IPM in the transgenic era: a review of the challenges from emerging pests in Australian cotton systems

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Abstract. The Cotton Catchment Communities Cooperative Research Centre began during a period of rapid uptake of Bollgard II® cotton, which contains genes to express two Bt proteins that control the primary pests of cotton in Australia, *Helicoverpa armigera* and *H. punctigera*. The dramatic uptake of this technology presumably resulted in strong selection pressure for resistance in *Helicoverpa* spp. against the Bt proteins. The discovery of higher than expected levels of resistance in both species against one of the proteins in Bollgard II® cotton (Cry2Ab) led to significant re-evaluation of the resistance management plan developed for this technology, which was a core area of research for the Cotton CRC. The uptake of Bollgard II® cotton also led to a substantial decline in pesticide applications against *Helicoverpa* spp. (from 10–14 to 0–3 applications per season). The low spray environment allowed some pests not controlled by the Bt proteins to emerge as more significant pests, especially sucking species such as *Creontiades dilutus* and *Nezara viridula*. A range of other minor pests have also sporadically arisen as problems. Lack of knowledge and experience with these pests created uncertainty and encouraged insecticide use, which threatened to undermine the gains made with Bollgard II® cotton. Here we chronicle the achievements of the Cotton CRC in providing the industry with new knowledge and management strategies for these pests.

Additional keywords: cotton, ecology, *Helicoverpa*, integrated pest management, mirids, transgenic, whitefly.

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Introduction

Insecticidal transgenic technology has revolutionised pest control in many parts of the world, including Australia. In Australian cotton systems through the 1960s to early 2000s, pest management was highly reliant on use of insecticides, mostly broad-spectrum organophosphates, carbamates, and pyrethroids, as well as endosulfan. Crops were sprayed ~12–16 times per season (Fitt and Wilson 2005). One of the primary pests, the larvae of the noctuid moth *Helicoverpa armigera*, had developed significant resistance to pyrethroids, carbamates, and endosulfan, rendering control difficult and necessitating rigorous sampling at least bi-weekly and very timely application of insecticides. The first insect-resistant transgenic cotton varieties were commercially released in Australia in 1996 and began to revolutionise pest management (Constable *et al.* 2011). These were known as Ingard® and they expressed a protein (Cry1Ac) from the bacteria *Bacillus thuringiensis* var. *kurstaki* that provided control of the primary pests, *H. armigera* and *H. punctigera*. To reduce the risk

that *H. armigera* would develop resistant to this protein, a comprehensive resistance management plan (RMP) was put in place (described later), and in addition, Ingard® could only be planted on a maximum of 30% of the cotton area. Insecticide use was reduced by ~50% in the Ingard crops compared with non-Bt cotton crops (Fitt and Wilson 2005; Fig. 1).

In 2003, varieties containing genes to express two Bt proteins (Cry1Ab and Cry2Ab) were released commercially as Bollgard II®. Over the next year, Ingard was phased out and the 30% cap on area was relaxed so Bollgard II® could occupy 100% of the cotton area on a farm. There was dramatic uptake of Bollgard II® by industry (~96% of the 2012–13 crop by area; Monsanto Australia, unpubl. data), which led to substantial reductions in insecticide use (Fig. 1), with benefits to the environment. Bollgard II® provided an ideal platform for integrated pest management (IPM), as it greatly reduced the need to apply pesticides for the target insect pests (*H. armigera* and *H. punctigera*) (Fitt and Wilson 2005; Fitt
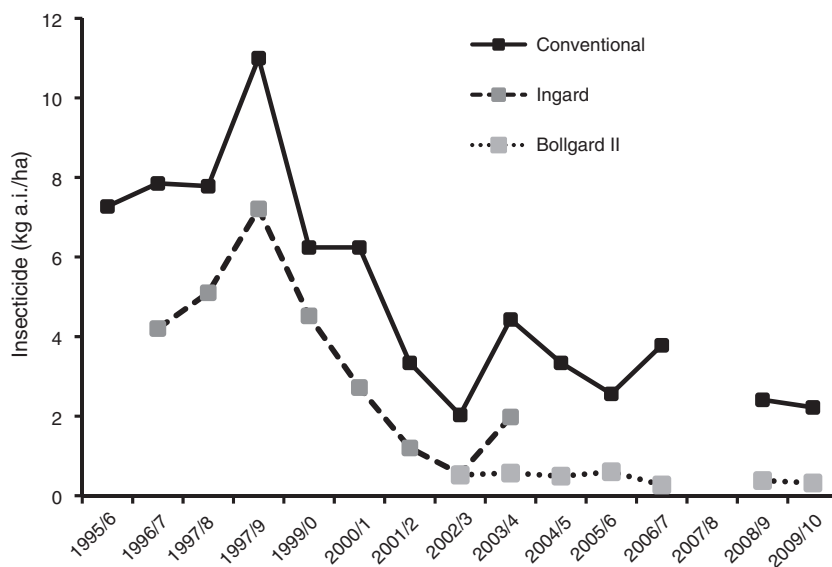
Fig. 1. Insecticide use on *non-Bt* (conventional), Ingard[®], and Bollgard II[®] *Bt* cotton in Australia. Data from Knox *et al.* (2006) and Constable *et al.* (2011) updated with recent industry surveys. No data were collected in 2007–08 because the cotton area was very small due to drought.

et al. 2009). This also reduced selection for pesticide resistance in *H. armigera* (Rossiter *et al.* 2008).

Transgenic technology has, however, created new challenges. First, prolonged exposure of *H. armigera* and *H. punctigera* to the Bt proteins means that there is strong selection for development of resistance (Roush *et al.* 1998). Second, reduced insecticide sprays against *Helicoverpa* spp. allowed some pests, formerly coincidentally controlled by these sprays and not affected by the Bt proteins, to increase to damaging levels (Lei *et al.* 2003; Naranjo *et al.* 2008). The most important of these emergent pests have been the green mirid (*Creontiades dilutus*) and the green vegetable bug (*Nezara viridula*). Lack of knowledge around the ecology and management of these emergent pests, and the broad-spectrum nature of the insecticides registered for their control (Wilson *et al.* 1998, 2012), subsequently undermined existing IPM strategies for traditional secondary pests (e.g. spider mites (*Tetranychus* spp.), aphids, and silverleaf whitefly (*Bemisia tabaci*); Mensah and Wilson 1999).

The research of the Cotton Catchment Communities CRC (hereafter Cotton CRC) from 2005 to 2012 had a strong emphasis on developing solutions to these challenges to maintain the susceptibility of *Helicoverpa* spp. to transgenic cotton (Bollgard II[®]) and to develop and adapt the cotton IPM system to account for the changed pest complex. A central tenet of the cotton IPM system has been to manage on-farm operations to support effective management of pests, with insecticide use being a last resort. This includes managing pest alternative and overwintering hosts (weeds), conservation of beneficial populations, effective crop scouting and use of economic thresholds, optimising crop management, and industry-wide adherence to a resistance management plan for both Bollgard II[®] and insecticides (Deutscher *et al.* 2005). Below we review the relevant research of the Cotton CRC that addressed these challenges.

The cotton pest complex

The larvae of *H. armigera* and *H. punctigera* damage the growing terminals of cotton, sometimes causing excessive branching and destroying the developing flower buds (squares) and fruit (bolls), potentially reducing yield (Fitt 1994). Unsprayed, non-*Bt* cotton can yield near zero as a result of damage caused by *Helicoverpa* spp. Thrips (*Thrips tabaci*, *Frankliniella schultzei*, and *F. occidentalis*) are early-season pests that damage seedling cotton, delay growth and maturity, and occasionally reduce yield (Wilson and Bauer 1993; Sadras and Wilson 1998), although they are also predators of spider mites (Wilson *et al.* 1996). Spider mites damage leaves, reducing photosynthesis and leading to reduced yield potential and fibre quality (Wilson 1993). Cotton aphids (*Aphis gossypii*) are vectors for Cotton Bunchy Top Virus (CBT, Reddall *et al.* 2004; Ellis *et al.* 2012), which stunts plant growth and yield. They also feed on plants, reducing photosynthesis, and secrete honeydew, which contaminates lint, reducing its value. Silverleaf whitefly also secretes a honeydew rich in the sugar trehalulose, which creates lint-processing difficulties (Williams *et al.* 2011). Sucking bugs (green mirid and green vegetable bug) feed on young squares and bolls, causing their abortion or damage to developing bolls (10–20 days old), resulting in reduced lint yield (Khan and Murray 2001; Khan *et al.* 2004*a*).

Pest management before Bollgard II[®]

Management of insect pests has been a key challenge for the Australian cotton industry since its inception in the mid-1960s. From the 1960s to 1990s, pest managers relied almost exclusively on regular application of synthetic insecticides, generally of a limited range of modes of action (Fitt 1994). This inevitably led to selection for resistance in some key pests and associated problems of secondary pest outbreaks, especially of spider mites, induced by destruction of natural enemies of the pests

(Fitt 1994; Wilson *et al.* 1998), as well as risks from off-farm movement and environmental contamination of the active ingredients (Wilson *et al.* 2004).

Pesticide resistance has especially been a major challenge in *H. armigera*, which developed resistance to organochlorines in the early 1970s (Wilson 1974), to endosulfan (cyclodiene) in the late 1970s, to pyrethroids in the early 1980s (Forrester *et al.* 1993), and to carbamates in the mid-1990s (Gunning *et al.* 1996). This had led to development of an insecticide resistance management strategy (IRMS) by the cotton industry. The IRMS focussed on *H. armigera*, since this was the species in which resistance had repeatedly developed. The IRMS was designed to manage resistance through limiting use of any mode-of-action group temporally (stages of the cotton season), by rotation (non-consecutive use), or by restricting the number of applications (Forrester *et al.* 1993; Bird *et al.* 2012). It was thought that this species tended to be more associated with cropping areas, and hence was exposed to successive selection for resistance with relatively low levels of dilution by migration of moths from unsprayed areas outside. *Helicoverpa punctigera*, by contrast, did not develop resistance, and this was thought to be due to dilution of resistance in cotton regions by immigration of large populations of moths from central Australia. These moths had not been exposed to insecticides and would mate with any resistant moths and dilute resistance (discussed below).

Deployment of Ingard® cotton in the mid-1990s reduced insecticide use on those crops by ~50% (Fig. 1), but efficacy was limited due to the declining expression of Cry1Ac through the growing season, and impact was limited as the area was capped at 30% of the cotton crop to avoid selection of resistance to this protein before a second protein became available (Roush *et al.* 1998). During this period, strong reliance on insecticides continued on the remaining 70% of non-*Bt* cotton and led to ongoing selection for resistance to insecticides in *H. armigera*, especially to the relatively new classes of insecticides such as spinosad, emamectin, and indoxacarb (Rossiter *et al.* 2008) and selection of pesticide resistance in secondary pests. For instance, by the early 2000s, spider mites were resistant to organophosphates (Herron *et al.* 1998), the pyrethroid bifenthrin (Herron *et al.* 2001*b*), and chlorfenapyr (Herron *et al.* 2004). Cotton aphids were also resistant to organophosphates, the carbamate pirimicarb and pyrethroids (Herron *et al.* 2001*a*).

The development of the first Integrated Pest Management Guidelines by the Australian Cotton CRC in 1999 was a response to this challenge (Mensah and Wilson 1999; Wilson *et al.* 2004). Among the strategies recommended was use of the newly registered, more selective control options (spinosad, indoxacarb, and emamectin) to control *Helicoverpa* spp. with reduced negative effects on beneficial insects (Wilson *et al.* 2012). However, despite the cotton industry developing voluntary restrictions on the use of these compounds as part of its IRMS (e.g. Gunning *et al.* 2003), their preferential use meant that by the early 2000s *H. armigera* showed incipient resistance to all three insecticides and pest management was on the verge of crisis (Rossiter *et al.* 2008) where control of *Helicoverpa* spp. would have to revert to older, less-selective compounds, with negative implications for IPM, costs, and the environment.

The advent of Bollgard II® dramatically reduced insecticide use and removed the strong selection pressure on both the newer and older insecticide groups. However, the challenges of resistance management for Bollgard II® and the management of emergent pests remained.

The pre-emptive resistance management plan for Bollgard II®

To slow the evolution of pest resistance, a pre-emptive RMP that was based on a 'high-dose' plus 'refuge' strategy (Fitt 2000; Tabashnik *et al.* 2004) was adopted by the Australian cotton industry, beginning with the release of Ingard® in 1996. The basis for the high-dose strategy is that heterozygote individuals are phenotypically similar to susceptible individuals. Therefore, administering a dose of Bt in the plant that was several times greater than that required to kill all susceptible insects would also kill all heterozgotes, which would therefore be functionally susceptible (Gould 1998). The refuge component would be hosts on which *Helicoverpa* could develop without selection by the Bt proteins. The role of the refuge component was to produce large numbers of homozygous susceptible moths that would presumably mate, at random, with the occasional homozygous resistant insects that survive in the transgenic *Bt* crop (Tabashnik *et al.* 2004). The offspring of such matings would be heterozygous and therefore functionally susceptible to a high dose of Bt toxin (Gould 1998). While there is a range of unstructured refuges in the cotton landscape, it is mandatory that growers plant a structured refuge crop to produce Bt-susceptible moths. The many 'unstructured' refuges are other crops that host *Helicoverpa* spp., such as sunflower, and also a range of weeds and native hosts. However, due to rainfall variability (drought and flood), the occurrence of unstructured refuges is unreliable. A structured refuge is a crop that is a host for *Helicoverpa* spp. and that would not contain or be sprayed with any product that contained the Bt proteins present in Bollgard II®. Hence, moths developing in the 'refuge' would not have had selection for resistance to the Bt proteins. The structured refuge has to be within 2 km of the associated Bollgard II® crop, to increase the chance that some of the moths produced in the refuge will service this Bollgard II® cotton crop.

The amount of land that must be planted to a structured refuge was based on the work of Roush *et al.* (1998), who calculated that sufficient moths would be produced by refuges to delay resistance by at least 20 generations if 10% of the *Helicoverpa* population was exposed to non-*Bt* plants (assuming that the only difference in mortality of *Helicoverpa* between *Bt* and non-*Bt* cotton was due to the presence of the Bt toxins). Therefore, 10% of all *Helicoverpa* eggs need to be laid on non-*Bt* refuges. Early work compared the productivity of different potential refuges, including unsprayed cotton, maize, sorghum, and pigeon pea, and sprayed cotton, and found, for instance, that pigeon pea was twice as productive as cotton with respect to the number of moths produced (Baker *et al.* 2008). Consequently, the amount of refuge that had to be planted for a given area of *Bt* cotton was set at 10% for unsprayed cotton and other refuges scaled accordingly (Table 1). Because pigeon pea refuges require less land, they are the most popular form of refuge (>70%; Monsanto Australia, unpubl. data); hence, on many farms all of the cotton is Bollgard II®.

Table 1.   Areas, as a percentage of the area planted to Bollgard II®, that must be planted as a structured refuge
Extracted from Cotton Pest Management Guide 2008–09 (Farrell 2008) and 2012–13 (Maas 2012). Sprayed refuges must not be sprayed with any Bt-based products that control *Helicoverpa* spp. Unsprayed refuges must not be sprayed with any products that control *Helicoverpa* spp. including Bt products

Refuge for:	Refuge option	Refuge irrigated or dryland	Sprayed/ unsprayed	% of Bollgard II®
Irrigated cotton	Cotton	Irrigated	Sprayed	100
	Cotton	Irrigated	Unsprayed	10
	Pigeon pea	Irrigated	Unsprayed	5
	Sorghum[A]	Irrigated	Unsprayed	15
	Corn[A]	Irrigated	Unsprayed	20
Dryland cotton	Cotton	Either	Sprayed	100
	Cotton	Either	Unsprayed	10
	Pigeon pea	Irrigated	Unsprayed	5

[A]Removed as options in 2010–11.

Additional management tactics were put in place to support the RMP. These included a defined 'planting window' restricting the spread of sowing dates and thereby limiting the period of exposure of the technology, and therefore the number of generations of *Helicoverpa* exposed to the Bt toxins, across regions. In addition, volunteer non-*Bt* cotton plants were required to be removed. These are plants that survive from one cotton season to the next within the field either by re-shooting from rootstock (ratoon) or from fallen seed that has germinated (volunteer). Non-*Bt* volunteers and ratoons are a problem in Bollgard II® crops as they could allow heterozygous Bt-resistant larvae to survive to a size where they can then complete development and contribute resistance genes to the next generation. Finally, growers were required to cultivate the soil to a depth of 10 cm in the winter following a Bollgard II® crop to destroy any diapausing *H. armigera* pupae, a practice known as 'pupae busting'. *Helicoverpa armigera* pupae diapause in the soil of cotton crops through winter. These over-wintering pupae are the last of five or so generations of moths that developed in the crop during the summer under heavy selection pressure from the insecticides used on the cotton or from the proteins in Bollgard II®. Consequently, overwintering pupae may carry resistance to insecticides (conventional insecticides or Cry proteins) from one season to the next (Fitt and Daly 1990). Pupae busting reduces survival of these pupae, which may carry insecticide- or Bt-resistance genes.

Testing assumptions upon which the RMP is based

A key assumption of the RMP, that the plants would produce a high dose, has been evaluated critically as part of the Cotton CRC's Farm Program. Fitt *et al.* (1998) showed that the expression of Cry1Ac declines within the plant through the cotton season and is generally too low to provide reliable control of *Helicoverpa* for the last half of the season. Expression of the second protein, Cry2Ab, is higher and more consistent throughout the season. However, the assumption of high dose for Cry2Ab is challenged by differential expression between different structures, variability of overall expression levels through the season in response to environmental conditions (Sivasupramaniam *et al.* 2008), and a naturally high tolerance to Bt of *Helicoverpa* spp. (Liao *et al.* 2002).

Another assumption of refuge efficacy is that moths derived from refuges and *Bt* cotton mate randomly. This was tested using moths collected within Bollgard II® crops, either individually or as mated pairs, which were analysed using stable carbon isotopes ($\delta^{13}C$) to test whether they originated from a C_3 (e.g. cotton) or a C_4 (e.g. maize, sorghum) host. This research found random mating between individuals originating from C_3 and C_4 crops, which provided evidence that random mating is likely to occur between moths produced in refuges and Bollgard II® crops (Baker and Tann 2013). Additional laboratory tests have demonstrated that moths emerging from pigeon pea and cotton crops showed no bias in mate preference (G. Baker, unpubl. data), thus also supporting the assumption of random mating.

Survey results from the Ingard® era (Baker *et al.* 2008), wherein the relative numbers of *Helicoverpa* emerging from pigeon pea refuges were demonstrated to be higher overall than those from unsprayed non-*Bt* cotton refuges, have generally remained consistent throughout subsequent years, i.e. during the deployment of Bollgard II® (Baker *et al.* 2013). However, Whitehouse *et al.* (2012) reported no difference in attractiveness (oviposition) and moth production between these two refuge crop options in a recent season. This is not surprising given the great variability (in time and space) commonly observed in *Helicoverpa* dynamics (Baker *et al.* 2013). Such variability may be attributed to differences in crop management, *Helicoverpa* moth dispersal to oviposition sites, local environmental conditions (e.g. moisture, wind direction), natural enemy populations, etc. (Baker *et al.* 2013).

While pigeon pea refuges are not always twice as attractive as cotton, cotton refuges may also be less attractive than *Bt* cotton. This difference could also be caused by differences in the management of *Bt* cotton and refuge cotton, greater larval feeding in refuges rendering them less attractive, or simply chance events dictating where oviposition occurs (Baker *et al.* 2013). Nevertheless, Baker *et al.* (2008) showed that, despite variability between refuges, the productivity of moths from refuges as a whole was as expected. Work is in progress to establish whether high productivity of a few refuges within a landscape can compensate for other underperforming refuges (G. Baker and C. R. Tann, unpubl. data). In the meantime, researchers recommend that growers strive to manage refuges

to maximise chances of producing non-Bt-selected moths (Ceeny *et al.* 2012; Baker *et al.* 2013).

Supporting the RMP and advancing knowledge of *Helicoverpa* spp. ecology

In the interval since the release of Bollgard II®, research funded by the Cotton Research and Development Corporation has found higher than expected levels of resistance to one of the Bt proteins (Cry2Ab) in *H. armigera* (Mahon *et al.* 2007). However, more surprising was the finding of similar resistance to Cry2Ab in *H. punctigera* (Downes and Mahon 2012). Previous experience with insecticide resistance and understanding of the ecology of both *Helicoverpa* species led to the expectation that the risk of development of resistance to the Bt toxins was much greater in *H. armigera* than *H. punctigera*; hence, the RMP and choice of refuge options was oriented more towards *H. armigera*. The reports of resistance to Cry2Ab in both species raised concerns about the durability of the technology, and the effectiveness of the RMP, especially the productivity of refuges, especially for *H. punctigera*. As a result, two refuge options, maize and sorghum, which were good hosts for *H. armigera* but poor hosts for *H. punctigera*, were removed from the RMP in 2010–11.

As part of the Cotton CRC program, methods to increase the productivity of refuges were explored. Baker *et al.* (2013) tested a range of crop options including: (*i*) crop mixes such as 10% mungbean + 90% cotton, 10% pigeon pea + 90% cotton; (*ii*) timing, such as splitting the planting of unsprayed cotton refuges to generate a wider spread in fruiting period, or 'ratooning' cotton, which involves allowing the cotton to stay in the ground many years; and (*iii*) testing different varieties of cotton. While some options had potential to increase attractiveness and productivity (Baker *et al.* 2013), any affect seemed likely to be overshadowed by management issues, such as not supplying enough water and nutrients to the crop. The importance of water and nutrients to the survival of larvae on refuges has been confirmed in the Cotton CRC-initiated PhD project of Mr Dominic Cross (D. Cross, unpubl. data).

The advent of Bollgard II® and presence of resistance alleles in both *Helicoverpa* species has also challenged current understanding of pest ecology. Historically, populations of *H. armigera* increase in successive generations in the cotton regions, resulting in large populations in cotton regions by late summer. Based on data from the USA and China, respectively, Carriere *et al.* (2003) and Wu *et al.* (2008) argued that wide-scale growing of the single-gene *Bt* cotton led to area-wide suppression of noctuids (including *H. armigera*). There was no evidence of this result in Australia with the single-gene Ingard® cotton, but these varieties were restricted to ≤30% of the Australian cotton area. However, with plantings of Bollgard II® now comprising >96% of the area planted, there is evidence emerging that the abundance of *H. armigera* late in the season may be suppressed (G. Baker and C. R. Tann, unpubl. data) but data for more years are required to confirm this pattern.

In addition, *H. punctigera* was believed to develop large populations in inland areas, which migrated into cotton regions during late spring where they were thought to be obligate migrants (Oertel *et al.* 1999; Gregg *et al.* 2001). As these

moths had originated from non-cultivated habitats and not been exposed to agricultural insecticides, they should be susceptible and hence interbreed with potentially resistant remnant populations of *H. punctigera* that may have remained in the cotton regions from the previous season, hence diluting any resistance. However, Baker *et al.* (2011) analysed long-term *Helicoverpa* spp. pheromone and light trap data from the cotton-producing areas within the Namoi Valley in NSW. They found no consistent correlations between winter rainfall patterns in central Australia and subsequent catches of moths in the Namoi Valley in spring, questioning the findings of earlier studies. The result that initial resistance frequencies in *H. punctigera* to Cry2Ab were higher than expected, and have subsequently increased over time, has further challenged the theory that this species is an obligate migrant (Downes *et al.* 2009, 2010). Surveys of moth and larval abundance in recent drought years suggested that the scarcity of hosts in these inland areas due to dry conditions probably prevented the build-up of migrant moth populations, which may have limited migration into cotton regions, thereby reducing dilution (Gregg *et al.* 2012). Furthermore, surveys that continued following rains in 2010 found widespread populations of *H. punctigera* larvae in these inland areas, but as rains continued to allow host growth in spring a second generation of larvae was produced, indicating that moths did not migrate, which again challenged the concept of obligate migration of this pest and spawned the ongoing research in the Cotton CRC-originated PhD project of Kris La Motee at University of New England.

Bollgard II® cotton is highly resistant to *Helicoverpa* spp. infestation (Lu *et al.* 2012*a*). Nevertheless, these crops are regularly checked for the presence of surviving larvae, and in some fields *Helicoverpa* spp. larvae survive to emerge as adults. From work undertaken as part of the Cotton CRC summer scholarship program, Whitburn and Downes (2009) reported that from 2005 to 2008, on average 15% of the area planted to Bollgard II® in any season carried *Helicoverpa* spp. larvae at levels that exceed recommended thresholds (2 larvae >3 mm long/m^2 cotton in two consecutive checks or 1 larva >8 mm long/m^2). Testing showed that these larvae were not physiologically resistant to the two proteins in Bollgard II® (S. Downes, unpubl. data), which raised questions as to why larvae were surviving. Behavioural studies identified that during test feeding some neonate larvae regurgitate cotton tissue containing Bt proteins, which enables some larvae to test feed until they locate plant structures such as flowers with lower expression of Bt proteins (Lu 2011). However, a comparison of larvae from a 'random' colony and a colony derived from 'survivors' on Bollgard II® showed no difference in the preferences for different plant structures. This indicated that the 'survivor' larvae from Bollgard II® had not evolved a behavioural-based mechanism for Bt protein avoidance on Bollgard II® cotton (Lu *et al.* 2011). Instead, normal foraging behaviour combined with seasonal variations in the phenotypic expression of proteins allows some susceptible larvae to survive (Lu 2011). Experiments with real and simulated pest damage, based on densities and distributions of surviving larvae seen in the field, showed that current thresholds are valid, and probably perhaps slightly conservative in terms of the impact of damage on yield (Lu *et al.* 2012*a*, 2012*b*).

Emergent pests: mirids the new *Helicoverpa*?

Green mirids (*Creontiades dilutus*) were historically a seedling pest, but in Bollgard II® crops, in the absence of broad-spectrum insecticides, populations started to build and cause crop damage during the growing season (Lei *et al*. 2003; Khan *et al*. 2006; Whitehouse 2009). This raised concerns about effective on-farm management because sampling protocols and insecticide application thresholds were lacking while control options were generally based on application of more toxic, broad-spectrum compounds (see below). Spraying mirids at non-economic levels with broad-spectrum insecticides would exacerbate the risk of outbreaks from secondary pests such as mites and aphids (Wilson *et al*. 1998). Green mirids are fast moving and often cryptic in the crop, leading to poor confidence among pest management practitioners about the reliability of sampling estimates of abundance. This led to a tendency for crops to be sprayed in the belief that some mirids may have been missed during sampling (Gregg and Wilson 2008; Whitehouse 2011).

Earlier research had shown that lucerne was more attractive to mirids than was cotton (Mensah and Khan 1997). This characteristic could be manipulated to manage mirids; planting strips of lucerne within cotton attracted mirids away from the cotton, and alternating mowing of portions of the lucerne meant that a portion of each strip was always highly attractive to mirids. Lucerne also served as an in-crop refuge for beneficial species, and a beneficial attractant spray was developed to attract beneficial species from outside the field (Mensah 1997, 2002*a*) and from the lucerne into the cotton crops to aid pest control (Mensah and Khan 1997; Mensah 2002*b*). The foodspray also reduced oviposition by *Helicoverpa* spp., valuable in non-*Bt* cotton (Mensah 1996). This approach was integrated with the use of biological and synthetic insecticides using a predator to pest ratio to determine when intervention in the control of *Helicoverpa* was required (Mensah 2002*a*). Unfortunately, severe drought from 2000 to 2010 meant that industry was unwilling to use water on a 'non-productive' crop, so there was very low uptake of this approach.

Hence, the Cotton CRC had to develop a new strategy to manage mirids and approached this from several angles. First, a range of sampling techniques was compared to establish which were better at estimating mirid abundance. The techniques included: visually searching the crop, the use of suction samplers, sweep netting, and using beat sheets (drop cloths). This research determined that beat-sheet and sweep-net methods were more effective at locating mirids than visual searches or suction sampling (Wade *et al*. 2006), and use of these methods has been extended widely and well adopted by industry (Threlfall *et al*. 2005). Further, calibrations between the different methods were derived using regression and used to adjust thresholds for sampling method (see below). At the same time, Cotton CRC PhD student Sam Lowor identified a sex pheromone released by females that attracted males and showed that the optimum blend of 5 : 1 hexyl hexanoate and (*E*)-2-hexenyl hexanoate consistently caught males in traps (Lowor *et al*. 2009*a*). The potential to use this pheromone in monitoring and management (mating disruption or attract and kill) of mirids was explored (Lowor *et al*. 2009*b*) and further work is continuing.

Development of valid economic thresholds for mirids was also a priority. Wilson *et al*. (2003, 2009) showed that seedling cotton could withstand considerable damage from pests without suffering delayed harvest or reduced yield. Duggan *et al*. (2007) showed that cotton could compensate for simulated mirid damage up to a density of ~4 mirids per m². Compensation occurred through a combination of: (*1*) instantaneous fruiting site substitution—cotton produces more fruit than it can retain so excess fruit are shed, hence a damaged boll may be shed and replaced by another boll that would have otherwise been shed; and (*2*) increased boll size for remaining undamaged bolls. This research was developed further by Khan *et al*. (2006), who evaluated the effect of mid-season mirid populations on yield. They assessed both pest abundance (using beat-sheet sampling) and plant fruit retention (proportion of total fruit produced that are retained by the plant—damaged fruit will usually shed) as measures of potential yield loss and developed thresholds that incorporated both measurements (Khan *et al*. 2006). This information was combined with that from Duggan *et al*. (2007) and Wilson *et al*. (2003, 2009) to provide comprehensive thresholds. These were calibrated for the different sampling methods, thereby providing flexibility in the choice of sampling method (Table 2). The greater effectiveness of beat sheets and sweep nets at finding mirids meant that these methods had a higher threshold than for visual checks. For instance, beat sheets are difficult to do in freshly irrigated fields, where visual searches or sweep nets may be more appropriate (Leven *et al*. 2012).

Despite the provision of scientifically valid sampling protocols and thresholds, further Cotton CRC work suggested

Table 2.   Thresholds for mirids in warm cotton regions (Central Queensland, western Darling Downs, St George, Bourke, McIntyre Valley, Gwydir Valley, lower Namoi Valley)

Extracted from the Cotton Pest Management Guide 2012–13 (Maas 2012). Tip damage: light, embryo leaves within the terminal are black; heavy, terminal and 2–3 uppermost nodes are dead

Stage	Mirid threshold			Plant damage threshold		
	Visual (mirids/m²)	Beat	Sweep (mirids/ 20 sweeps)[A]	Tip damage	Fruit retention (%)	Boll damage (% of all bolls damaged)
Planting–1 flower/m²	1.3	4	4 adults + 2.1 nymphs	50% (light) 20% (heavy)	60%	–
Flowering–1 open boll/m²	1.0	3	3 adults + 1.6 nymphs	–	60–70%	20%
1 open boll/m²–harvest	–	–	–	–	–	20%

[A]After plants have 9–10 nodes.

that many crops (≈54%) were being sprayed at sub-threshold levels, as a precautionary measure due to incorrect matching of sampling and thresholds (Whitehouse 2011). This occurred because some pest managers sampled using beat sheets but then conservatively used the lower thresholds that had been calibrated for visual sampling. The reasons for this are not clear but highlight the importance of acknowledging that researchers and growers perceive the world differently; whereas researchers noticed that beat sheets caught more mirids, growers noticed the insects that left the beat sheets before being counted (you do not see the insects missed in a visual survey). Hence, growers preferred to use the lower threshold for visuals with beat sheets. Recognising that people with different backgrounds perceive the world differently is important in developing successful extension tools. In addition, unexplained fruit loss (physiological or weather-related), which is difficult to differentiate from mirid damage, further complicated assessment of risk; hence, even when fruit retention was satisfactory (>60%), sprays would be applied. Nevertheless, there was no significant difference in yield of crops sprayed below threshold for pest abundance and when retention was high compared with those sprayed when mirid densities were above thresholds and retention declined below 60%. This information was extended widely to industry and was important in changing attitudes to mirid management (Whitehouse 2011).

Most insecticide options for mirids, such as pyrethroids, organophosphates, and fipronil, were detrimental to populations of beneficial arthropods (Wilson *et al*. 1998, 2012). Application of these pesticides against mirids was found to increase the risk of outbreaks of secondary pests, especially silverleaf whitefly (see below). In addition, Mansfield *et al*. (2008) and Whitehouse *et al*. (2009, 2011) found that lynx spiders (Oxyopidae) and yellow night stalkers (Clubionidae, *Cheiracanthium* sp.) were important natural enemies of mirids that could reduce crop damage by mirids. Further, the mere presence of lynx spiders on cotton plants reduced the likelihood that mirids would attack bolls (Whitehouse *et al*. 2011). Petroleum spray oils (PSOs) were developed as an option for control of mirids, and were selective on beneficial species (Wilson *et al*. 2012) and effective on low populations of mirids but not on higher populations, so provided a partial solution (Khan *et al*. 2004*b*).

One way to increase selectivity of insecticides to conserve beneficial species is to reduce the applied rate; however, this usually also results in reduced efficacy against the target pests. Research in grain legumes has shown that the selectivity of broad-spectrum compounds such as dimethoate could be improved and efficacy maintained by reducing rates and adding table salt (NaCl) (Brier *et al*. 2008). Khan (2003, 2009) and Khan *et al*. (2008*b*) investigated this option and showed that, for several compounds, reduced rates with salt provided comparable efficacy to full rates—a win-win that was extended widely through the industry. Similarly, reduced rates of insecticides mixed with PSO showed acceptable efficacy against mirids with reduced negative affects against beneficial species compared with full rates (Khan *et al*. 2004*a*). Independent testing confirmed the selectivity of these insecticide + salt and insecticide + PSO combinations (Wilson *et al*. 2012).

The ecology of mirids was also a gap in knowledge and potentially very important for developing management strategies. If mirid populations infesting cotton crops were of a local origin, then the opportunity for exposure of successive generations to pesticide may be high, which could constitute a risk for resistance. Anecdotal observations in inland Australia whilst surveying for *Helicoverpa* suggested that mirids were active in these regions and might possibly be migrants to eastern cropping regions. The Cotton CRC PhD project of James Hereward (Hereward *et al*. 2012*b*) showed that populations of mirids do develop in inland regions, particularly using the weed annual verbine (*Cullen cinereum*) as a host (Hereward and Walter 2012), and molecular studies using microsatellites showed that these mirids migrate to cotton regions. Within the cotton regions, mirids use a wide variety of hosts for survival and development (Khan *et al*. 2004*a*), and mirid movement between these hosts and cotton is very common. Hereward *et al*. (2012*a*) found that the mirid populations on hosts such as cotton and lucence are genetically the same, suggesting further localised exchange and dilution, which bodes well for resistance management for mirids. The evidence for significant levels of short- and long-range movement may assist with minimising the potential selection of resistance genes within mirid populations.

Other emerging pests

The altered spray regime of Bollgard II® cotton also allowed other pests to build. Jassids (*Austroasca viridigrisea*) and thrips (*Frankliniella schultzei* and *F. occidentalis*) in particular were occasionally reported at high levels in some crops in the mid and late season causing extensive damage to leaves, the economic consequences of which were unknown. In addition, thrips are also valuable predators of mites (Wilson *et al*. 1996) and provide significant suppression of mite populations especially in Bollgard II® crops; therefore, insecticide disruption of thrips in cotton could jeopardise natural mite control. To establish whether control of jassids and thrips was necessary, the Cotton CRC supported a project testing the response of cotton to both real damage from these pests and simulated damage—either by removing leaves or by applying a vinegar-based 'burn' spray that simulated pest damage. These studies showed that damage occurring during fruit-set could reduce yield, but late-season damage, after cut-out (when the crops stops producing new fruiting structures), rarely reduced yield (Wilson 2010). While this information helps better manage jassids and thrips, it has also been valuable in managing similar damage from other foliage feeders such as spur-throated locusts (Acrididae, *Austracris guttulosa*) and cluster caterpillar (Noctuidae, *Spodoptera litura*).

The Cotton CRC also played a key role in providing information to industry at times of unforseen outbreaks of pests. Over the past 10 years, B-biotype *Bemisia tabaci* (silverleaf whitefly) has gradually become a pest across the Australian cotton industry. This pest excretes sticky honeydew that contaminates cotton lint and reduces its value (Gunning *et al*. 1995; De Barro *et al*. 2011). It was first reported in cotton regions in 1994 (Gunning *et al*. 1995), and the first major outbreak occurred in the northern production regions of

central Queensland in 2001–02. The reasons for the incremental rise in pest status of this species are complex, but essentially the invasive B-type silverleaf whitefly displaces the endemic non-pest *Bemisia tabaci* biotype (De Barro *et al.* 2011), with resultant pest outbreaks occurring once the B-biotype becomes the dominant strain. Since 2001, outbreaks have been reported from virtually all cotton regions. Honeydew contamination effects processing of the lint as it causes the lint to stick to spinning machinery and results in severe price and reputation penalties (Hequet and Abidi 2002; Hequet *et al.* 2005)).

Sequeira and Naranjo (2008) developed comprehensive sampling and threshold recommendations for this pest (see the Cotton Pest Management Guide, Farrell 2008). A concerted research and extension effort by Cotton CRC scientists and the extension team across agencies and states allowed rapid dissemination of this and other key information about species identification and control options, preventing any cotton from receiving penalties. Responding to these outbreaks, the CRC initiated new research to quantify breakdown or wash-off of honeydew, to identify the factors causing mortality of immature silverleaf whitefly, and to identify alternative control options. Local research has shown no difference between *non-Bt* cotton and Bollgard II® in attractiveness for silverleaf whitefly, but okra leaf cultivars tend to harbour about half as many silverleaf whitefly (Maas *et al.* 2009). However, the silverleaf whitefly problem has not yet caused growers to plant okra leaf varieties, and the bulk of cotton grown is normal leaf shape.

Another emergent pest is the pale cotton stainer (*Dysdercus sidae*), which is a rare pest of cotton that has been found in greater numbers in recent years. In 2008, populations of this pest were at high levels, and damage to bolls resulting in tight-locking (bolls that do not fluff out) and staining of the lint was obvious. A collaborative effort between CRC scientists and extension staff ensured information on sampling, damage, and management of this pest was quickly assembled from both local and international sources and published for industry, resulting in better control decisions (Khan *et al.* 2008a; Wilson *et al.* 2008b). Since then, further research has shown that the bugs can damage bolls of all ages, but only females damage the seeds and cause reduced yield and seed germination (Khan *et al.* 2010).

Outbreaks of mealy bug occurred in 2008–09 in the Burdekin and 2009–10 in the Emerald area, in both cases resulting in extensive damage to plants and dramatically reduced yield in affected areas. Solenopsis mealy bug (*Phenacoccus solenopsis*) was identified from both regions, and it is believed to be introduced, although this is now being questioned (Khan *et al.* 2012a). Cotton CRC scientists and extension officers developed research to understand the ecology, especially over-winter survival strategies, of this pest, as well as to evaluate control options. An initial response focussed on limiting spread of this pest to other regions, especially via contract pickers, and a strict de-contamination protocol was developed and extended to contract harvesters. This has been effective, as only a few individual *P. solenopsis* have been identified from other regions. A survey of affected farms commissioned by the CRC found that the abundance of alternative hosts for the mealy bugs, mainly weeds, in and around a field was associated with increased risk. Recommendations from the CRC encouraging good control of alternative hosts of *P. solenopsis* on-farm and

conservation of natural enemies have contributed to reduced outbreaks in subsequent years (Miles *et al.* 2010; Khan *et al.* 2012a). Recently a parasitoid, *Aenasius bambawalei*, has been identified that will further strengthen IPM for mealy bug (Khan *et al.* 2012b).

The green vegetable bug, *Nezara viridula*, is another minor pest of cotton that has become more abundant in recent years. It tends to live low in the crop canopy, and it lays eggs in dense clusters or rafts, which hatch into nymphs that tend to remain in that area, gradually spreading as they grow (Khan and Murray 2001). This means the distribution of the pest in the crop is patchy, and it is hard to control with insecticides. Research has shown that this pest uses a range of common weed species as hosts throughout the year (Wilson 2010). The bugs are present in non-cultivated areas near cotton, especially in remnant native vegetation areas along rivers that have been disturbed and contaminated with weeds. During winter, most of the adult bugs become bronze-coloured and live under the bark of trees such as river red gums, which are common flora in many cotton regions. Two important natural enemies of this bug are a wasp that parasitises the eggs and a fly that parasitises late instars and adults (Khan and Murray 2002). Unfortunately in the Namoi Valley, the abundance of the parasites was generally low. Crop sequence experiments showed that although adult bugs would colonise cotton they preferred to oviposit in legume crops such as mungbean, soybean, and pigeon pea (Wilson 2010). A key outcome is that clearing weed species from native vegetation areas may help reduce background populations of this pest.

Aphids (*Aphis gossypii*) and spider mites (mainly *Tetranychus urticae*) have historically been frequent and damaging secondary pests, typically exacerbated by poor on-farm weed control and the disruption of natural enemies by insecticide use. During the drought years of 2000–09, lack of alternative hosts limited overwinter survival of these pests and few problems were experienced in cotton. However, widespread rainfall in 2010 led to proliferation of hosts for these pests and significant populations in cotton in 2010–11, and with aphids this led to increased incidence of the disease cotton bunchy top (see below).

Lack of understanding about aphid ecology and effect on crop yield from feeding has hampered management. Cotton aphid reproduces parthenogenetically in Australia, so populations may consist of one or more 'clones'. Understanding of the clonal structure is import for management, as clones may have different host preferences and insecticide-resistance profiles. Indeed, Najar-Rodriguez *et al.* (2009) have suggested that there may be cryptic species within the current definition of *A. gossypii*, based on differences in host settling behaviour and genetic structure. A study of the clonal structure of aphids using microsatellites to understand host use and resistance by different clones revealed 13 multi-locus groups (MLGs) from 936 aphids (Chen *et al.* 2013). Of these, three MLGs, or 'superclones', accounted for >78% of aphids tested, and two of these MLGs were strongly linked to resistance to dimethoate, omethoate and pirimicarb via target site insensitivity in the acetylcholinesterase encoded by the *Ace*1 gene (Toda *et al.* 2004). These two MLGs were found on cotton and many other hosts, emphasising the need for vigilant farm hygiene to minimise carry-over of these MLGs through winter. Aphid feeding was shown to cause reduction in photosynthetic rate of infested leaves,

and this was directly related to aphid density. Contamination of lower leaves with aphid honeydew was also show to significantly reduce photosynthesis, probably through blockage of stomata, and this was exacerbated by the presence of dust that adhered to the honeydew and reduced light penetration to the leaf surface (Heimoana 2012).

Aphid feeding was also found to reduce cotton lint yield and was related to the density and duration of aphid infestation. This information has been incorporated into an extension document (Wilson *et al*. 2008*a*, 2008*c*) and into new tools developed to assist with management decisions for both of these pests, as part of the CottASSIST suite (http://cottassist.cottoncrc.org.au/Default.aspx). Further, cotton aphids developed resistance to the neonicotinoid class of insecticides, most likely due to long-term use of these products as prophylactic seed treatments, where they select for resistance in aphids (Herron and Wilson 2010, 2011). Strategies for managing this resistance have been developed and incorporated in the industry's IRMS (Bird *et al*. 2012). Amongst the options for selective control of aphids are the PSOs (Mensah and Austin 2011). CRC PhD student Adriana Najar-Rodrriguez investigated the mode of action of a C24 oil against aphids and found that those directly contacted by spray droplets died due to suffocation (anoxia) as predicted (Najar-Rodriguez *et al*. 2007*a*). However, it was also demonstrated that this oil was directly toxic, as aphids that walked over deposits also died. This confirmed the potential role of the PSOs in IPM strategies to manage aphids. The authors suggest that, although the PSOs alone are unlikely to control high aphid infestation through inability to achieve sufficient leaf coverage, consecutive applications of PSO will ensure many leaves have residues on them that will help suppress aphid populations (Najar-Rodriguez *et al*. 2007*b*).

The role of beneficial arthropods

Insecticide selection can strongly influence the risk of secondary pest outbreaks. The IPM Guidelines for Cotton Production Systems emphasise conservation of beneficial species through use of the most selective control options against target pests when required (Deutscher *et al*. 2005). The dramatically reduced spray regime associated with *Bt*-cotton has led to increased survival of beneficial populations, both in Australia (Mansfield *et al*. 2006) and in China (Lu *et al*. 2012*c*). Further, recent research has also shown the value of native vegetation remnants on farms as a source of beneficial species that can migrate into cotton and other crops (e.g. Schellhorn and Silberbauer 2003; Smith *et al*. 2012; Bianchi *et al*. 2013). To further support the cotton industry in the selection of insecticides, a look-up chart was developed that identifies the potential for negative effects of each insecticide against key beneficial groups (Wilson *et al*. 2012). This is based on field-based evaluation of the effects of pesticides on beneficial populations using a standardised protocol, and the utility of this approach was validated in commercial cotton (Mansfield *et al*. 2006). The chart has been updated annually with research information for all new pesticides, and also for a range of the insecticides at reduced rates with or without salt or PSO, and is made available to industry in the Cotton Pest Management Guide (Maas 2012). Complimenting this, CRC scientists also assisted in the compilation and contents of revised 'Pests and

beneficials in Australian Cotton Landscapes' to provide pest managers with information to accurately identify pest and beneficial species (Williams *et al*. 2011). A range of biological and semiochemical approaches, including 'attract and kill', were also explored by the Cotton CRC and other agencies (e.g. Del Socorro *et al*. 2010*a*, 2010*b*; Gregg *et al*. 2010; Mensah and Austin 2012) and these are reported by Mensah *et al*. (2013, this issue).

Managing insect-born disease

The disease Cotton Bunchy Top Virus (CBT), which is spread by the cotton aphid (*Aphis gossypii*), was first recorded in cotton in 1999 (Reddall *et al*. 2004). It has been identified as a polerovirus, and a molecular test (RT-PCR) has been developed to allow identification of the disease in plants and aphids (Ellis *et al*. 2012). Besides cotton, the only other confirmed host for CBT is the weed marshmallow (*Malva parviflora*). Changes in the cotton system, however, have increased risks from this disease. The move towards reduced tillage on cotton farms, to help conserve soil moisture, has allowed cotton plants to survive in fallows, providing host continuity between seasons. With the widespread adoption of glyphosate-tolerant cotton, many surviving plants that reshoot (ratoons) and volunteers that germinate from fallen seed cotton are difficult to control. As a result these 'weed' cotton plants are now much more abundant on farms, and serve as a reservoir for CBT, and a host for cotton aphids. Research confirmed that CBT was common in these plants (Sharman *et al*. 2012). This increases the risk from CBT on farms, and in 2010–11 the mild, wet winter promoted build-up of volunteers/ratoons as well as aphids, resulting in a significant increase in incidence of CBT, with several fields badly affected (Allen *et al*. 2011). The disease surveys confirmed the increase in the incidence of CBT as well as widespread presence of volunteers/ratoons on farms. Recent extension efforts have strongly targeted control of volunteers as a key factor in reducing risks from pests and diseases, and this is also a core practice emphasised in the industry's on-line Best Management Practice program, myBMP (www.mybmp.com.au).

Conclusions

The Cotton CRC has played a fundamental role in identification of challenges to cotton production, development of strategic research, and rapid delivery of outcomes to industry. The many multi-agency publications attest to the effectiveness of the Cotton CRC at bringing the combined skills of the participant agencies to bear on problems, breaking down perceived institutional and state barriers. The outcome is an industry that has reaped the rewards from the *Bt*-cotton technology and avoided poor management of green mirids or silverleaf whitefly undermining the benefits of the *Bt*-cotton technology. In addition, the CRC's research and extension efforts have provided information to industry on a range of other key and occasional pest species, contributing to more rational management and an ongoing emphasis on IPM (Fitt and Wilson 2012). The improved management practices resulting from the deployment of Bollgard II® cotton, including control of emergent pests, have resulted in substantial reductions in pesticide found in river systems in cotton-growing areas

(Mawhinney 2011). RMPs are understood and adopted by cotton growers, despite their additional costs or inconvenience.

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