Preamble to the Resistance Management Plan (RMP) for Bollgard II 20010/11

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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 recent data for *H. punctigera* also demonstrate significant increases over seasons in the frequency of Cry2Ab resistance genes (for more details see below). 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 2010/11 season is predicted to increase considerably from recent years, and the Bollgard II acreage will still represent around 80% 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, Genuity 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 Genuity 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 Genuity 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 Genuity 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. F_2 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 F_2 screens using a shorter method called an F_1 test. F_1 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 1 in 1,000,000 (<0.000001 or 0.0001%). 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 (I&I NSW) 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

H. armigera. (Melina Miles, DEEDI)

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.

F_2 tests indicated that the frequency of the gene for resistance to Cry2Ab in 2009/10 was less than 10 in 1000 (0.010, 1%).

In 2004 CSIRO developed protocols for testing the frequency of resistance using a modified and shorter version of the F_2 method called an F_1 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 F_1 tests in *H. armigera* in earnest.

Results with *H. armigera* show that the estimate of Cry2Ab resistance frequency for F_1 screens is higher than for F_2 tests. Of particular concern is the high frequency of Cry2Ab resistance genes which at the end of the 2009/10 season is approximately 2 in 100 (0.02, 2%). Currently, we believe that the frequencies obtained from the F_1 screens are likely to most accurately reflect the situation in the field. Both Monsanto and CSIRO are working together to better understand the differences between the F_2 and F_1 screens but to date have not determined the reason for this discrepancy.

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, in 2008/09 a gene was isolated from field populations of *H. punctigera* that confers resistance to Cry1Ac. F_2 tests indicate that the frequency of this gene in 2009/10 was less than 1 in 1000 (0.001, 0.1%). It is not cross-resistant to Cry2Ab. Researchers are currently attempting to establish a colony containing this gene for further characterisation.

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. F_2 tests indicated that the frequency of this gene in 2009/10 was less than 5 in 1000 (0.005, 0.5%).

In 2007/08 and 2009/10 CSIRO and Monsanto respectively began F_1 tests in *H. punctigera*. As with *H. armigera*, the Cry2Ab resistance frequency in *H. punctigera* for F_1 screens is higher than that determined with the F_2 tests. At the end of the 2009/10 season, the frequency of Cry2Ab genes in *H. punctigera* was approximately 1 in 100 (0.01, 1%).

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. F_1 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 F_1 resistance frequency for Cry2Ab in *H. punctigera* prior to the widespread adoption of Bollgard II. However, in both years that we have F_2 and F_1 screen data for *H. punctigera* in the cropping regions the difference in the estimated frequencies is four-fold. We can estimate an F_1 screen frequency for a time close to the introduction of Bollgard II (2004/05) by multiplying the F_2 screen frequency for that season by four. This gives a frequency of 6 in 1000 (0.006, 0.6%) which is similar to the current frequency in inland regions. Hence it is likely that the background resistance in *H. punctigera* to CryAb was common even before Bollgard II was released.

Is the frequency of *cry2Ab* genes increasing in field populations of *H. armigera*?

F_2 data for *H. armigera* do not demonstrate a significant change in frequency of Cry2Ab resistance over time. Since 2004/05 Monsanto has used the F_1 protocol developed by CSIRO to screen for resistance to Cry2Ab. CSIRO also have F_1 screen data for *H. armigera* since 2007/08. Despite both organisations utilising identical robust protocols, and sampling from the same hosts and regions, the frequencies scored by Monsanto are consistently lower than those scored by CSIRO. Since the difference is not consistent among data sets it is not possible to apply a simple correction. Both data sets analysed independently show that there is no significant difference in the frequencies of Cry2Ab resistance alleles over time. 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 F_2 and F_1 data sets from CSIRO demonstrated significant increases in the frequency of *cry2Ab* resistance genes in field populations of *H. punctigera*. CSIRO began collecting F_2 screen data for *H. punctigera* in 2002/03. Each year since 2006/07 there has been an increase in the frequencies of resistance to Cry2Ab. This gradual increase over time became statistically significant in 2007/08 and remained highly significant in 2008/09. The 2009/10 CSIRO data demonstrate a Cry2Ab resistance frequency which is not significantly different from the frequency obtained in 2008/09. While the 2009/10 data do not follow the exponential growth curve that was fitted at the end of 2008/09, the complete data set shows a highly significant linear increase over time. Monsanto began F_2 screens with *H. punctigera* in 2007/08 and since then have not detected any significant shifts in Cry2Ab resistance frequency.

The 2008/09 F_1 data set for *H. punctigera* demonstrated a 5 fold increase in frequency compared to 2007/08. The frequency obtained in 2009/10 is significantly lower than that detected in 2008/09 and there is no significant difference in the frequencies obtained in 2007/08 and 2009/10. The shifts in F_1 screen data from 2007/08 to 2009/10 mirror those of the F_2 screen data, however since the data set is restricted to the last three years only, it is not possible to look for longer term shifts over time.

Why is *H. punctigera* 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, DEEDI)**

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

Monitoring for resistance to Vip3A protein is in its infancy however genes allowing survival against this toxin have been isolated in *H. punctigera* and *H. armigera*. The early 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 F_2 screens and F_1 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. At this early stage the frequencies of *vip3A* resistance alleles in *H. armigera* obtained from F_2 screens are significantly 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. From 2010/11 Monsanto will include screens for *vip3A* resistance genes in their Bt resistance monitoring program.

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 F_1 tests than previously detected using F_2 screens is a major concern. Of even more concern is the demonstrated significant increase in Cry2Ab resistance frequency in *H. punctigera*. 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, the TIMS Bt Technical Panel have developed a working document entitled 'Contingency Plan for Mitigating Resistance to the Toxins Within Bollgard II Cotton' which provides background information and recommendations for the Cotton Australia convened TIMS Committee. This document includes possible measures to be taken in response to further 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. This document is undergoing a process of consultative stakeholder review prior to ratification by the Cotton Australia TIMS Committee. The Contingency Plan will be reviewed annually in light of new information on resistance frequencies in *Helicoverpa spp.* and knowledge of and tactics for Bt resistance management. 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 contains 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. As detailed above, this production is especially critical in a drought year because there is reduced contribution from non-cropping vegetation and dryland crops. 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. For this reason, refuge crop options (sorghum, corn) which have shorter periods of producing moths than cotton, need to have several staggered plantings to extend the period over which moths are produced. 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 will be removed from the RMP as refuge options.

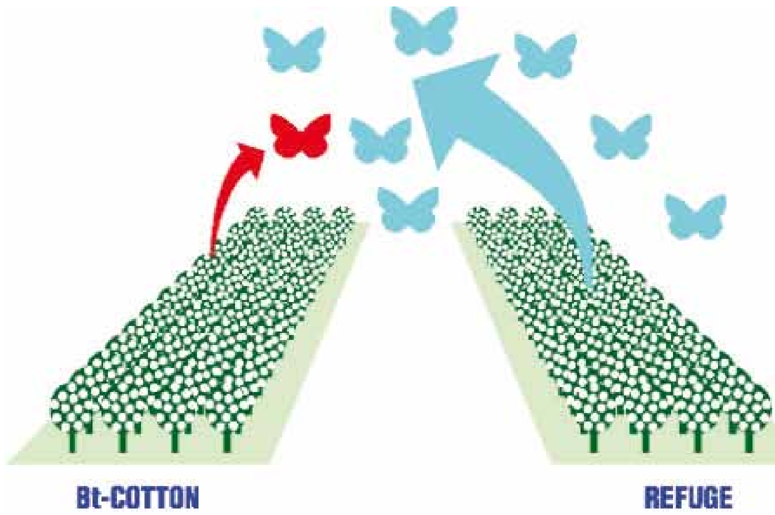
Is there a minimum size to a refuge crop?

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.



Moths produced from refuges dilute resistance genes in the population.

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 options available in irrigated Bollgard II (pigeon pea, sorghum, corn) tend to be planted after the cotton and in dryland situations there is uncertainty about whether soil moisture will be adequate to successfully establish future crops. However CSIRO and Monsanto are currently conducting work on the suitability of pigeon pea as a dryland refuge option for dryland Bollgard II cotton. CSIRO and Monsanto are also working with growers in several valleys to investigate the suitability of 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. Especially during a drought season 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, at least 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?

The same logic applies as in the previous question. The presence of Bollgard II volunteer plants in a conventional crop 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 happens to be 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 76**. 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. It is possible that the survival is due to a modified or existing behaviour of larvae that enables them to target sites of the plant that do not express at high levels (e.g., pollen in flowers). It is also possible that the survival is due to a temporary decline in the expression of toxins in the whole plant. Work is underway to investigate these possibilities.

Irrespective of how survival occurs, it is recommended that if larvae reach thresholds in Bollgard II fields they should be controlled by spraying. Work conducted by Monsanto suggests that it is unlikely that there will be a yield penalty associated with larvae survival in Bollgard II fields. An experiment conducted by PhD student Baoqian Lu within Bollgard II fields at threshold involved removing larvae from plots and leaving larvae in other plots. In a field that was sprayed for Helicoverpa after setting up the trial there was no difference between the two types of plots. However in a field that was not sprayed for Helicoverpa the plots with larvae had significantly less cotton lint compared to the plots where larvae were removed. Additionally, in some instances the surviving larvae are able to pupate and emerge. This poses a threat because the larvae will presumably have been exposed to Bt toxin at a low level which may select for resistance if some dominance is present.

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.

Collection kits are also available from your Regional Cotton Extension Officer.

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 61–69). The predator/pest ratio (described on page 8) 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 58–59. 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 6.

GUIDELINES FOR AMENDING BOLLGARD II PLANTING WINDOWS 2009/10

Developed by the Transgenic and Insect Management Strategies Committee of Cotton Australia

Note: Requests for variation to the Bollgard II planting window only affect the planting window component of the RMP for the requestee/s for the current season.

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 (emergencies).

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 the first week of September.

It is essential that there has been wide consultation regarding the proposal including; CGA members, local consultants, Regional Cotton Officers and researchers and the local Monsanto Accounts & Stewardship Specialist. 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 local Cotton Growers Association (CGA) must request and approve the change with a majority vote and advise all growers of the outcome of the vote. The majority decision affects TUA compliance for all licensed growers. Evidence of this process will be required from the CGA in writing together with the information requested below.
2. The region (or individual grower) requesting the variation is more than 100 kms from any other significant Bollgard II planting.
3. Planting of Bollgard II in the region has not exceeded 10% of the anticipated Bollgard II cotton area.
4. No Bollgard II cotton has been planted in excess of 21 days prior to the opening of the new window.
5. There are no known threats to the efficacy of refuges in the region (e.g., plague locust pressure).
6. The requested planting window variation must be a 42 day window that falls entirely within the period September 1 to December 15.

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

1. Describe 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 approval and agreement of all cotton growers in the region to abide by the requested changes to the window.
9. Statement acknowledging capacity to still meet pupae busting requirements in the RMP when a later planting window is requested.

SCHEDULE A - RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2010/2011

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 1 and 2, 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.

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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 1: 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 2: 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 conventional cotton refuge is at risk due to early season pressure by *Helicoverpa spp.*, and with prior approval from the Monsanto Compliance and Stewardship Manager, a non-Bt heliocide can be applied up to the 4th true leaf stage. 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.

RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2010/2011

New South Wales, Southern Queensland & Central Queensland (continued)

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 should 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 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.*

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.

RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2010/2011

New South Wales, Southern Queensland & Central Queensland (continued)

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.

NB: If any grower encounters problems in complying with the Resistance Management Plan please contact your local Monsanto Regional Business Manager.

Section 3: Central Queensland only

1. Planting Windows

Emerald: 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: All Bollgard II crops are to be planted into moisture or watered-up in the period between October 10 and November 20, 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 Helicoverpa 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.
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 Helicoverpa spp. until after the cotton is defoliated.
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 (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).

* 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 resistance management plan, 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" in the current Cotton Pest Management Guide.

SCHEDULE A - RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2010/2011

Ord River Irrigation and Burdekin Bowen Basin 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

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 the 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:

TABLE 1: Irrigated Bollgard II cotton refuge options

Crop	Conditions	% of Bollgard II	Regions permitted
Conventional Cotton	Irrigated, unsprayed conventional cotton	10	Ord River, Burdekin Bowen
Pigeon pea	Fully irrigated, unsprayed	5	Ord River
Chickpea	Fully irrigated, unsprayed	5	Ord River

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 conventional refuge is at risk due to early season pressure by *Helicoverpa spp.*, and with prior approval from the Monsanto Compliance and Stewardship Manager, a non-Bt heliocide can be applied up to the 4th true leaf stage.

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.

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: The refuge must be planted within the 2 week period prior to planting Bollgard II.

(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).

SCHEDULE A - RESISTANCE MANAGEMENT PLAN FOR BOLLGARD II COTTON 2010/2011

Ord River Irrigation and Burdekin Bowen Basin areas (continued)

(c) Once the Bollgard II cotton begins to flower the corresponding refuge should 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 (g) 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.

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. Table 2 shows the requirements for the chick pea trap crop.

TABLE 2: 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 Helicoverpa spp. from 2 weeks before defoliation of the Bollgard II cotton. It must remain attractive to Helicoverpa 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 Helicoverpa 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 Helicoverpa 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).

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 moth, it is also crucial that the timing of production of moths from refuges matches that of Bollgard ll cotton crops.

The following information is intended to assist growers establish and maintain effective pigeon pea refuges. It is not part of the Resistance Management Plan (RMP) and growers should also 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 prone 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 ll 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 ll cotton crops by a sufficient distance to minimise drift but not more than 2km from the Bollgard ll 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 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 choosing 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 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 the table below, 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 ll cotton begins to flower the corresponding refuge should not be cultivated. The presence of Bollgard ll 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 be 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 aerial parts of a pigeon pea refuge should only be carried out after Bollgard ll 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 ll 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 dessication may be required.

HERBICIDES AVAILABLE FOR USE IN PIGEON PEA (REGISTERED OR PERMIT NUMBER PER1021)

Active Ingredient	Mode of Action	Concentration and formulation	Application rate of product	Comment
Prometryn	C	500 g/L:	Apply up to 4.5 L per hectare.	Apply up to the maximum rate pre planting and incorporate, or as a post emergent directed spray towards the base of established plants.
Prometryn	C	900 g/kg:	Apply up to 2.5 kg per hectare.	
Trifluralin	D	480 g/L:	Apply up to 2.3 L per hectare	Apply up to the maximum rate pre planting and incorporate.
Butroxydim	A	250 g/L:	Apply 180 grams per hectare.	Apply the specified rate as a post emergence spray over the top of the pigeon pea crops.
Fluazifop-p	A	212 g/L:	Apply 1 L per hectare.	
		128 g/L:	Apply 1.6 L per hectare.	
Sethoxydim	A	186 g/L:	Apply 1 L per hectare.	
Haloxyfop	A	130 g/L:	Apply 600 mls per hectare.	
Haloxyfop	A	520 g/L:	Apply 150 mls per hectare.	
Clethodim	A	240 g/L:	250 to 375 mL/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 or Uptake at 500 mL/100L 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.
Quiazalofop		99.5 g/L	250-750 mL/ha (dependent on growth stage and species of weed)	Refer to permit for growth stages of species and critical comments.
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.
		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	780 g/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.
		750 g/L	470 g/ha	
		700 g/L	500 g/ha	

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.