burdekin Bowen Basin Area, Queensland
- Richmond Area, Queensland

1. Refuges

Growers planting Bollgard II cotton will also be required to grow a refuge crop that is capable of producing large numbers of *Helicoverpa* spp. moths which have not been exposed to selection with Bt proteins Cry1Ac and Cry2Ab. These unselected moths are expected to dominate matings with any survivors from Bollgard II crops and thus help to maintain resistance to Bt proteins Cry1Ac and Cry2Ab at low levels.

All refuge options are based on the requirement of a 10% unsprayed cotton refuge or its equivalent as determined by the relative production of *Helicoverpa* spp. from each of the refuge types as described in tables below.

For each area of irrigated Bollgard II cotton planted, a grower is required to plant a minimum of one, or a combination of, the following:

Irrigated Bollgard II cotton refuge options			
Crop	Conditions	% of Bollgard II	Regions permitted
Conventional Cotton	Irrigated, unsprayed conventional cotton	10	All regions
Pigeon pea	Fully irrigated, unsprayed	5	All regions

Note: Unsprayed means not sprayed with insecticides that target any life stage of *Helicoverpa* spp.

Bt products must not be applied to any refuge.

If the viability of an unsprayed refuge is at risk due to early or late season pressure by *Helicoverpa* spp., or any other caterpillar species, contact Monsanto immediately. With prior approval from the Monsanto Compliance and Stewardship Manager, a non-Bt heliocide can be applied.

An unsprayed refuge should not be planted in the same field as any crop sprayed with a rate of insecticide that is registered for *Helicoverpa* spp, with the exception of Bollgard II unless a sufficient buffer is in place to prevent insecticide drift.

Sprayed crops and unsprayed refuges that are planted in adjacent fields must also be separated by sufficient distance to minimise the likelihood of insecticide drift onto the unsprayed refuge.

For the purposes of this Resistance Management Plan, conventional cotton includes any cotton varieties that do not have Bt proteins in the plant that control *Helicoverpa* spp. larvae.

General conditions for all refuges:

(a) Refuge crops are to be planted and managed so that they are attractive to *Helicoverpa* spp. during the growing period of the Bollgard II cotton varieties.

Ord River Irrigation Area: It is preferable that all refuge is planted within the 2 week period prior to planting Bollgard II. If this is not possible, refuge planting must be completed within 3 weeks of the first day of sowing of Bollgard II. At this time, sufficient refuge must have been planted to cover all of the Bollgard II cotton proposed to be planted for the season (including Bollgard II already planted and any that remains unplanted). Should additional Bollgard II planting be made after this date, which is not already covered by refuge, additional refuge must be planted as soon as possible and no more than 2 weeks after sowing of the additional Bollgard II.

Burdekin Bowen and Richmond Areas: Refuges must be sown within the 2 weeks prior to planting any Bollgard II. This timing attempts to mitigate wet season planting risks.

SCHEDULE A – RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2014–15

- (b) Group J legume inoculant should be used to treat pigeon pea planting seed just prior to sowing to ensure effective root zone colonisation by nitrogen fixing rhizobium bacteria.
- (c) Once the Bollgard II cotton begins to flower the corresponding refuge must not be cultivated.
- (d) Insecticide preparations containing Bt may be used on Bollgard II cotton throughout the season BUT NOT on any refuge crops.
- (e) All refuges are to be planted within the farm unit growing Bollgard II cotton. Subject to clause (f) below, all reasonable effort should be taken to plant the refuge either on one side of, or next to, a Bollgard II cotton field, and all Bollgard II fields must be no more than 2 km from the nearest Bollgard II refuge.
- (f) To minimise the possibility of refuge attractiveness being affected by herbicide drift, non-herbicide tolerant refuges should be separated from herbicide tolerant Bollgard II cotton crops by a sufficient distance to minimise such drift, but no more than 2km from the Bollgard II cotton.
- (g) To account for possible insecticide drift, Bollgard II refuge crops must be at least 24 metres wide and 24 metres long. Different unsprayed refuge options may be planted in the same field as a single unit.
- (h) Slashing of plants within the refuge should only be carried out after Bollgard II cotton lint removal has been completed. Soil disturbance of refuge crops can only occur 2 weeks after Bollgard II cotton plants have been harvested.
- (i) Refuges for Bollgard II crops must be planted in the same row configuration as the Bollgard II crop.

2. Control of volunteer and ratoon cotton

Volunteer and ratoon cotton may impose additional selection pressure on *Helicoverpa* spp. to develop resistance to the Bt proteins Cry1Ac and Cry2Ab produced by Bollgard II cotton.

Growers must make all reasonable efforts to remove volunteer and ratoon plants as soon as possible from all fields – including fallow areas, Bollgard II crops, conventional cotton crops and all refuges. **The presence of Bollgard II volunteers/ratoon cotton in any refuge will diminish the value of the refuge and must be removed as soon as possible.**

Note: The refuge should preferably be planted into fallow or rotation fields that have not been planted to cotton in the previous season.

3. Post-harvest crop destruction

As soon as practical after harvest, Bollgard II cotton crops must be destroyed by cultivation or herbicide so that they do not continue to act as hosts for *Helicoverpa* spp. Unsprayed refuges must be left uncultivated for two weeks after harvest to allow emergence of any pupating *Helicoverpa* spp.

4. Planting windows

All Bollgard II crops and cotton refuges are to be planted into moisture or watered-up in a five week window.

In each region, the start date of the planting window will be determined by TIMS in consultation with local growers and reflected in a regionally amended "Bollgard II Planting Window Variation Notice".

The planting window will occur within the following periods:

- **Ord River Irrigation Area:** March 1 and May 1.
- **Burdekin Bowen Basin Area:** December 1 and April 1.
- **Richmond Area:** December 1 and April 1.

5. Refuge

Unsprayed Pigeon Pea refuge should preferably be planted into a fallow or rotation field that has not been planted to cotton in the previous season.

6. End of season chick pea trap crop

An end of season chick pea trap crop must be planted. The planting configuration of the trap crop should be the same as that of the Bollgard II crop. The table below shows the requirements for the chick pea trap crop.

End of season chick pea trap crop requirements

Criterion	End of season chick pea trap crop
Minimum area & dimensions	A trap crop of 1% of planted Bollgard II crop area is required. This planting must be at least 24 m x 24m wide.
Planting time	In April for Burdekin Bowen Area. In July/August for Ord area. The trap crop is to be planted such that it is attractive to <i>Helicoverpa</i> spp. from 2 weeks before defoliation of the Bollgard II cotton. It must remain attractive to <i>Helicoverpa</i> spp. until at least 2 weeks after defoliation of the Bollgard II cotton.
Insect control	The trap crop should be monitored and sprayed with insecticide if the larval pressure threatens the viability of the crop.
Irrigation	The trap crop is to remain attractive to <i>Helicoverpa</i> spp. until after defoliation of cotton. In some cases this may require one additional irrigation after the cotton is defoliated. The trap crop must be planted into an area where it can receive the additional irrigation required to ensure the trap crop remains attractive to <i>Helicoverpa</i> spp.
Weed control	The trap crop should be kept free of weeds and particularly volunteer Bollgard II cotton.
Crop destruction	The trap crop must be destroyed 2-4 weeks after defoliation of the Bollgard II cotton crop, but not before 3 weeks (slash and pupae bust – full soil disturbance to a depth of 10 cm across the entire trap crop area). All Bollgard II cotton and associated trap crops must be destroyed by: Burdekin Bowen Basin/Richmond Area – August 31; Ord River Irrigation Area – December 10

NB: If any grower encounters problems in complying with the resistance management plan, please contact your Monsanto Regional Business Manager.

Unsprayed pigeon pea refuge agronomy

Establishing and growing an attractive, refuge is a critical, mandatory component in the Resistance Management Plan for Bollgard II. The aim of a refuge is to generate significant numbers of *Helicoverpa* spp. moths which have not been exposed to selection pressure from either of the Bt proteins. Attractive, fully irrigated, unsprayed pigeon pea will, on average, produce twice as many moths as the same area of unsprayed cotton. As well as producing high numbers of moths, it is also crucial that the timing of production of moths from refuges matches that of Bollgard II cotton crops.

The following information is intended to assist growers establish and maintain effective pigeon pea refuges. Although it is not part of the Resistance Management Plan (RMP), growers should refer to the RMP for guidance on refuge requirements.

While pigeon pea is a hardy, deep-rooted crop typically grown in dryland situations, it is not currently offered as a dryland refuge option because establishment and timing and duration of flowering can be problematic. Research is exploring the use of pigeon pea within a dryland environment as a refuge option.

Planting

Field selection

Pigeon pea can be grown on a wide range of soils, however is very susceptible to waterlogging, so select fields with good surface and internal drainage. Avoid areas where water tends to back up after irrigation and/or heavy rainfall.

The presence of Bollgard II volunteers/ratoons cannot be tolerated in refuge crop areas. This will diminish the value of the refuge and may

impose additional selection pressure to *Helicoverpa* species. All refuges should preferably be planted into a fallow or rotation fields that have not been planted to cotton in the previous season so as to avoid the likelihood of ratoon or volunteer cotton in refuges. Avoid fields where Bollgard II was the most recent crop and there is a high risk of ratoon cotton (ie there were difficulties with crop destruction).

Refuges should be planted on one side of, or next to, a Bollgard II field. Sprayed crops and unsprayed refuges that are planted in adjacent fields must be separated by sufficient distance to minimise the likelihood of insecticide drift onto the unsprayed refuge. To minimise the possibility of herbicide drift, pigeon pea refuges should be separated from herbicide tolerant Bollgard II cotton crops by a sufficient distance to minimise drift but not more than 2km from the Bollgard II cotton.

Nitrogen fixation by legumes such as pigeon pea is optimal in soils with very low residual soil N. Field selection should take this into consideration.

As with many other legumes, pigeon pea has been shown to have allelopathic properties which may inhibit the growth and performance of the following season's crop. This should be taken into account if large fields are planted.

Timing

Pigeon pea requires a minimum soil temperature of 17°C and rising (similar to mungbeans and soybeans). Depending on location, this will normally occur in October-November. Pigeon pea is a photoperiod sensitive plant, and there is a wide range of flowering times among varieties. Therefore, choice of variety and sowing date will strongly affect when it flowers.

Variety

Quest is currently the only variety available for refuge purposes. There is on going research to identify improved varieties, particularly for Northern cotton growing areas.

Given the usual planting time for cotton refuges, Quest takes 65 to 80 days to flower. With the right conditions it will continue to flower for a long period. To ensure Quest is attractive to *Helicoverpa* spp. during the same period of time that cotton is attractive (flowering), refuges should be planted



Larvae in pigeon pea refuge. (Photo: Johnelle Rogan)

within the two week period prior to planting Bollgard II, or if not possible, completed within 3 weeks of the first day of sowing Bollgard II*.

*See RMP for details.

Row spacing

As pigeon pea is only available for use as a fully irrigated refuge option in the RMP, the maximum row spacing is 1.0 metre. Where cotton is grown on a row spacing narrower than 1.0 metre, the row spacing for pigeon pea should match that of the cotton for which it is a refuge.

Seeding rates

To maintain attractiveness, it is important to comply to the required plant stand of not less than 4 plants per square metre. Higher plant populations tend to produce plants with thinner stalks, making crop residues easier to handle. Evenly spaced, lower plant populations can still be attractive and tend to produce larger plants that flower for longer and can cope better with water stress.

Seed germination percentages can vary greatly (<30% to >80%). Growers are advised to have a current germination test for either purchased or farm-saved seed. The proportion of hard seed can also influence the number of plants established, often above expectations.

Seed size is normally in the range of 6,000–10,000 seeds/kg. Generally a sowing rate of 25–40 kg/ha is used, but allowances must be made for planting conditions and seed quality.

Seed bed preparation and planting

Ensure seedbed preparation is reasonable to avoid replants. Reasonable preparation is described as that in which seed is sown to a depth of no more than 5cm. Levelling of any seed trenches created during planting is important, particularly when residual herbicides have been used and/or the field is to be watered up. The use of press wheels with light pressure has been shown to improve emergence.

Pre-irrigation

Pre-irrigation and planting into moisture is generally recommended over watering up. Some growers choose to water up the refuge with the rest of the field, then replant into this moisture if a replant is required.

Inoculum and fertiliser

Pigeon pea requires inoculation with Group J inoculant. To ensure efficacy of inoculant, follow all label requirements and directions regarding storage, handling and application. Nodulation will be limited in high nitrogen soils. A well-grown crop of pigeon pea can add up to 38kg/ha

TABLE 22: Herbicides available for use in pigeon pea (registered or permit number Per13758)

Active Ingredient	Mode of Action	Concentration and formulation	Application rate of product	Comment
Prometryn*	C	500 g/L 900 g/kg	Apply up to 4.5 L/ha Apply up to 2.5 kg/ha	Apply up to the maximum rate pre planting and incorporate, or as a post emergent directed spray towards the base of established plants (Per13758)
Trifluralin	D	480 g/L*	Apply up to 2.3 L/ha	Apply up to the maximum rate pre planting and incorporate. Rate dependent on soil type, refer to label or Per13758
		500 g/L	Apply up to 1.6 L/ha	
		530 g/L	Apply up to 1.5 L/ha	
		600 g/L	Apply up to 1.35 L/ha	
Butoxydim*	A	250 g/L	Apply 180 g/ha	Apply the specified rate as a post emergence spray over the top of the pigeon pea crops. (Per13758)
Fluazifop-p*	A	212 g/L 128 g/L	Apply 1 L/ha Apply 1.6 L/ha	
Haloxyfop*	A	130 g/L	Apply 0.6 L/ha	Apply specified rate as a post emergence spray over the top of the pigeon pea crops. (Per13758)
Haloxyfop*	A	520 g/L	Apply 0.150 L/ha	
Sethoxydim*		186 g/L	Apply 1 L/ha	
Clethodim*	A	240 g/L	0.250–0.375 L/ha (2–3 leaf stage)	Always apply with D-C-trate at 2 L/100 L or Hasten or Kwickin at 1 L/100 L Uptake at 500 mL/100 L spray volume. The lower doses will provide effective control if applied under ideal conditions to weed that are smaller, actively growing and free from temperature or water stress. (Per13758)
Quizalofop*	A	99.5 g/L	0.25–1 L/ha (dependent on growth stage and species of weed)	Refer to permit for growth stages of species and critical comments. (Per13758)
Flumetsulam	B	800 g/kg	25–50 g/ha + wetter	Post plant, pre emergent. Minimum spray volume 150 L water.
Diquat	L	200 g/L	2–3 L/ha	Harvest aid
Diquat/paraquat	L	135 g/L + 115 g/L	0.8–2.4 L/ha	Apply pre-sowing, in minimum 50–100 L water
Pendimethalin	D	330 g/L	2.5–3 L/ha	Incorporate into the soil within 24 hours of application. Use higher rate on heavy textured soils or those high in organic matter. May be applied by aerial or ground spraying. In Macquarie Valley area, only apply by air when ground is too wet for ground application.
		435 g/L	1.9–2.3 L/ha	
		440 g/L	1.9–2.25 L/ha	
		455 g/L	1.8–2.2 L/ha	
		475 g/L	1.74–2.11 L/ha	
Metribuzin	C	480 g/L	750 mL/ha	Furrow irrigated: apply after furrowing out, within 2 weeks before sowing and incorporate. For post-emergence: apply to actively growing seedling stage weeds provided crop plants have at least 2 trifoliate leaves. Do not spray if rain is likely to fall within several hours. Overhead irrigated: apply pre emergence then irrigate.
		700 g/kg	470 g/ha	
		750 g/kg	470 g/ha	

*Use of these products is under permit (Per13758).

NOTE: Only apply to pigeon pea crops that are to be destroyed at the end of the season or to be harvested for seed for refuge replanting only. No crop product or crop residue is to be fed to livestock. Refer to all labels and permit conditions. Please go to www.apvma.gov.au to check allowable usages.

of nitrogen. However grown in soils with moderate to high background nitrogen, pigeon pea can leave the soil depleted of nitrogen. Pigeon pea is much more sensitive to phosphorus deficiency than cotton. In soils with long cropping histories where soil P may be depleted, pigeon pea is likely to respond to addition of phosphorus and zinc. Like cotton, pigeon pea is highly VAM dependent and in long fallow situations, it may even be more responsive to P and Zn.

Weed management

Pigeon pea grows slowly, particularly when planted into low soil temperatures. Therefore will be a poor competitor with weeds.

While there are a number of herbicides available for use under permit, as seen in Table 22, inter-row cultivation can be a useful tactic. However cultivation can inadvertently kill (the Bt-susceptible) *Helicoverpa* pupae present in the soil at the time. For this reason it is a requirement that once Bollgard II cotton begins to flower the corresponding refuge should not be cultivated. The presence of Bollgard II volunteers/ratoon cotton in any refuge will diminish the value of the refuge and must be removed as soon as possible.

Irrigation

Pigeon pea is extremely sensitive to waterlogging, and flood irrigation is generally not ideal for this crop. However it is the most common form of irrigation and growers need to manage this carefully, for example, it is advisable to delay irrigating if heavy rain is predicted. Practices such as watering every second row, can be useful in supplying water to the crop, while reducing the risk of waterlogging by leaving room in the soil profile to make use of rainfall.

While pigeon pea generally requires less irrigation water than cotton, it is important to ensure crops do not become water stressed as this will impact on attractiveness. Flowering will be delayed under periods of extreme moisture stress and this situation appears to be one of the biggest problems facing an efficient refuge system. If there is moisture present, pigeon pea will respond very quickly with attractive regrowth after insect attack.

Destruction and harvest of pigeon pea refuge crops

Harvest or destruction of a pigeon pea refuge should only be carried out after Bollgard II lint removal has been completed. In NSW and Southern Qld, soil disturbance should only occur after Bollgard II cotton fields have been pupae busted, (to ensure maximum emergence of pupae from refuges), and preferably be left uncultivated until the following October to enable the emergence of overwintering pupae. In Central Queensland soil disturbance of refuge crops can only occur 2 weeks after final defoliation of the Bollgard II cotton. Growers in Central Queensland using pigeon pea for trap crop purposes should refer to the late summer pigeon pea trap crop requirements of the RMP for full details.

The pigeon pea refuge can be harvested with the aim of recouping refuge planting seed for the following season. No crop product or crop residue is to be fed to livestock. To ensure viability for planting, focus on preserving quality. Harvest at 13.0% grain moisture for optimum seed quality. Rotary harvesters with low drum speeds (350–400 rpm) give best results. Crop desiccation may be required.

III

Every refuge counts. Moths don't recognise farm boundaries.



Photo: Annie Johnson

**Grow a great refuge to slow down resistance
Because we are in this together**
www.mybmp.com.au

Best Practice

**This document is part of a larger publication -
The Cotton Pest Management Guide for Cotton 2014 - 15**

The complete document can be found on the CRDC or myBMP web sites during the 2014-15 Australian cotton season

www.crdc.com.au

or

www.mybmp.com.au

DISCLAIMER

This document has been prepared by the authors for CRDC in good faith on the basis of available information.

While the information contained in the document has been formulated with all due care, the users of the document must obtain their own advice and conduct their own investigations and assessments of any proposals they are considering, in the light of their own individual circumstances.

The document is made available on the understanding that the CRDC, the authors and the publisher, their respective servants and agents accept no representation, statement or information whether expressed or implied in the document, and disclaim all liability for any loss, damage, cost or expense incurred or arising by reason of any person using or relying on the information claimed in the document or by reason of any error, omission, defect or mis-statement (whether such error, omission or mis-statement is caused by or arises from negligence, lack of care or otherwise).

Whilst the information is considered true and correct as at 31 August 2014, changes in circumstances after the time of publication may impact on the accuracy of the information. The information may change without notice and the CRDC, the authors and the publisher and their respective servants and agents are not in any way liable for the accuracy of any information contained in this document.

Recognising that some of the information is provided by third parties, the CRDC, the authors and the publisher take no responsibility for the accuracy, currency, reliability and correctness of any information included in the document provided by third parties.

The product trade names in this publication are supplied on the understanding that no preference between equivalent products is intended and that the inclusion of a product does not imply endorsement by CRDC over any other equivalent product from another manufacturer.

ISSN 1442-8462

Production by Greenmount Press, 2014

Liberty® and Liberty Link® are Registered Trademarks of Bayer.
Bollgard II®, Roundup Ready Flex® and PLANTSHIELD® are registered trademarks of Monsanto Technology LLC used under licence by Monsanto Australia Ltd.