The seasonal dynamics of arthropods in conventional, INGARD® and Bollgard II® cotton genotypes in a winter production system at Kununurra.

GR Strickland & AJ Annells
Department of Agriculture
Locked Bag 4
Bentley Delivery Centre WA 6983

Background:

In recent seasons, cotton research at Kununurra has focussed on developing integrated pest management (IPM) systems for INGARD® cotton production in a winter growing system. Encouraging naturally occurring beneficial arthropods into the production system has been viewed as an important aspect of IPM because it enhances sustainability by reducing the reliance on the INGARD® gene for lepidopteran pest control.

INGARD® cotton varieties contain a single Bt (*Bacillus thuringiensis*) gene that produces the Cry1Ac protein that controls several lepidopteran pests, especially the key pests *Helicoverpa armigera*, *H. punctigera* and *Pectinophora gossypiella*. However the field performance of INGARD® was sometimes erratic and Monsanto has since replaced INGARD® with an improved two Bt gene product, Bollgard II®. In addition to expressing the Cry1Ac protein, Bollgard II® lines also express the Cry2Ab protein which improves efficacy against lepidopteran pests and also greatly reduces the risk of resistance development by key pests.

The 2002 season was the first opportunity to evaluate Bollgard II® cotton in the experimental program at Kununurra.

Aims:

The aim of the experiment was to compare the arthropod fauna in conventional, INGARD® and Bollgard II® cotton genotypes with the same parental background.

Methods:

Deltapine Australia provided seed of its commercial cotton variety “Emerald” in three forms; conventional, INGARD® and an experimental Bollgard II® line. Paddock 3A1 on the Frank Wise Institute was used for the trial and 1 hectare blocks of each of the three genotypes was planted on 9 May using standard district practices for cotton growing (Diagram 1).

Diagram 1. Layout of paddock 3A1, Frank Wise Institute, 2002.

Irrigation supply channel

Conventional "Emerald" (~ 1 hectare)	INGARD® "Emerald" (~ 1 hectare)	Bollgard II® "Emerald" (~ 1 hectare)

Each of the three cotton genotypes was sampled for arthropod abundance using a blower vac machine fitted with a fine gauze "sock" to prevent escapes. At each sampling event, five metres of row was "suctioned" whilst walking at approximately 1 metre/second with the machine set at maximum revs. This was repeated five times in each block so that a total of 25 metres was sampled from each block at each date. Sampling occurred at approximately 3-week intervals.

Each suction sample containing arthropods was immediately placed into a jar containing a rich atmosphere of ethyl acetate to quickly kill the catch. The collections were later returned to the laboratory where they were either sorted and identified immediately or frozen until time permitted to process the samples.

The arthropods were identified and counted according to the list in table 1 showing the 61 arthropod forms recorded. The arthropods were broadly categorised as pests, minor pests, predators of pests, parasitoids of pests or incidental insects, such as stilt bugs and pollen beetles that inhabit cotton crops. (See appendix 1 for scientific names).

Table 1. The 61 arthropod forms counted from suction samples at Kununurra, 2002.

Arthropods counted	**Classification**	**Category**
Jassid -adult	Hemiptera: Delphacidae	Pest
Jassid-nymph	Hemiptera: Delphacidae	Pest
Green mirid -adult	Hemiptera: Miridae	Pest
Green mirid -nymph	Hemiptera: Miridae	Pest
Brown mirid -adult	Hemiptera: Miridae	Pest
Brown mirid -nymph	Hemiptera: Miridae	Pest
Other mirids	Hemiptera: Miridae	Pest
Aphid	Hemiptera: Aphididae	Pest
Apple dimpling bug	Hemiptera: Miridae	Pest
Assassin bug	Hemiptera: Reduviidae	Predator - beneficial
Big eyed bug	Hemiptera: Lygaeidae	Predator - beneficial
Broken back bug	Hemiptera: Miridae	Predator - beneficial

Table 1. (Continued).

Arthropods counted	**Classification**	**Function**
Flatid	Hemiptera: Flatidae	Incidental
Lygaeid bug	Hemiptera: Lygaeidae	Incidental
Other leafhoppers	Hemiptera: various	Incidental
Rutherglen bug	Hemiptera: Lygaeidae	Incidental
Stilt bug	Hemiptera: Berytidae	Incidental
Other Lepidoptera	Lepidoptera: various	Incidental
Flea beetle	Coleoptera: Chrysomelidae	Incidental
Weevil	Coleoptera: Curculionidae	Incidental
Other Coleoptera	Coleoptera: various	Incidental
Ant	Hymenoptera: various	Incidental
Other Hymenoptera	Hymenoptera: various	Incidental
Thrips	Thysanoptera: various	Incidental
Grasshopper	Orthoptera: Acrididae	Incidental
Cricket	Orthoptera: Gryllidae	Incidental
Cotton seed bug	Hemiptera: Lygaeidae	Minor pest
Cotton stainer	Hemiptera: Pyrrhocoridae	Minor pest
Green vegetable bug	Hemiptera: Pentatomidae	Minor pest
Redbanded shield bug	Hemiptera: Pentatomidae	Minor pest
Whitefly	Hemiptera: Aleyrodidae	Minor pest
Helicoverpa egg (parasitised)	Lepidoptera: Noctuidae	Parasitoid - beneficial
Tachinidae	Diptera: Tachinidae	Parasitoid - beneficial
Chelonus	Hymenoptera: Braconidae	Parasitoid - beneficial
Ichneumonidae	Hymenoptera: Ichneumonidae	Parasitoid - beneficial
Microplitis	Hymenoptera: Braconidae	Parasitoid - beneficial
Trichogramma	Hymenoptera: Trichogrammatidae	Parasitoid - beneficial
Helicoverpa egg	Lepidoptera: Noctuidae	Target pest
Helicoverpa larvae	Lepidoptera: Noctuidae	Target pest
Helicoverpa adult	Lepidoptera: Noctuidae	Target pest
Pink bollworm	Lepidoptera: Gelechiidae	Target pest
Rough bollworm	Lepidoptera: Noctuidae	Target pest
Spodoptera larvae	Lepidoptera: Noctuidae	Target pest
Brown smudge bug	Hemiptera: Miridae	Predator - beneficial
Damsel bug	Hemiptera: Nabidae	Predator - beneficial
Glossy shield bug	Hemiptera: Pentatomidae	Predator - beneficial
Pirate bug	Hemiptera: Anthocoridae	Predator - beneficial
Predatory shield bug	Hemiptera: Pentatomidae	Predator - beneficial
Ladybird adult	Coleoptera: Coccinellidae	Predator - beneficial
Ladybird larvae	Coleoptera: Coccinellidae	Predator - beneficial
Predatory carab	Coleoptera: Carabidae	Predator - beneficial
Red and blue beetle	Coleoptera: Melyridae	Predator - beneficial
Syrphidae (hover fly)	Diptera: Syrphidae	Predator - beneficial

Table 1. (Continued).

Arthropods counted	Classification	Function
Predatory wasps	Hymenoptera: various	Predator - beneficial
Brown lacewing adult	Neuroptera: Chrysopidae	Predator - beneficial
Brown lacewing larvae	Neuroptera: Chrysopidae	Predator - beneficial
Green lacewing adult	Neuroptera: Chrysopidae	Predator - beneficial
Green lacewing larvae	Neuroptera: Chrysopidae	Predator - beneficial
Predatory thrips	Thysanoptera: various	Predator - beneficial
Preying mantis	Mantodea: various	Predator - beneficial
Spider	Arachnida: Araneida	Predator - beneficial

The entire paddock was managed for pests as if it was an INGARD® crop. The blocks were scouted for insect abundance according to CottonLOGIC protocols but pest control action was only triggered when the INGARD® thresholds were breached. By using INGARD® as the "standard", all the genotypes received the same insecticide applications on the same day, thus eliminating the problem of vastly different insecticide applications on the three genotypes. Previous studies with conventional cotton showed that at least 10-15 insecticide applications were required to produce good yields and this level of spraying was highly deleterious to arthropod fauna generally.

Similarly, all other crop management decisions, including irrigation, nutrition, defoliation and picking, were based on the INGARD® crop's requirements.

Results:

Due to the large number of arthropods collected by suction sampling, most of the data presented has been grouped according to ecological/taxonomic function. For example, "pest lepidoptera" includes the key pests *Helicoverpa* spp, *Spodoptera litura* (cluster caterpillar), *Earias huegeliana* (rough bollworm) and *Pectinophora gossypiella* (pink bollworm). Other groupings can be derived from the "function" column in table 1.

Two insecticide applications were made during the season. The first spray was an application of Regent 200SC at 60 ml/ha on 15 August, selectively targeting mirids. The second spray was on 12 September and was a mix of Larvin 800WG at 1.2 kg/ha and Bulldock 25EC at 800 ml/ha. At this stage the crop had reached "cutout" and the purpose of the broad spectrum spray was to protect fruiting structures and enable the crop to mature.

Total arthropod abundance

Most arthropods were distributed throughout the whole paddock in a reasonably uniform manner, with the exception of the aphid population. An *Aphis gossypii* population developed as a "hot spot" in the INGARD® block and this had the effect of skewing some of the data to indicate high numbers of some arthropod groups, such as pest hemiptera in INGARD® cotton. The dominance of the aphid counts in the INGARD® block is shown in figure 1

(aphid counts excluded) compared to the total arthropod counts in figure 2. Aphid “hot spots” are a very common phenomenon in cotton and other crops and are caused by winged aphids landing in a target crop and reproducing rapidly in favourable conditions. Typically aphid populations are attractive to beneficial insects that prey on them but there is often a lag between aphid colonisation and the more gradual build-up of beneficial species, such as lady beetles and hover flies.

Due to the strong clump of aphids in one area, their counts have been excluded from the pest hemiptera chart shown as figure 4.

Figure 1. The seasonal dynamics of all arthropods (except aphids) collected in three cotton genotypes at Kununurra, 2002.

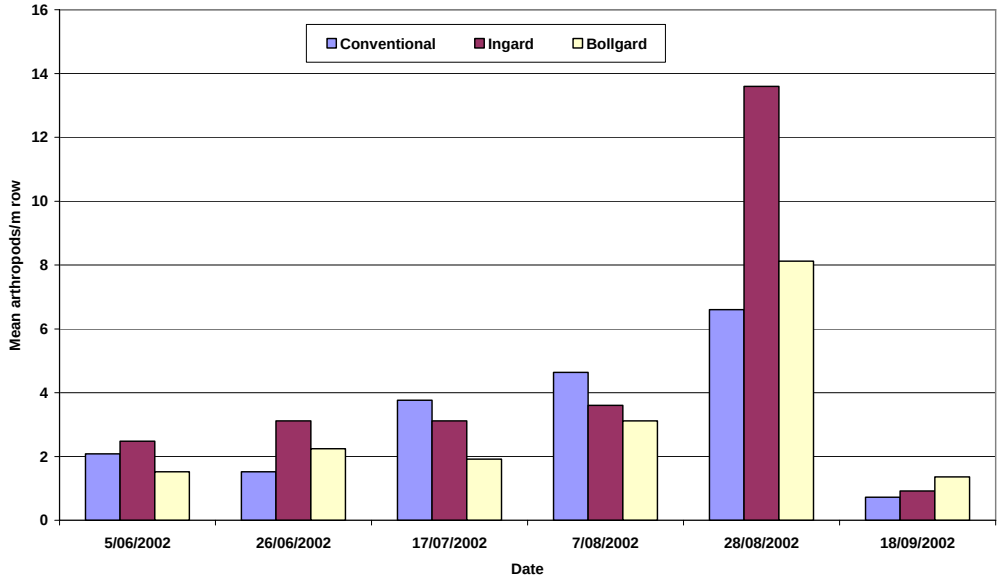
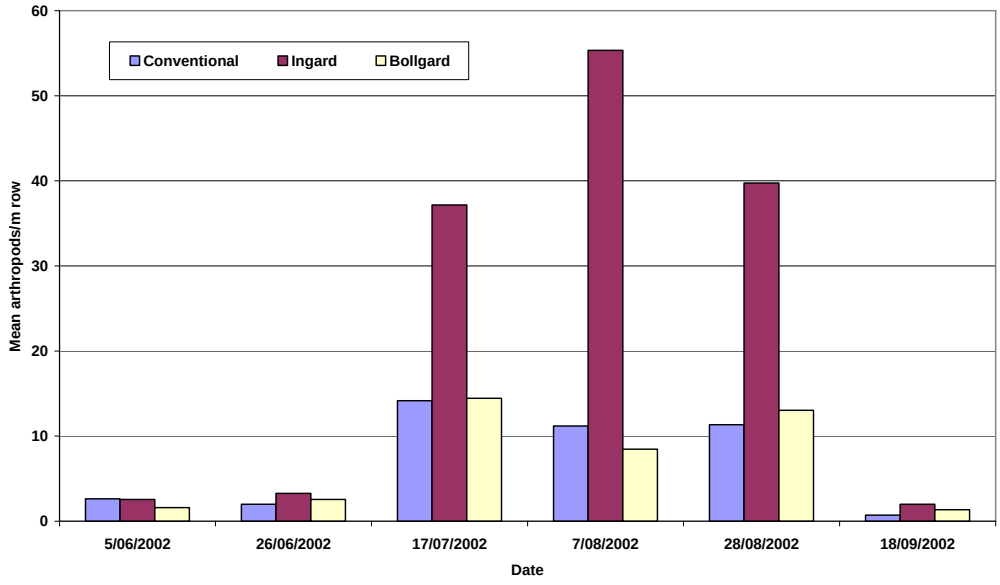


Figure 2. The seasonal abundance of all arthropods collected in three cotton genotypes at Kununurra, 2002.



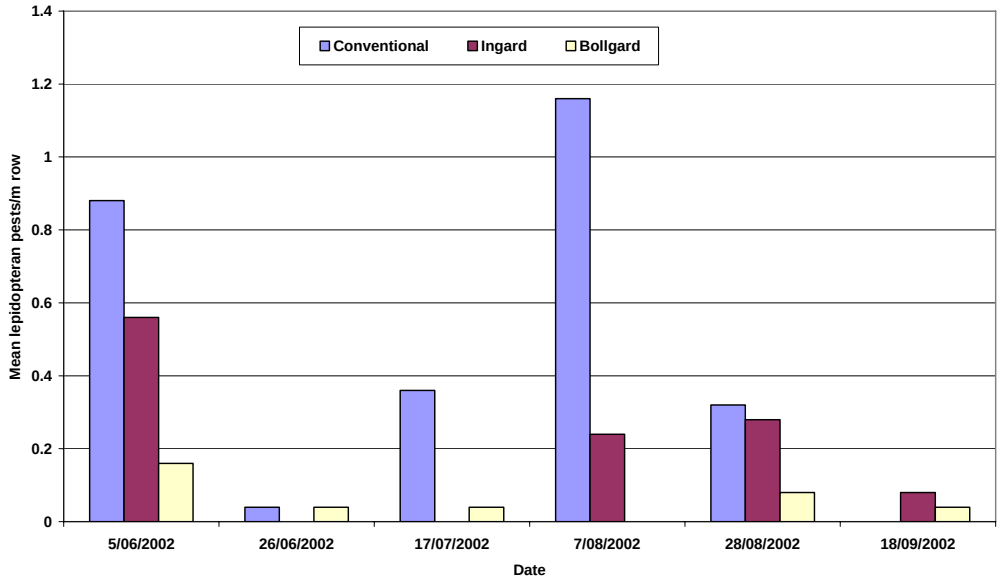
Target pest abundance

Lepidopteran pests are the target species for INGARD® and Bollgard II® gene technology. Figure 3 summarises the seasonal dynamics of lepidopteran larvae collected by suction sampling. Visual scouting data (not presented) show that suction sampling underestimates the populations of lepidopteran pests. This is because the larvae of *Helicoverpa* spp, pink bollworm and rough bollworm all entrench themselves within the plants' fruiting structures and growing points, and are not readily dislodged by suction devices.

Despite the limitations of the suction sampling technique, the data presented in figure 3 show consistently higher numbers of larvae in conventional cotton relative to the cotton expressing GM traits. This result was, of course, expected and is consistent with previous research demonstrating the efficacy of INGARD® cotton against lepidopteran pests.

The low numbers of larvae collected from the INGARD® block were predominantly *Helicoverpa* spp but no *Helicoverpa* spp were recorded from Bollgard II® cotton. *Spodoptera litura* larvae were also collected in the INGARD® block and less frequently in the Bollgard II® block. *S. litura* are only moderately susceptible to Bt toxins and this explains their low abundance in the GM varieties. The species is also more readily collected by the suction sampling technique because the larvae feed on leaf surfaces, rather than entrenching in fruiting structures.

Figure 3. The abundance of lepidopteran pest larvae collected by suction sampling in three cotton genotypes at Kununurra, 2002.

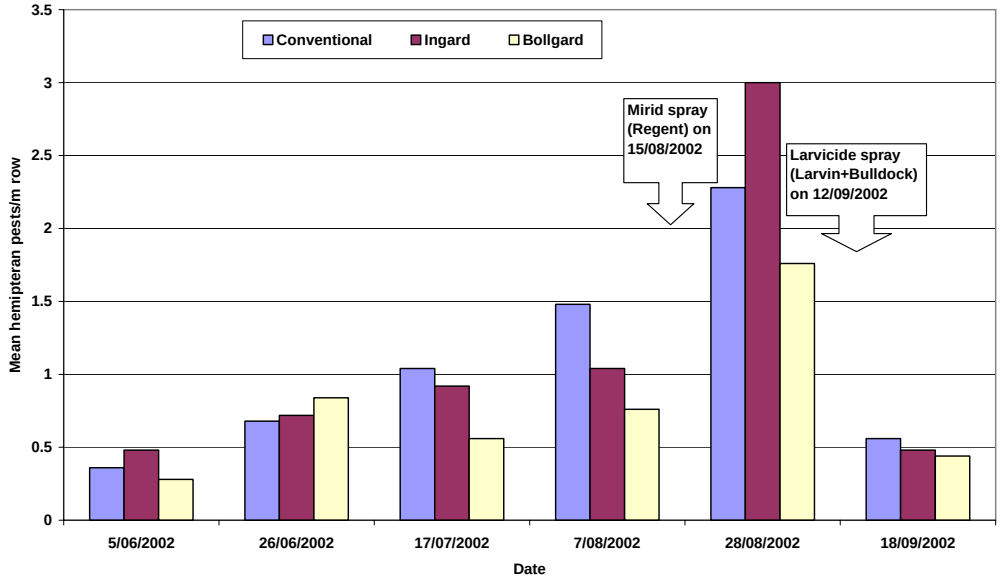


Non-target pest abundance

The main group of pests that are non-target pest species for the GM traits in INGARD® and Bollgard II® are the hemiptera. In this study the hemipterans counted included the green mirid, brown mirid, apple dimpling bug, redbanded shield bug, green vegetable bug, cotton seed bug, jassids, aphids and cotton stainer. Figure 4 shows the seasonal distribution of hemipteran pests (except

aphids) in the three cotton genotypes. The data show uniform populations of pests up to mid-season, after which there is a trend for more pests to occur in the conventional cotton compared to the GM varieties. However, following the broad spectrum insecticide application on 12 September, uniform populations of hemipteran pests were again recorded across all genotypes.

Figure 4. Total hemipteran pests (except aphids) suction sampled in three cotton genotypes at Kununurra, 2002.



Non-target arthropod abundance

Cotton crops are occupied by a large number of arthropods that are not pests. Some of them, such as *Apion* spp weevils, pollen beetles and lygaeid bugs, may feed on the crop but their damage is insignificant and they are deemed to be "incidental" rather than minor pests. Similarly, bees and lepidopterans visit cotton crops to feed on nectar or sugary exudates secreted by the crop or insects such as aphids.

Apart from incidental arthropods there is a range of arthropods that are considered to be "beneficial" species because they exert some control of pests. Beneficials can be broadly defined as either predators or parasitoids of pest species. Table 1 lists many of the arthropods classified as beneficials in this study. Important predators include lady beetles, hover flies, lacewings, assassin bugs, spiders and big eyed bugs. Significant parasitoids in the cotton ecosystem include the moth egg parasitoid *Trichogramma pretiosum*, and larval parasitoids such as *Microplitis demolitor*.

The abundance of beneficial arthropods is shown in figure 5 and figure 6. Figure 5 shows the abundance of all beneficial species throughout the season. In the early season, beneficial arthropods tended to be in higher abundance in the cotton with GM traits compared to conventional cotton. However, this trend was reversed in the mid-season although numbers

fluctuated between sampling events and there was no block that consistently maintained a difference from the others.

Predatory arthropods are the largest component of the beneficial population and shown in figure 6. For this reason the data follows a very similar trend to that in figure 5 with more predators being collected from the GM cotton early and late in the season but with fluctuating distributions in mid-season. The number of predators in Bollgard II® cotton tended to be lower than the other genotypes during the mid-season, probably due to the lower levels of lepidopteran prey available (see figure 3).

Figure 5. The seasonal dynamics of all beneficial arthropods collected by suction sampling in three cotton genotypes at Kununurra, 2002.

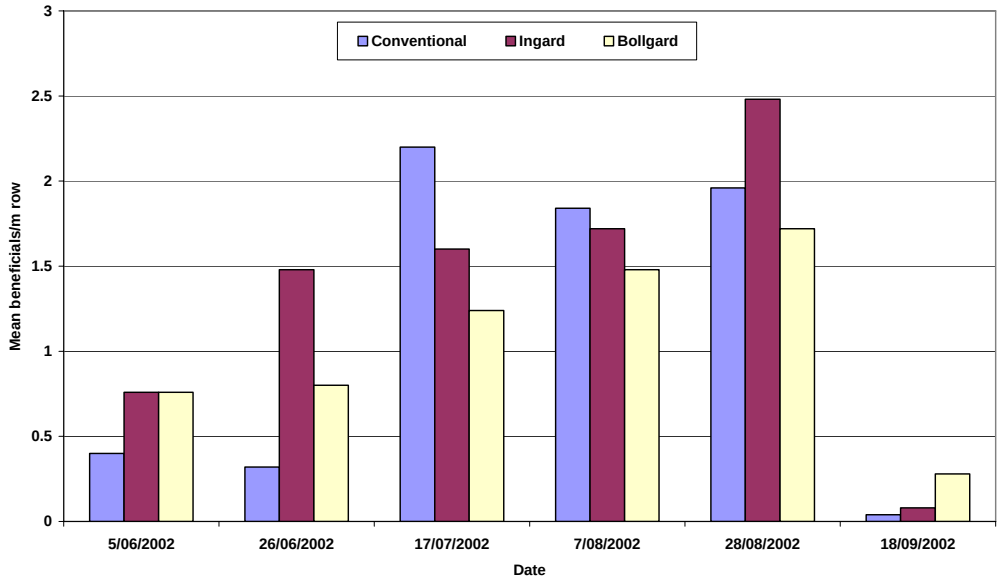
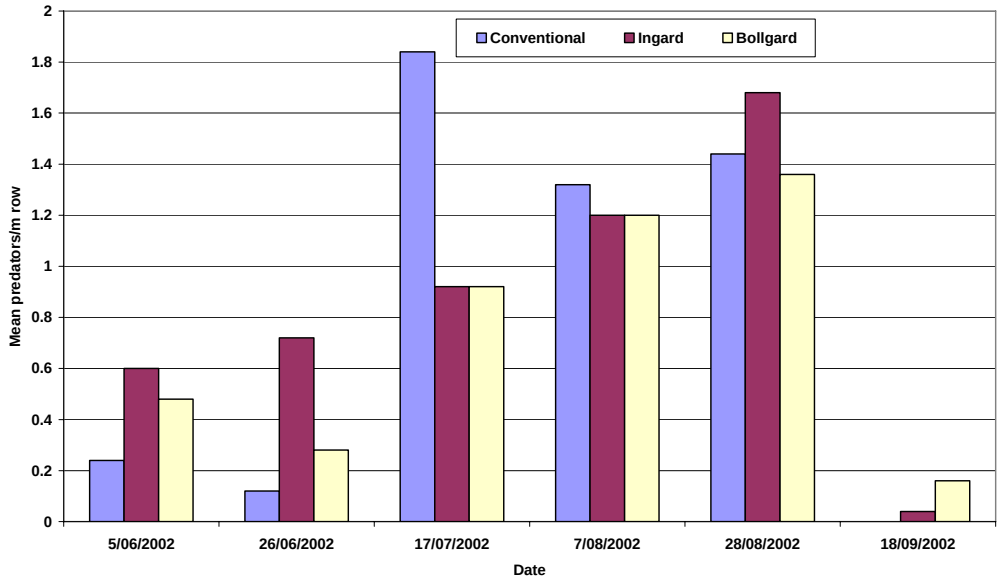


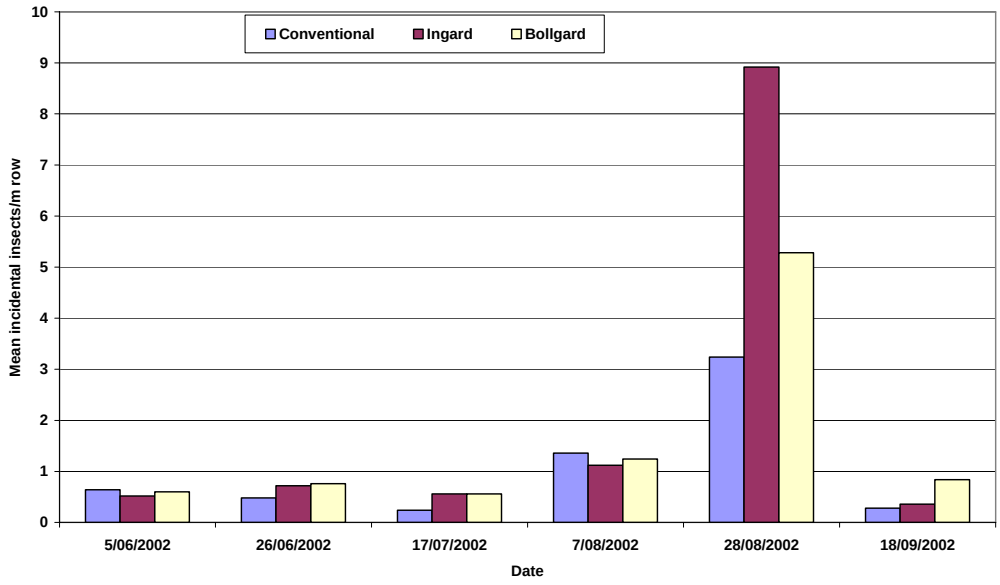
Figure 6. The seasonal abundance of predatory arthropods collected by suction sampling from three cotton genotypes at Kununurra, 2002.



The final category of insects considered in the study is referred to as "incidental" species because they are neither pests nor beneficial species in

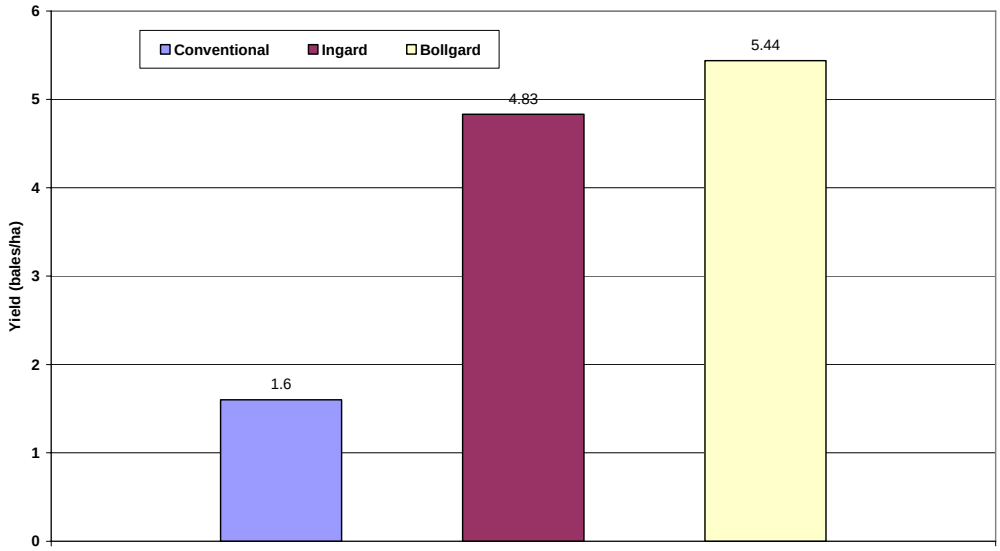
the cotton ecosystem. More than 13 species were allocated to this grouping. Figure 7 shows that these insects were generally uniformly distributed amongst the three genotypes except at the 28 August sampling date when large numbers of incidental insects were captured in the GM blocks, especially the INGARD® block.

Figure 7. The seasonal dynamics of "incidental" insects collected by suction sampling from three cotton genotypes at Kununurra, 2002.



Yield potential in this study was low due its late sowing date in May, rather than the optimum, which is late March. However the yield differences illustrated in figure 8 confirm the efficacy of the GM traits in controlling lepidopteran pests and ultimately increasing yield. All genotypes received a single application of Regent 200SC for mirid control and a broad spectrum "cleanup" spray after cutout.

Figure 8. Yield of two GM cotton genotypes relative to their conventional parent at Kununurra, 2002.



Conclusions:

This study was the first and only opportunity to compare arthropod communities in conventional, INGARD® and Bollgard II® genotypes, bred from the same parent line, in the Kimberley. OGTR policy actively discourages mixing cotton expressing different GM traits at the same site. Although plots were not replicated, each of the genotypes was planted in a large 1 hectare block adjacent to each other so that each block developed its own ecosystem. Sampling within the blocks is likely to be more representative of a commercial cropping situation than numerous small plots, which can be compromised by mobile insects "plot hopping" ahead of sampling.

The effect of Bollgard II® cotton on both target and non-target arthropods was the main aim of this study. More than 60 species were scored from extensive suction sampling so that broad conclusions concerning Bollgard II® impact could be inferred. Major conclusions follow.

Target pest abundance

Figure 3 confirms the excellent efficacy of Bollgard II® against lepidopteran pests. The figure shows very low larval numbers in the Bollgard II® block compared to conventional and INGARD® cotton blocks. The only lepidopteran pest surviving in the Bollgard II® block was low numbers of the cluster caterpillar, *Spodoptera litura*, which is known to be poorly controlled by Bt proteins. The otherwise excellent efficacy of Bollgard II® was reflected in its yield performance (figure 8) which was 240% higher than the conventional block and 12.6% higher than the INGARD® yield with the same insecticide inputs.

Non-target pest abundance

The major non-lepidopteran pest group assessed in the study was the hemipterans, which include mirids, whitefly, green vegetable bug and others. A hot spot of *Aphis gossypii* developed in the INGARD® block and because the large population dominated the sample counts, the aphid counts have been excluded from this discussion. The impact of the aphid population, predominantly in the INGARD® block, is shown in figures 1 and 2.

The hemipteran pest data is shown in figure 4. Early and late in the season the Bollgard II® block had very similar hemipteran pest numbers as the other genotypes. During the mid-season however, the conventional and INGARD® blocks had higher pest populations. This may be explained by the high levels of "tipping out", caused by mirids and *Helicoverpa* in these genotypes. Tipping out causes multiple branches and leafier crops that may be more favourable to the sucking insect pests. Despite this trend, there was a steady increase in the abundance of hemipteran pests in the Bollgard II® block, albeit at a lower rate than the other genotypes.

Beneficial insect abundance

Arthropods that prey or parasitise pest species in the cotton ecosystem are very important to sustainability because they reduce the reliance on manmade insecticides whether GM or conventional. Data for all beneficial arthropods are summarised in figure 5 and for predatory species in figure 6. These charts show that higher numbers of beneficial species occurred in the GM cotton blocks both early and late in the season. However, during the mid-season there was a trend for slightly higher beneficial abundance in conventional and INGARD® blocks. This is to be expected because higher pest populations also occurred in those blocks and predatory and parasitic arthropods are well adapted to locating prey. Importantly, despite lower prey numbers in the Bollgard II® cotton, beneficial arthropod populations were only slightly lower and continued to build throughout the season.

Incidental arthropod abundance

Apart from pest and beneficial arthropods there is a large number of species that simply live within cotton crops or visit the crop to feed on nectar, pollen or honey dew excreted by sucking insect pests. In this study more than 13 species, described as “incidentals”, were counted. Figure 7 shows the seasonal abundance of incidental species throughout the season. Generally there were very few large differences between the genotypes, with the exception of a peak in the INGARD® block at the 28 August sampling point. This was probably a response to the abundance of honey dew created by the large population of aphids in the INGARD® block (see figure 2) at that time.

The population of incidental species in the Bollgard II® block was the same, or higher, than those found in conventional cotton throughout the season.

Summary

The broad conclusion from this study is that Bollgard II® cotton is unlikely to have significant effects on non-target arthropod species. Some beneficial arthropod species were in lower abundance than conventional and INGARD® cotton but this can be explained as a response to the lower prey (pests) availability in Bollgard II®. Incidental arthropod species not dependent on prey species were found to be in equal or greater abundance in Bollgard II® cotton than conventional cotton.

Acknowledgements

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Appendix 1*. The common and scientific names of arthropods collected from suction samples at Kununurra, 2002.

Common name	**ORDER: Family**	**Species name**
Spider	Arachnida: Araneida	*??*
Apion	Coleoptera: Brentidae	*Apion spp.*
Predatory carab	Coleoptera: Carabidae	*Calosoma*
Flea beetle	Coleoptera: Chrysomelidae	*Chaetocnema sp.*
Transverse ladybird	Coleoptera: Coccinellidae	*Coccinella transversalis*
Maculate ladybird	Coleoptera: Coccinellidae	*Harmonia octomaculata*
Red and blue beetle	Coleoptera: Melyridae	*Dicranolaius bellulus*
Syrphidae (hover fly)	Diptera: Syrphidae	*Ischiodon?*
Tachinidae	Diptera: Tachinidae	*??*
Whitefly	Hemiptera: Aleyrodidae	*Bemisia tabaci*
Pirate bug	Hemiptera: Anthocoridae	*Orius spp.*
Cotton aphid	Hemiptera: Aphididae	*Aphis gossypii*
Stilt bug	Hemiptera: Berytidae	*Metacanthus sp.*
Jassid	Hemiptera: Delphacidae	*Amrasca sp.*
Jassid	Hemiptera: Delphacidae	*Austroasca sp.*
Flatid	Hemiptera: Flatidae	*Siphanta spp*
Big eyed bug	Hemiptera: Lygaeidae	*Geocoris lubra*
Lygaeid bug	Hemiptera: Lygaeidae	*Graptostethus*
Rutherglen bug	Hemiptera: Lygaeidae	*Nysius vinitor*
Grey cluster bug	Hemiptera: Lygaeidae	*Nysius clevelandensis*
Cotton seed bug	Hemiptera: Lygaeidae	*Oxycarenus luctuosus*
Coon bug	Hemiptera: Lygaeidae	*Oxycarenus arctatus*
Green mirid	Hemiptera: Miridae	*Creontiades dilutus*
Brown mirid -adult	Hemiptera: Miridae	*Creontiades pacificus*
Apple dimpling bug	Hemiptera: Miridae	*Campylomma liebknechti*
Broken back bug	Hemiptera: Miridae	*Taylorilygus pallidulus*
Brown smudge bug	Hemiptera: Miridae	*Deraeocoris signatus*
Damsel bug	Hemiptera: Nabidae	*Nabis spp.*
Green vegetable bug	Hemiptera: Pentatomidae	*Nezara viridula*
Redbanded shield bug	Hemiptera: Pentatomidae	*Piezodorus hybneri*
Glossy shield bug	Hemiptera: Pentatomidae	*Cermatulus nasalis*
Predatory shield bug	Hemiptera: Pentatomidae	*Oechalia schellenbergii*
Cotton stainer	Hemiptera: Pyrrhocoridae	*Dysdercus cingulatus*
Assassin bug	Hemiptera: Reduviidae	*??*
Chelonus	Hymenoptera: Braconidae	*Chelonus sp.*
Microplitis	Hymenoptera: Braconidae	*Microplitis*
Wasp parasite	Hymenoptera: Ichneumonidae	*Lissopimpla sp.*
Egg parasite	Hymenoptera: Trichogrammatidae	*Trichogramma pretiosum*
Pink bollworm	Lepidoptera: Gelechiidae	*Pectinophora gossypiella*
Native budworm	Lepidoptera: Noctuidae	*Helicoverpa punctigera*
Cotton bollworm	Lepidoptera: Noctuidae	*Helicoverpa armigera*

Rough bollworm	Lepidoptera: Noctuidae	*Earias spp.*
Spodoptera larvae	Lepidoptera: Noctuidae	*Spodoptera litura*
Praying mantis	Mantodea: various	*??*
Brown lacewing	Neuroptera: Chrysopidae	*Micromus spp.*
Green lacewing	Neuroptera: Chrysopidae	*Chrysopa spp.*
Grasshopper	Orthoptera: Acrididae	*Stenocatantops angustifrons*
Cricket	Orthoptera: Gryllidae	*Madasumma sp*
Flower thrips	Thysanoptera: Thripidae	*Frankliniella spp.*
Thrips	Thysanoptera: Thripidae	*Thrips spp*
Predatory thrips	Thysanoptera: various	*Scolothrips sp.*

* Numerous other species were counted into categories but not identified. For example all spiders were bulked as “spiders”, bees, wasps and hornets were bulked as “other hymenoptera, etc.