**Background**

*Helicoverpa spp.* are major pests of cotton worldwide – Australia being no different. With conventional cotton (non-genetically modified cotton) the normal control method of these pests is to spray insecticides. The decision to spray is based on information gained from the constant monitoring of crops throughout the growing season for the presence of *Helicoverpa*, with the aim of managing the damage caused by these species.

*Bacillus thuringiensis* or *Bt* is a naturally occurring but commercially cultured bacterium that kills *Helicoverpa.* For the past thirty years it has been formulated into a biopesticide (insecticide) for spray applications, with variable results. The variability is because the bacterial proteins in the biopesticide need to undergo an activation process upon ingestion into the gut of the caterpillar.

Genetic modification has enabled the *Bt* protein to be inserted into the genome of cotton in a form that does not require additional activation. Provided the plant is growing actively *and* the *Bt* gene is expressing normally, susceptible *Helicoverpa* that feed on the plant should be killed.

The initial release of *Bt* cotton in Australia was known as Ingard and only had one *Bt* gene in its genome. The next stage of release was Bollgard II which has two *Bt* genes in its genome, increasing its potency and delaying the development of resistance by *Helicoverpa* to the bacterial proteins.

# *A Comparison of Arthropod Communities in Transgenic Bt and Conventional Cotton in Australia*

Prior to the full scale release of Bt Bollgard II cotton by the Australian Cotton Industry, it was important to have a thorough understanding of the impact this technology would have on the wider arthropod communities, and whether its adoption would require altering existing pest resistance management strategies.

M. Whitehouse, L. Wilson and G. Fitt undertook this study and published the results in the magazine "Environmental Entomology" (vol.34; pages 1224-1241) in October 2005. The research methodology, analysis, results and discussion below are a summarised version of this research.

**Aim:** To determine if the diversity and population of insect communities differ between *Bt* cotton, unsprayed conventional cotton and sprayed conventional cotton.

This study aimed to use statistical analysis of data collected to thoroughly explore whether some functional groups (insects with similar characteristics) were more affiliated with *Bt* or conventional cotton and whether the overall community structure of functional groups in *Bt* and conventional cotton differ.

**Methodology:** To gather the required data set the scientists set up study sites across three farms in the Macquarie and Namoi valleys. Particular fields were selected that were relatively isolated from other cotton fields to minimise the impact of any spray drift. All fields were treated as commercially grown crops i.e. nutrition and irrigation, scouting and control where appropriate for the trial according to industry best practice, trying to minimise the seasonal variability. The data was collected over three years from replicated treatments of:

- unsprayed conventional cotton (control)
- unsprayed *Bt Ingard* cotton
- unsprayed *Bt.* Stacked cotton (the precursor to Bollgard II™)

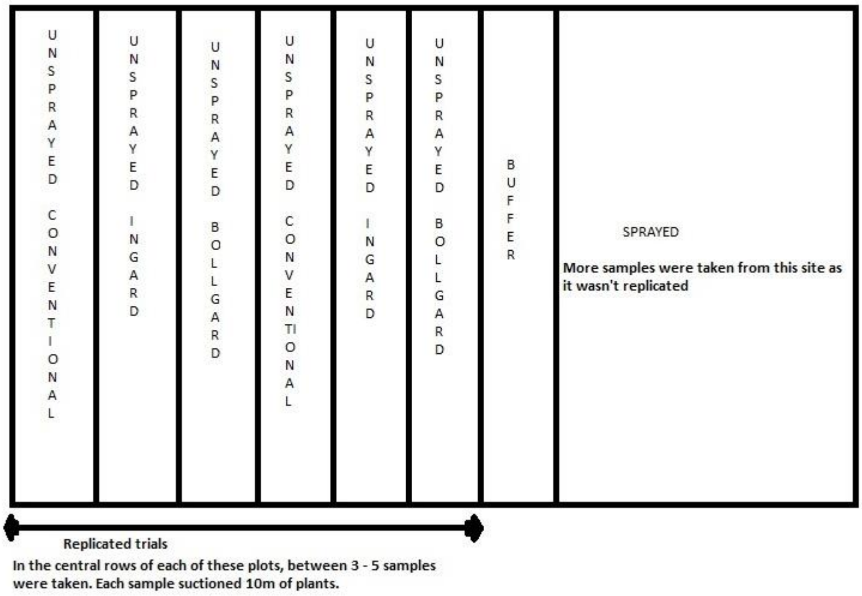
- sprayed conventional cotton.
  The sprayed conventional treatment was not replicated at all to minimise the chance of any spray drift onto any other treatments –rather the sampling effort was replicated.

**Sampling**: Insects were collected using a suction sampler (blower vac). Samples were taken at regular intervals throughout the growing seasons. Sampling began at seedling emergence and continued until 20% of the bolls had opened.

At each trial plot, suction samples were taken weekly or fortnightly from the central rows. At each trial plot 3-5 suction samples were taken along a 10 metre section of row.

As the sprayed trial plots weren't replicated, more samples were taken within the plots to get the desired data. Sampling was carried out the same as above, 10m of plants were suction sampled.

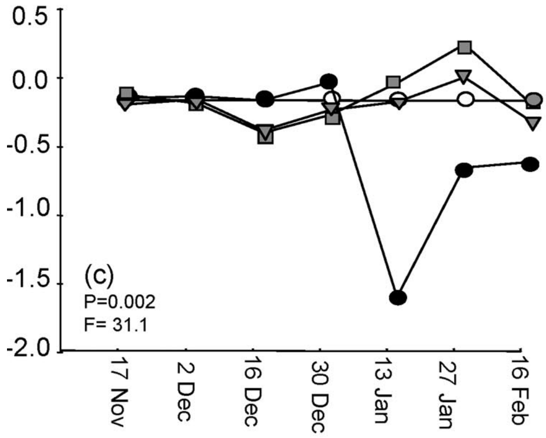
See figure below for example.




In younger plants a single pass with the suction sampler was made, in older plants several diagonal sweeps were made from the base to top of plant to ensure the entire plant was sampled.

**Classification:** Identification of insects was carried out using a reference collection of cotton insects at Australian Cotton Research Institute Narrabri or sent for identification to the Australian National Insect Collection, Commonwealth Scientific and Industrial Research Organisation Entomology, Canberra.

**Statistical Analysis:** To compare the biodiversity of the Bt and conventional communities, two different indices were used to analyse the data. The two indices used were The Simpson Index and the Shannon Weaver Index. The Simpson index is more sensitive to dominant species in the dataset, whereas the Shannon Weaver Index is more sensitive to rare species. The function of these indices is to measure species diversity (species richness and abundance or population number). Rarefaction curves are another way of measuring biodiversity; it also measures species richness and abundance. The data was then examined using principle response curves (PCR- figure 1) which display differences between the communities of the different crop types (treatments) over time. The significance of the community differences shown in the PRCs was tested using the Monte Carlo permutation test.




This PRC displays arthropod communities found in different crops (treatments) across time. The closer the points are to each other at any date, the more similar the communities. The diagram shows that from the 13th of January, the sprayed community was very different from the others.

**Key:**

Conventional unsprayed = white circle (Control)

Conventional sprayed = black circle

Ingard *Bt*. = Black square

Stacked *Bt*= grey triangle

**Figure 1: An example of a principle response curve used in the statistical analysis.**

**Results:**

**Community differences in *Bt.* and conventional Cotton**: The results indicated that all sites had significant differences in their communities between crop types i.e. unsprayed conventional, Bollgard II etc. To test whether the arthropod communities in Ingard and Stacked Bt cotton differed significantly from conventional cotton, the sprayed treatment was removed from the analysis. Removing the sprayed treatment revealed significant differences between the communities of the crop types in three of the four sites tested, confirming that the arthropod communities varied slightly (c.4.5%) but significantly between unsprayed *Bt.* and unsprayed conventional crops.

**Are any species more affiliated with *Bt.* or conventional cotton**: The results indicated that the make-up of the communities in Bt and conventional cotton was slightly different. For example, there are fewer lepidopteron (caterpillars) in Bt cotton; and fewer Drosophila and Chloropidae (Frit flies), that usually feed on frass or decaying matter.

**Abundance, diversity and species richness of beneficial arthropods**: The results found that there was no consistent difference in the diversity of Bt and conventional cotton communities over the four sites.

**Discussion:** Insecticide sprays caused the greatest change in arthropod communities. *Bt c*otton caused less changes in arthropod communities. By using *Bt*, there was a 56% reduction in chemical applied and 50% reduction in active ingredient (the chemical/ingredient that affects the insect) applied by the cotton industry.

It is imperative to have a thorough understanding of the changes *Bt.* cotton could bring about to arthropod communities and be able to alter management practices if needed.

The results showed that there was only a subtle shift in the arthropod communities between *Bt* and conventional cotton, with some of this shift due to the reduction in the lepidopteron species which are the species that *Bt* toxin target. The analysis showed there was no significant or consistent effects that would warrant a different approach to pest management

**Further Information:** This paper is a rewrite of the original paper by Cotton Australia for schools. It has been rewritten with the consent of the authors. For the full and original report see 'Whitehouse Et. Al.: Arthropod Communities in Bt Cotton in Australia, 2005, Environmental Entomology; 34:1224-1241 which can be found at http://www.biosicherheit.de/pdf/aktuell/davos_bt-cotton-australia.pdf. A short version of this report was published in *Outlooks on Pest Management*, October 2005. This article is reproduced below by permission of Research Information Ltd. See website www.pestoutlook.com"

# COMPARING INVERTEBRATE COMMUNITIES IN TRANSGENIC BT AND CONVENTIONAL COTTON

Louise Lawrence[a], Mary Whitehouse[a], Lewis Wilson[b] and Gary Fitt[a], Australian Cotton CRC, Narrabri compare and contrast the beneficial arthropod populations found in GM and conventional cotton
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**Keywords**
Bt cotton, transgenic, invertebrate, beneficials, parasitoids, communities, conventional

Bt has been used against *Helicoverpa* in cotton for over 30 years, first as a spray and now in transgenic Bt cottons. It is useful in Integrated Pest Management (IPM) strategies because it affects only lepidopteran (moth) pests, does not harm predatory insects and spiders (hereafter referred to as "beneficials") or the environment and it does not poison mammals.

## Bt cotton is more useful in IPM than Bt sprays

As a spray, Bt had a major drawback. Its efficacy was not as good as conventional insecticides and so it required careful and well-timed applications to be useful in IPM. Bt sprays break down quickly in sunlight, and coverage can be variable. By contrast, the Bt in Bt cotton is present throughout the growing season. The new generation of two-gene Bt cottons provide consistent control of *Helicoverpa* which has lead to further dramatic reductions in the use of chemical sprays. This has made these cottons a valuable platform for IPM as very few insecticides are applied to them for the control of *Helicoverpa*, allowing beneficials to contribute to the control of a range of other pests.

However, because of the differences between Bt sprays and Bt expressed by plants, there may have been unknown side effects of transgenic Bt cotton on beneficials, or indeed the whole invertebrate community which includes all insects and spiders. Obviously Bt cotton will affect populations of *Helicoverpa* larvae directly – that is what they are meant to do – and this will indirectly affect predators and parasites that are specialist feeders on these larvae. But it was not clear whether such indirect effects extend to other non-target species.

## Bt cotton has little effect on non-target organisms

As part of a comprehensive environmental impact assessment of Bt cottons conducted prior to the commercial release of Bt cotton, scientists from CSIRO looked for effects on the total invertebrate community in cotton growing areas of northern NSW. Over three seasons and on three commercial cotton farms, more than 100 species groups in the canopies of different types of cotton crops were studied. At each site there were three or four treatments:

***Cheiracanthium* species eating a mirid. In Australia, these spiders are known as "yellow night stalkers" while in the UK they are called "sac spiders"**

- sprayed conventional cotton
- unsprayed conventional cotton
- unsprayed Bt cotton (Ingard®) and/or unsprayed two Bt cotton (a forerunner of Bollgard®II)

Invertebrates in the crop canopy were sampled using a suction sampler and sampling began at seedling emergence and continued until about 20% of the bolls had opened. Collected samples were taken back to the laboratory where they were killed and counted under a dissecting microscope. All the experiments involved fertilised, irrigated cotton grown on beds 1m apart with agronomic practices which followed commercial "best practice".

The following questions were asked:

- Does the overall community structure differ between Bt and conventional cotton?
- Are there species groups or individual species that are more associated with Bt or conventional cotton?

There were slight but significant differences in the whole community. Statistical methods which reveal relationships within communities indicated that seasonal changes accounted

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**Table 1. Insects and spiders whose numbers are lower in unsprayed Bt cotton compared to unsprayed conventional cotton. Only *Helicoverpa* is strongly affected, the others are only slightly lower (see Fig. 1) in Bt cotton.**

| Common name | Role in cotton |
|---|---|
| *Helicoverpa* larvae | Major pest damaging all parts of the cotton plant |
| Jassids or leafhopper | Pest which sucks sap. Unknown whether jassids cause economic damage in cotton. Jassids can transmit diseases. |
| Damsel bugs | Predator in cotton. |
| Frit flies | None are known to be pests in cotton. Maggots feed on a range of material, including bacteria, insect eggs, plant material. |
| Fruit flies | Not a pest in cotton. Maggots attack fruit in other crops. |
| Spiders | Predators in cotton. |

for 43% to 60% of the community variability. Crop type (i.e. whether the crop was sprayed, Bt or conventional) accounted for 9% to 17% of the variability. If sprayed plots were discounted, then whether the crop type was Bt or conventional accounted for only 4% to 6% of the variability in the community.

As in overseas trials, no difference was found in the diversity or species richness of beneficials in the unsprayed Bt and conventional crop types. However, there were differences in diversity between unsprayed and sprayed crops, as would be expected.

Some difference between the insects and spiders found in unsprayed conventional and Bt cotton is to be expected given that the number of moth larvae has been greatly reduced in Bt cotton. It is possible that lower numbers of parasitoids or predators which specialize on larvae could be causing this difference.

However, there were no consistent differences between the number of egg and larval parasitoids of moths throughout the season, although there were slightly lower numbers of beneficial and pest bugs (Hemiptera) in Bt cottons in comparison to unsprayed conventional cotton. These bugs include damsel bugs and jassids (leafhoppers) both of which were in lower numbers in Bt cotton and may have driven this effect (Fig.1).

Observations in commercially grown Bt cotton crops in Australia have also shown lower numbers of damsel bugs compared to conventional crops (M. Dillon, CSIRO Entomology, unpublished data). Why there should be lower numbers of damsel bugs is unclear. It may be that they are more dependent on moth larvae for food than was thought, which could partially explain their reduced abundance. Likewise the drop in larvae numbers may also account for the slight drop in spider numbers in Bt crops (Fig.1).

Jassid densities were slightly lower in Bt compared to unsprayed conventional cotton (Fig. 1). As jassids are sometimes considered a pest, this could be a bonus for growers.

The numbers of two groups of small flies, frit flies and fruit flies, were lower in Bt cotton compared to unsprayed conventional cotton. Why is unclear. The Bt cottons used in this study produced Bt proteins which are specifically toxic to moths, and it is unlikely that the Bt protein in cotton had a direct effect on these flies.

The role of frit flies in cotton is unclear. Larvae of this family are reported to feed on a diverse range of organisms, including bacteria, vegetative matter (both living and rotting), the eggs of other insects and spiders, beneath the skins of living frogs and as parasites of wasps and bees. One species is a pest of wheat in Europe. As frit flies do not appear to be pests or beneficials in cotton, their role from an IPM perspective is probably limited to providing an alternative food source for some predators.

## Implications for cotton management

The greatest influences on invertebrate communities in cotton are insecticide sprays, and the advent of Bt cotton has seen a large drop in insecticide applications (56% in Ingard® and 86% in Bollgard®II). Nevertheless, when managing Bt cotton it is important to understand how the dynamics of pest and beneficial species may be affected so that management practices can be adjusted if necessary.

The results of this research indicated only a subtle shift in the invertebrate community between Bt and conventional cotton, some of which was probably driven by the reduction in *Helicoverpa* and other moths. There was no indication of changes in key species which would warrant a different pest management approach.

This research highlights the value of pre-emptively looking at issues such as non-target effects for these new technologies. Such studies of non-target impacts will be important for any new transgenics. The uptake of Bt cotton has led to dramatic reductions in insecticide use. As the area of Bt-cotton increases (it is estimated to be about 80% for 2005–06) the effect of reduced insecticide use and subtle non-target effects may necessitate changes to current pest management strategies. Research is under way to consider these issues.

## Acknowledgements

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Similar articles that appeared in *Outlooks on Pest Management* include: 2004 **15(5)** 215; **15(6)** 283; 2005 **16(4)** 164

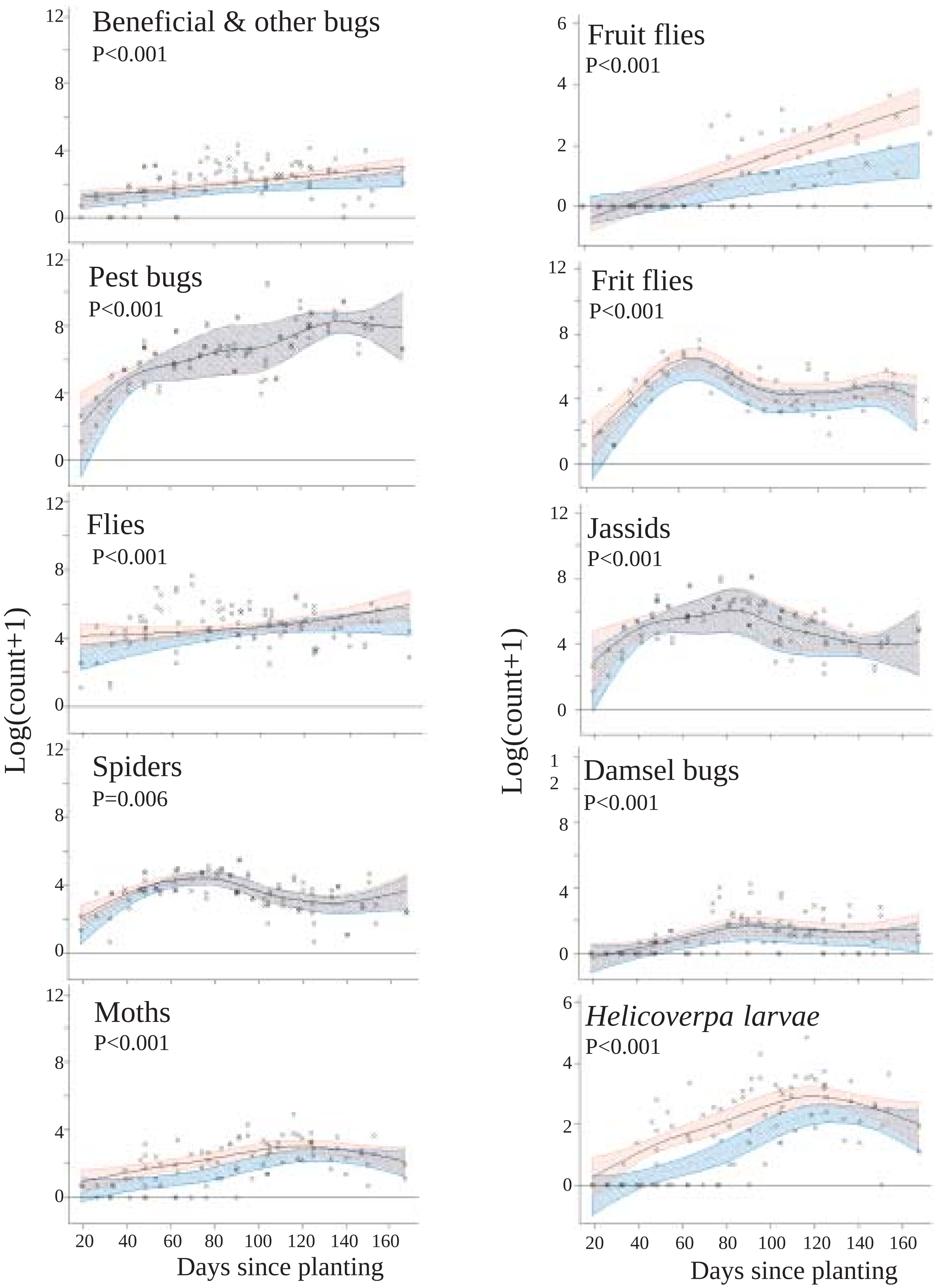


Figure 1. **The relative abundance of general groups (left) and specific insects (right) in Bt and unsprayed conventional cotton. Each diagram is the result of an analysis which combines the results from all four sites, and then plots the species overall abundance during the course of a season. Only groups in which there was a significant difference between Bt and conventional cotton are presented (the smaller the P value, the greater the significance). Blue shading indicates the species distribution in Bt cotton; red shading indicates the species distribution in conventional cotton. Overlap between the shading for Bt and conventional indicates similar relative abundance, whole separation of shading indicate less similar abundance. Crosses are conventional data points, and circles are Bt data points.**