REFUGE CROP OPTIONS FOR A BOLLGARD II® RESISTANCE MANAGEMENT STRATEGY AT KUNUNURRA, WESTERN AUSTRALIA

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Introduction

In Australia, the introduction of genetically modified (GM) cotton with lepidopteran insect protection traits has been highly successful. Farmers have adopted the technology readily and in 2005/06, ten years after the first INGARD® crops were planted, more than 80% of farmers grow Bollgard II® cotton, which expresses two genes from *Bacillus thuringiensis* (Bt), for caterpillar pest control (Monsanto, unpublished data).

The risk of pests, especially *Helicoverpa armigera*, developing resistance to the Cry1Ac and Cry2Ab genes expressed in Bollgard II® plants, was recognised prior to the commencement of commercial production and Resistance Management Plans (RMPs) put in place to mitigate against this concern. For example the early plantings of INGARD® were limited to 10% of the total cotton crop area in each production region and allowed to increase gradually to a final "cap" of 30%. Current RMP conditions for using Bollgard II® technology included "pupae busting" to help reduce the survival of diapausing resistant insects from one season to the next, tight planting windows, post-harvest crop and volunteer destruction and the growing of refuge crops to provide a source of moths not exposed to Bt genes.

Extensive mathematical modelling of refuge crop options led to the development of the current requirements, which aim to produce large numbers of Bt susceptible moths that are available to mate with the few Bt resistant types and therefore dilute the frequency of genetically resistant moths (Roush 1998). The models show that a standard refuge crop of unsprayed conventional cotton should be 10% of the Bollgard II® crop. Other crops are rated against this standard in terms of their capacity to produce viable *H. armigera* pupae. The currently approved alternatives to the unsprayed conventional cotton refuge, expressed as a percentage of the Bollgard II® crop area are; 100% sprayed conventional cotton, 5% unsprayed pigeon pea, 15% unsprayed sorghum or 20% unsprayed maize. Conditions apply to each of these alternatives and are detailed in Farrell and Johnson (2005).

Northern Australian regions could become significant cotton producers in the future if regulatory issues and negative industry perceptions can be overcome (Yeates 2001). In Western Australia, significant production-based research has been conducted at Kununurra and Broome in order to evaluate the feasibility of a winter cropping strategy for cotton. Sustainability has been a focus of research and the development of a RMP for Bollgard II® is an essential prerequisite to obtaining regulatory approval. For seven years, between 1997 and 2003, a range of crops was assessed for their usefulness as refuge crops in a future Ord Stage 2 cotton industry and a draft RMP produced.

Methods

Initially, a large number of crop species were compared in terms of their capacity to produce viable *H. armigera* pupae, relative to the standard conventional cotton crop. The crops included sorghum, maize, sweetcorn, lablab, borlotti beans, green beans, chickpea, watermelon, pumpkin, tomato, niger, lucerne, sunflower and millet. GM cotton, either INGARD® or Bollgard II®, was also included each season. In later years, crops such as the cucurbits, niger, beans and lucerne were discontinued because it was clear that they were poor producers of *H. armigera*.

In some years each of the candidate crops were planted as replicated plots within a single paddock, but usually as large single plots within a paddock. None of the crop species was sprayed to control *H. armigera*. In other seasons, commercial crops grown on farmer properties were assessed. Some of these crops were sprayed for pests and therefore probably underestimate the number of pupae that would be produced in an unsprayed situation.

In 2002, seven crops were planted as blocks in one paddock at the Frank Wise Institute on 8 May. The crops were conventional cotton, Bollgard II® cotton, chickpea, pigeon pea, maize, lablab and watermelon. Each crop plot was 32 m X 180 m (0.57 hectare), except the conventional cotton plot which was twice this size. Approximately every 3 weeks, in between irrigation cycles, the crops were sampled for *Helicoverpa* pupae. At least 15, 0.5 m^2 (1 m x 0.5 m) quadrats were placed in the soil surface under each crop. The loose dirt was scraped away and the hard ground examined for larval entrance holes. If holes in the soil were found they were carefully dug up with a trowel to expose the pupal chamber. The number of pupal chambers and pupae in each quadrat was recorded. Pupae were removed and returned to the laboratory where they were placed in individual rearing containers. The pupae were identified to species level and inspected regularly. The fate of each pupa was recorded as emerged, parasitised, diapausing or diseased. These data were then used to estimate the total number of viable pupae per hectare at the date of sampling for each of the candidate refuge crops.

Similar methods to calculate the number of viable *H. armigera* pupae per hectare were used in each of the seven years (1997 to 2003) that refuge crop assessments were conducted. In each year relatively large plots of 0.5 hectare, or commercial paddocks, were used for sampling. Small plot experiments were avoided due to concerns about “plot hopping” by key pests and other possible interactions caused by the close proximity of several crop species.

Results

The results for the 2002 season are summarised in Table 1, which shows the total number of lepidopteran pupae collected and the percentage that emerged in the laboratory as *H. armigera* at four sampling dates. On the first sampling date (10 July) the Bollgard II® and conventional cotton were at mid-squaring. The maize, chickpea, lablab and pigeon pea were flowering. Chickpea had the highest number of lepidopteran pupae collected from the soil but only one produced a *H. armigera* moth. The majority of the pupae were parasitised by tachinid flies and few pupae were found in any of the other refuge crops on this date.

Table 1. Summary of the total number of pupae found under several crop species and the percentage of pupae that were *Helicoverpa armigera*, Kununurra, 2002.

Date	10 July		30 July		11 September		2 October	
Crop	Total pupae	% *H. armigera*	Total pupae	% *H. armigera*	Total pupae	% *H. armigera*	Total pupae	% *H. armigera*
Bollgard II®	0	0	1	0	2	50%	3	0
Maize	0	0	1	100%	32	22%	17	23%
Pigeon Pea	1	100%	3	33%	42	62%	42	48%
Lablab	2	0	5	20%	36	30%	17	41%
Cotton	2	0	3	0	32	12%	12	58%
Chickpea	13	8%	15	27%	26	46%	28	57%
Watermelon	-	-	0	0	1	100%	0	0

On 30 July, the Bollgard II® crop had commenced flowering but the conventional cotton shed its early squares and flowers were not found on this date. The maize was heading and the watermelons were vegetative. The chickpeas had commenced pod development and the lablab and pigeon pea were flowering.

Chickpea had the highest number of lepidopteran pupal chambers in the soil and 27% produced viable *H. armigera* adults. An additional 30% of the pupae were *H. punctigera* and 20% of pupae collected from chickpeas had been parasitised by tachinid flies.

On 11 September the maize was ready for harvest. The Bollgard II® had produced its last effective flower but the conventional cotton was behind due to the early fruit loss. The pigeon pea and lablab had new flushes of growth and were continuing to flower. The greatest number of lepidopteran pupae was found on the pigeon pea and 62% emerged as *H. armigera* adults. Thirty percent of the pupae collected from lablab emerged as *H. armigera* adults, 16% of the total pupae were parasitised by tachinid flies and 44% of the pupae were identified as other lepidopteran species (*Spodoptera*, *Mythimna spp*, and others). Chickpeas also had a high number of *H. armigera* pupae on this sampling occasion with 46% of the lepidoptera collected emerging as *H. armigera* adults. The remaining pupae from the chickpeas were also identified as *H. armigera* but, unexpectedly, appeared to be in diapause because they failed to emerge after 12 weeks at 25°C. Large numbers of lepidopteran pupae were collected from maize and conventional cotton but few of these emerged as *H. armigera* adults, the majority of pupae being either *Spodoptera litura or Mythimna separata*.

On 2 October when the last sample was collected, the Bollgard II® and conventional cotton were showing cracked bolls. The maize, chickpea and watermelons had been harvested and the stubble was sampled. The pigeon pea and lablab were still flushing due to the management of flowering by slashing. In the maize, 23% of the pupae found emerged as *H. armigera* adults. The remaining pupae were either *S. litura* or *M. separata*. In the chickpea, 57% of the pupae collected emerged as *H. armigera*. One pupa that emerged was identified as *Helicoverpa hardwicki*, which has not been recorded on chickpea previously and the remaining pupae were classified as diapausing *H. armigera*. Forty two pupae were collected from the pigeon pea and 48% of these emerged as *H. armigera* adults. The remaining pupae were identified as *H. punctigera* (19%) or other species of lepidoptera. Forty one percent of the pupae collected from lablab and 58% of pupae from conventional cotton emerged as *H. armigera* adults.

Figure 1. Mean number of viable *Helicoverpa armigera* pupae collected from several crops grown at the Frank Wise Institute, Kununurra, WA, 2002.

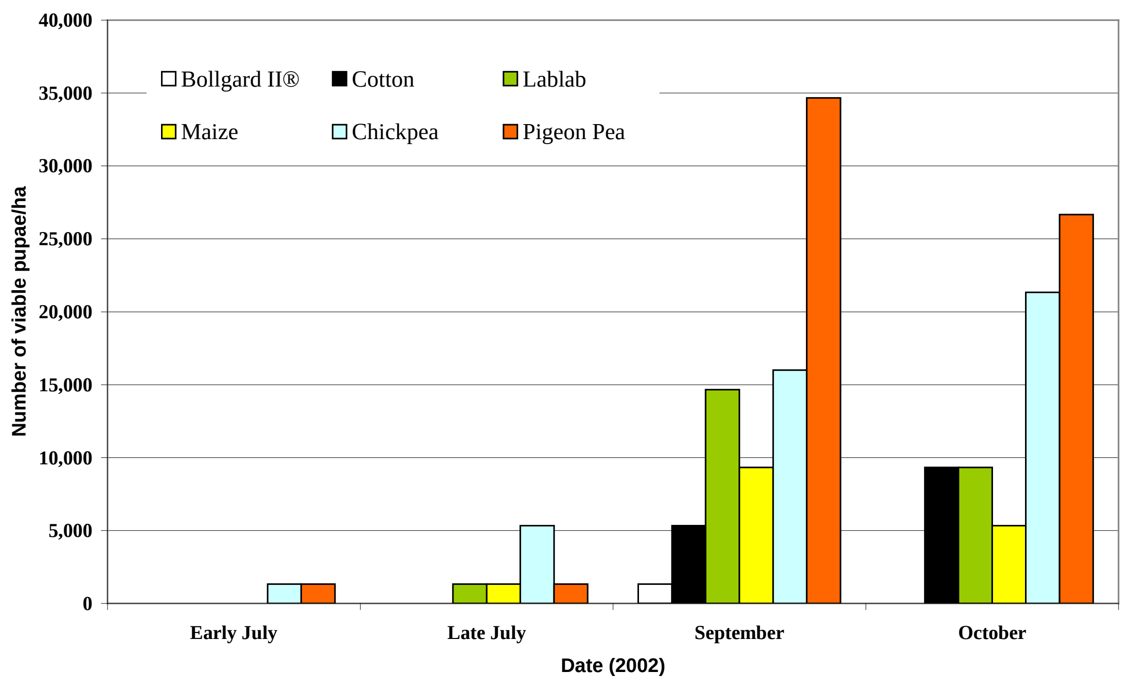


Figure 1 shows the abundance of viable *H. armigera* pupae per hectare for each crop (except watermelon) and for Bollgard II®. Very few *H. armigera* pupae were found in Bollgard II® throughout the season and pupae were found in watermelon on only one occasion. All of the other refuge options produced much higher levels of *H. armigera* in the second half of the season than the first half of the season. Chickpea produced the most *H. armigera* pupae early in the season and pigeon pea was the better refuge option later in the season. However, if diapausing pupae from chickpea are included, then chickpea and pigeon pea were similar in their production of pupae and both far exceeded conventional cotton in this regard.

Similar data was collected each year between 1997 and 2003 but are not presented here in detail. However a summary of the data is shown in Figure 2. A significant finding was that *H. armigera* in Bollgard II® plantings was extremely rare and was found only in early September.

At all times during the growing season, chickpea and pigeon pea produced many more *H. armigera* than the standard, conventional cotton. Maize also tended to produce more viable pupae than conventional cotton but the spread of pupae throughout the season was limited compared to chickpea and pigeon pea.

Figure 2. Mean number of viable *Helicoverpa armigera* pupae collected from several crops grown at Kununurra, 1997-2003.

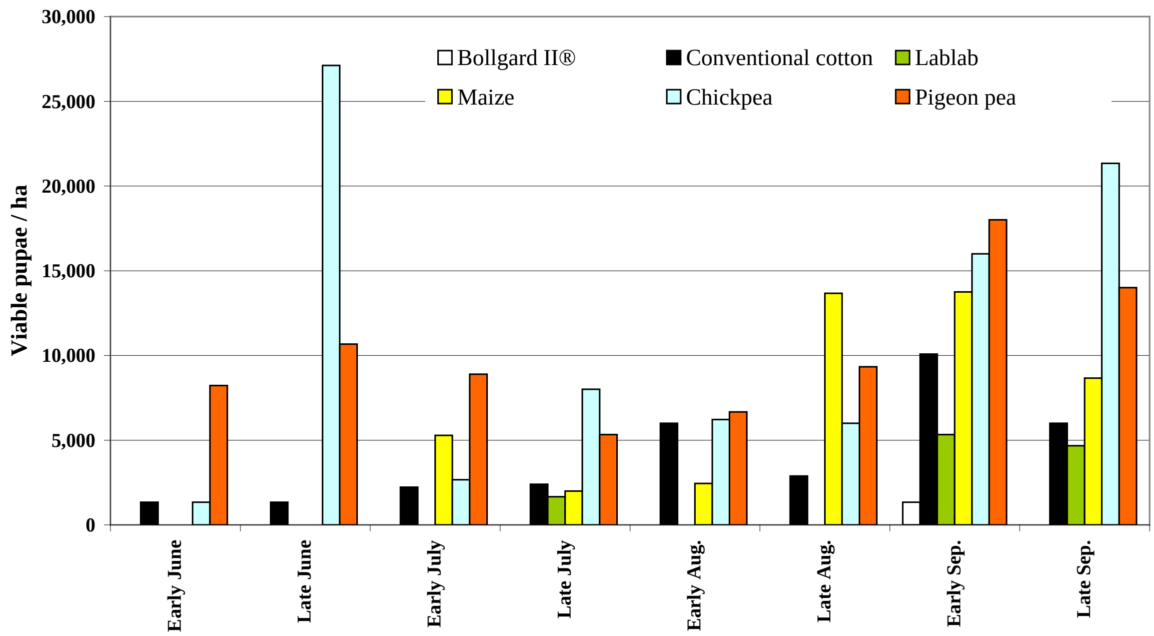
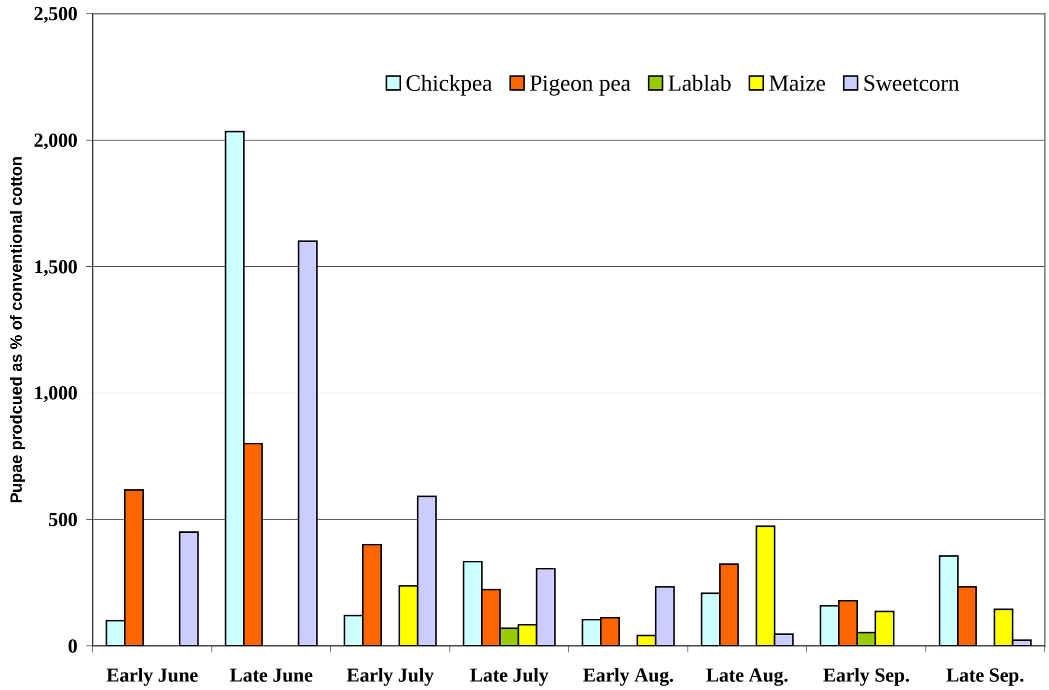


Figure 3 shows the relative pupae production levels from several crops compared to conventional cotton. Chickpea, pigeon pea, lablab, maize and sweetcorn frequently produced vastly more viable *H. armigera* pupae than conventional cotton. Peak seasonal pupae production from chickpea, pigeon pea and sweetcorn all exceeded conventional cotton by 500% or more.

Figure 3. Mean *Helicoverpa armigera* pupae production from several crops as a percentage of pupae from conventional cotton, Kununurra, 1997-2003.



Discussion

In Western Australia a long-running native title dispute between government and the traditional owners was resolved in October 2005, clearing the way for the future development of the Ord Stage 2 area. The Gene Technology Regulator is also considering an application by Monsanto Australia for the commercial release of Bollgard II® in northern Australia, above latitude 22^0S, with a decision expected by late 2006. For these reasons, the prospects of a cotton industry being re-established at Kununurra are relatively good, notwithstanding the government's current moratorium on all GM crops in Western Australia, including cotton.

An essential prerequisite to Bollgard II® production in new regions is the approval of an appropriate Resistance Management Plan (RMP) tailored to the conditions of the new area. Research described in this paper has enabled recommendations for the refuge crop component of a RMP to be developed through a subcommittee of the Australian Cotton Growers Research Association and submitted to the Australian Pesticides and Veterinary Medicines Authority for approval as a condition of Bollgard II® use in northern Australia. A summary of the proposed refuge crop options is shown in Table 2. In developing the options, the standard of 10% unsprayed conventional cotton, as used in eastern Australia, has been used as the benchmark. The data in Figures 1, 2 and 3 show that chickpea and pigeon pea consistently produce more than twice as many viable *H. armigera* pupae per hectare as conventional cotton and therefore a 5% refuge crop area is recommended for these options. Maize is deemed to be equivalent to the conventional cotton benchmark and therefore the standard 10% refuge crop area is recommended. It should be noted that detailed growing conditions for each refuge crop are applicable to ensure moth emergence that is synchronous with Bollgard II®.

Table 2. Summary of the proposed refuge crop options developed for commercial Bollgard II® production at Kununurra.

Refuge crop	**Conditions**	**% of Bollgard II® crop**
Cotton	Irrigated, unsprayed conventional or Roundup Ready® cotton	10
Pigeon pea	Irrigated, unsprayed – conditions apply	5
Chick pea	Irrigated, unsprayed – conditions apply	5
Corn	Irrigated, unsprayed – conditions apply	10

Other components of the Bollgard II® RMP include planting windows, post-harvest crop and volunteer destruction and a "suicide trap crop" at the end of the season. The latter strategy aims to reduce the likelihood of resistant genotypes surviving through summer to the next cotton season. It aims to replace the pupae busting strategy, which is unlikely to be effective in northern Australia where the proportion of moths entering diapause at the end of the season is low.

Acknowledgements

The authors acknowledge the dedication and hard work of technical officers and farm staff at the Frank Wise Institute, Kununurra during the period of this research. Funding and research support from the Cotton CRC and the Cotton Research and Development Corporation are also gratefully acknowledged, as are the authors' host organisations.

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