

# Cotton

RESEARCH & DEVELOPMENT

## UTILISING PARASITIDS IN SOUTH QUEENSLAND COTTON

CRDC Project DAQ 125C  
*(July 2002 to June 2003)*

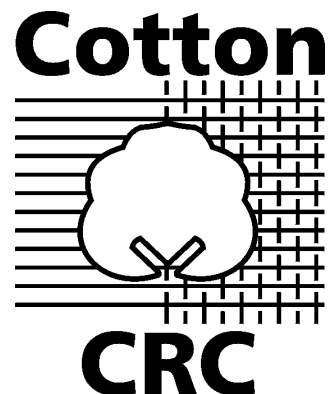
### Final Report

Brad Scholz

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**Queensland  
Government**  
Department of  
Primary Industries



# Utilising parasitoids in south Queensland cotton.

## Plain English Summary

The research focussed on evaluating the impact of the egg parasitoid *Trichogramma pretiosum* against heliothis on the Darling Downs. *Trichogramma* are small wasps that attack the egg stage of heliothis. They are excellent biological control agents because they kill heliothis before they hatch and start feeding. *T. pretiosum* is native to North America, and was established in the Ord River region of Western Australia during the 1970's. It was released on the Darling Downs in 1995. Here we report on its distribution over the Darling Downs, and the levels of heliothis egg mortality it caused in cotton and sorghum.

Field collections of heliothis eggs showed that *T. pretiosum* is widespread and abundant over the Darling Downs. *T. pretiosum* was also collected from sorghum at Goondiwindi, and identified in parasitised eggs that we were sent from Wee Waa and Gunnedah. The levels of heliothis egg parasitism were high in sorghum (80-90%) from the beginning of February until the end of April 2003. The main species of egg parasitoid collected was *Trichogramma pretiosum*, accounting for 99% of all eggs parasitised. This species is spreading and will increasingly impact on heliothis. It is likely to thrive in IPM cotton systems where *B.t.* crops and increasing use of selective insecticides reduces the need for broad spectrum insecticides that have previously had an adverse impact on *Trichogramma*.

We also recorded the levels of heliothis egg parasitism in irrigated cotton at Branchview on the Darling Downs. The levels of heliothis egg parasitism rose from 20% in December to 96% in February in irrigated conventional cotton at Branchview. The findings suggest that *Trichogramma* have an important role to play in managing heliothis in conventional cotton. High egg densities of 60 eggs/m were recorded in some blocks, however the same blocks had up to 96% egg parasitism. This highlights the value of understanding the mortality that *Trichogramma* can cause in cotton, and also the importance of not spraying heliothis based on egg densities alone.

We evaluated a DNA diagnostic test for detecting egg parasitism, and found a correlation between the DNA technique and standard developmental techniques. The DNA test consistently underestimated the levels of parasitism, with the average DNA estimate of parasitism being 74% that of the developmental standard. The DNA test has potential to estimate the levels of egg parasitism without having to wait for developmental changes, provided eggs can be collected and delivered for testing quickly.

# Utilising parasitoids in south Queensland cotton.

## Synopsis

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## Background

The project focussed on understanding more about the egg parasitoid *Trichogramma pretiosum*. This minute wasp attacks the egg stage of heliothis (*Helicoverpa* spp.) and causes significant mortality of heliothis on the Darling Downs.

*T. pretiosum* is a new species to the Darling Downs. It is native to North America, but was established in the Ord River region of Western Australia in the mid 1970's. The DPI obtained some *T. pretiosum* from scientists in Western Australia and released it onto the Darling Downs in 1995. It appears to thrive in agricultural environments, especially when selective pest management tactics are employed, e.g. *B.t.* cottons and the use of selective insecticides.

There is increasing grower interest in utilising beneficial insects and spiders in pest management, and thereby reducing the need to spray pests with insecticides. Cotton growers on the Darling Downs are interested in learning more about *Trichogramma* because of the high impact the wasps have had in dryland cotton. In particular, leading consultants and growers want to know if *T. pretiosum* can be utilised in irrigated cotton and conventional cotton.

The project built on the findings of previous research where *T. pretiosum* successfully managed heliothis in unsprayed dryland INGARD® cotton for three consecutive years, including a high pest pressure year when heliothis egg densities rose to 90 eggs/m. Previous DPI research found that *T. pretiosum* is a significant natural enemy of heliothis (DAQ 96C –“IPM in dryland cotton on the Darling Downs’). This work was carried out in dryland INGARD® cotton at Jimbour on the Darling Downs. The research reported here was conducted in other regions, and included trials in irrigated conventional cotton.

## Objectives

The primary objectives of the research were to:

- Record the distribution of *T. pretiosum* in other regions of the Darling Downs.
- Assess the impact of *T. pretiosum* against heliothis in irrigated conventional cotton.
- Evaluate sorghum as a nursery for *Trichogramma* and other natural enemies.
- Evaluate different release rates of *T. pretiosum* in sorghum and cotton.
- Evaluate the impact of paraffin oils on *T. pretiosum*.
- Validate a DNA test for assessing the levels of egg parasitism.
- Study aspects of the biology of *T. pretiosum*.

All of these objectives were met.

## Project Duration

Commencement Date: 1 July 2002.

End Date: 30 June 2003.

## Relevance to CRDC Outputs

The project addresses the ongoing output of “Sustainability of Natural Resources” by placing increasing emphasis on understanding and utilising beneficials in pest management, and reducing the need to use chemical sprays.

## Intellectual Property

There were no intellectual property rights associated with this project.

## Research Methods

Details of all of the research conducted in project DAQ125C, including the methodologies, are provided in the technical papers. The following key developments in scientific methodology were developed in the project:

- A protocol for evaluating the impact different release rates of *Trichogramma* against heliothis was developed (technical paper 2).
- Techniques for evaluating the impact of insecticides on *Trichogramma* were refined (technical paper 6).
- A DNA diagnostic test for detecting egg parasitism was validated (technical paper 7).

## Key Findings

### **The value of intercropping sorghum with cotton**

The impact of growing sorghum intercropped with cotton on heliothis egg parasitism and predator abundance was evaluated at Evanslea on the Darling Downs. A 7 ha block of sorghum was grown between two 13 ha blocks of conventional cotton. We found that intercropping cotton with sorghum encouraged the establishment and population growth of *Trichogramma* and two species of predatory beetles in the cotton.

Higher levels of heliothis egg parasitism were found in cotton adjacent to sorghum where *Trichogramma* were released, than in cotton adjacent to control sorghum. These differences were detected approximately one generation after the *Trichogramma* were released, suggesting that inoculative releases of wasps can supplement native populations, and enhance the overall impact of *Trichogramma* on heliothis.

Predatory beetles were more abundant in sorghum than adjacent cotton. Red and blue beetles and the white collared ladybird beetles were the most abundant predatory species found. Their abundance in cotton was greater downwind of intercropped sorghum than it was in upwind cotton, suggesting that there was some movement from the sorghum into the cotton.

### **Evaluating different release rates of *Trichogramma* against heliothis**

We evaluated different release rates of *Trichogramma pretiosum* against heliothis in sorghum and cotton. Releasing *T. pretiosum* increased the levels of heliothis egg parasitism in sorghum and cotton by 6-27%. Such increases are difficult to justify as an inundative tactic, but may have merit as an inoculative or supplementative approach.

The levels of egg parasitism achieved with low level release rates (ca. 30,000 wasps per hectare) were comparable with medium release rates (ca. 60,000 wasps/ha), and are worthy of additional investigation because of the associated cost saving.

High background levels of egg parasitism (66-70%) were found in sorghum in December. The activity of natural populations of egg parasitoids should be assessed before purchasing and releasing *Trichogramma*. The benefit obtained from releasing wasps may be small, and the impact of the natural populations may be sufficient to manage heliothis in some circumstances.

### **Natural levels of egg parasitism in irrigated cotton**

The levels of heliothis (*Helicoverpa* spp.) egg parasitism were recorded in irrigated cotton at Branchview on the Darling Downs to assess the distribution and impact of natural populations of egg parasitoids.

The levels of heliothis egg parasitism rose from 20% in December to 96% in February in irrigated conventional cotton at Branchview. A selective insecticide regime was used to manage pests, with the conventional cotton receiving 4-7 sprays for the season. The findings suggest that *Trichogramma* have an important role to play in managing heliothis in conventional cotton. High egg densities of 60 eggs/m were recorded in some blocks, however the same blocks had up to 96% egg parasitism. This highlights the value of understanding the mortality that *Trichogramma* can cause in cotton, and also the importance of not spraying heliothis based on egg densities alone.

The majority of egg parasitoids collected were *Trichogramma pretiosum* (99%). This species was introduced to the Darling Downs in 1995 and now seems widespread and abundant in agricultural environments.

The levels of heliothis egg parasitism were monitored in two adjacent 25 ha blocks of Sicot 80 cotton during January and February 2003. The blocks were managed using the softest possible options for heliothis management. One block used standard IPM spray recommendations employed throughout the farm, and the other was managed with a 'soft' approach, i.e. sprays were used as a last resort. At the end of the season the Standard and Soft blocks had received 5 and 4 sprays respectively, with no significant difference in yields (5.6 and 5.9 b/ha respectively).

### **Natural levels of egg parasitism in sorghum**

The levels of heliothis (*Helicoverpa armigera*) egg parasitism were recorded in sorghum at various locations on the Darling Downs to assess the distribution and impact of the egg parasitoid *Trichogramma pretiosum* that was introduced in 1995.

The levels of heliothis egg parasitism were high (80-90%) in sorghum across the Darling Downs from the beginning of February until the end of April 2003. The main species of egg parasitoid collected was *Trichogramma pretiosum*, accounting for 99% of all eggs parasitised. This exotic species is spreading and should be monitored in other cotton growing districts. The surveys were conducted in fields where *Trichogramma* had not been released, i.e. they reflected the activity of natural populations of wasps.

### **Parasitism and progeny production of *Trichogramma pretiosum***

The lifespan, parasitism levels and progeny production of *Trichogramma pretiosum* reared on *Helicoverpa armigera* eggs was studied in the laboratory. The data will be useful in population models to simulate the impact of *Trichogramma* on heliothis.

We found that *Trichogramma pretiosum* parasitised an average of 58 *Helicoverpa armigera* eggs per lifetime in the laboratory. Approximately 10 eggs were parasitised on the first day of an adult wasp's life, and female wasps lived for approximately 10 days.

*T. pretiosum* progeny production is female biased, with over 70% of offspring produced being females. This is a favourable trait for a biological control agent because only female wasps attack eggs. Each female wasp produced 51 female offspring in the laboratory.

The rapid generation time of *T. pretiosum* (8-10 days in summer) is undoubtedly important in rapid population growth, and probably explains how field populations of *Trichogramma* can develop quickly throughout the cotton season. If a female wasp always produced 51 female offspring, then one female wasp could theoretically produce 6.7 million female offspring in four generations (approx. 40 days in summer).

### **The effect of paraffin spray oils on *Trichogramma***

Paraffin oils are currently being explored as an option to control heliothis in cotton as they are comparatively softer on beneficial insects than some other insecticides. We evaluated the impact of two paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on *Trichogramma pretiosum* during larval, pupal and adult stages of development.

We found that sprayed paraffin oils were reasonably safe on *Trichogramma* at the rates and volumes likely to be used in cotton. However, both of the paraffin oils tested caused considerable mortality of developing *Trichogramma* when parasitised eggs were immersed in oil solutions for one second. This may be an issue in high volume spray applications, e.g. horticulture where 500-800 L/ha spray volumes are used, or in situations where good target coverage is achieved.

## **Validation of a DNA technique for assessing heliothis egg parasitism**

The Centre for Insect Diagnostics (CID), University of Queensland, has been developing a DNA diagnostic test for assessing the levels of heliothis egg parasitism. It is also possible to identify eggs to species, i.e. *H. armigera* or *H. punctigera*. These tests can be completed in four hours and have the potential to more rapidly assess egg parasitism levels than standard developmental methods.

We compared the levels of egg parasitism in paired egg collections from cotton and sorghum on the Darling Downs. For each paired collection a sample of eggs was sent to the CID for DNA diagnostic analyses of egg parasitism, and a matching sample was held at the DPI Toowoomba Entomology laboratories for standard developmental assessments of egg parasitism.

*Trichogramma pretiosum* parasitism was readily detected using the CID DNA test, and a correlation was found between the two techniques. The DNA test consistently underestimated the levels of parasitism, with the average DNA estimate of parasitism being 74% that of the developmental standard.

The DNA test has potential to estimate the levels of egg parasitism without having to wait for developmental changes, provided eggs can be collected and delivered for testing quickly. In addition, unparasitised eggs can be identified to species, i.e. *H. armigera* or *H. punctigera*, and this would be useful for managing insecticide resistance in cotton.

## **Implications for Future Work**

The research presented here highlights the significant impact that *Trichogramma pretiosum* is having on heliothis on the Darling Downs. We also collected *T. pretiosum* from sorghum at Goondiwindi, and were sent eggs parasitised by *T. pretiosum* from Wee Waa and Gunnedah. This species is now widespread and abundant during the cotton growing season, and can cause significant mortality of heliothis. A beneficial insect that can cause 90-100% mortality of heliothis eggs is something that should be treasured in an IPM program. This is especially the case when the beneficial insect builds up naturally in agricultural environments, as is the case with *T. pretiosum*.

*T. pretiosum* is a free natural resource. Farmers do not have to release wasps to obtain a benefit – there is much to be gained by incorporating the natural heliothis egg mortality caused by wild populations egg parasitoids into pest management decisions. Despite this, cotton consultants often overlook the impact of natural populations of *Trichogramma* when making spray decisions.

Additional effort should be made to utilise natural populations of egg parasitoids that build up as the season progresses. This is particularly the case for the Darling Downs where we have consistently found *Trichogramma* causing 90-100% mortality of heliothis eggs during January and February for the past four seasons. It is important to understand this pattern and be aware of *Trichogramma* in stages two and three of the cotton season. Historically broad spectrum insecticides have been used during these stages, but this may not be necessary when *Trichogramma* are active. The traditional IPM philosophy of ‘go soft early’ to conserve beneficials must now become ‘go soft for as long as you can’, as this will ensure that the natural populations *Trichogramma* can realise their potential in January-February.

It is likely that many cotton consultants are not aware of the impact of *Trichogramma* because they do not assess the levels of egg parasitism, or they don’t appreciate some of the trends in their pest count data. For example, when *Trichogramma* are active high egg counts do not result in high larval counts because egg mortality is high. It is important to take this into consideration before making a spray decision.

The research reported here has shown that *T. pretiosum* is prevalent in irrigated cotton in the heart of the Darling Downs. We are keen to explore the role of *Trichogramma* in irrigated cotton and conventional cotton further, because we believe there is scope to reduce the numbers of heliothis sprays when *Trichogramma* are active.

The trend over the past four seasons has been that populations of *Trichogramma* explode in January and February. Ideally it would be advantageous to have *Trichogramma* active earlier in the cotton season, e.g. during December. There are two possibilities for achieving this that deserve additional research, i.e.

1. Develop farming practices that utilise other crops and/or habitats as breeding sources for *Trichogramma* that are adjacent to, or near, cotton. This includes designing specific farmscapes that support populations of beneficial insects, including *Trichogramma*; or
2. Evaluate inoculative and supplemental releases of *Trichogramma* into cotton, or other crops (e.g. sorghum or maize), to boost the natural populations of wasps early in the season.

*Trichogramma* are very small wasps (approx. 0.5 mm long) and are difficult to study in the field. However, additional ecological and population dynamics studies would assist in understanding their role in the regional management of heliothis in the cotton farming system.

## Publications

- Cleary, A. and Scholz, B. (2002). Evaluating the toxicity of insecticides on immature and adult *Trichogramma pretiosum*. *Proceedings of the Eleventh Australian Cotton Conference*, Brisbane, Queensland, 13-15 August, 2002. pp. 251-257.
- Scholz, B., Parker, N. and Lloyd, R. (2002). An evaluation of unsprayed INGARD strips as nurseries for beneficials in dryland cotton on the Darling Downs. *Proceedings of the Eleventh Australian Cotton Conference*, Brisbane, Queensland, 13-15 August, 2002. pp. 297-306.
- Scholz, B., Cleary, A. and Lloyd, R. (2002). The value of unsprayed strip crops as nurseries for beneficials in dryland cotton on the Darling Downs. *Proceedings of the Eleventh Australian Cotton Conference*, Brisbane, Queensland, 13-15 August, 2002. pp. 307-314.
- Parker, N., Scholz, B., and Lloyd, R. (2002). A survey of predatory arthropods in a range of dryland refuge crops on the Darling Downs. *Proceedings of the Eleventh Australian Cotton Conference*, Brisbane, Queensland, 13-15 August, 2002. pp. 315-320.
- Lloyd, R., Parker, N., Scholz, B., Hopkinson, J. and Wade, M. (2002). The efficacy of AMINO-FEED UV® in manipulating beneficial abundance in dryland cotton. *Proceedings of the Eleventh Australian Cotton Conference*, Brisbane, Queensland, 13-15 August, 2002. pp. 353-357.

## In Press

- Scholz, B. and Parker, N. (2004). The nursery value of sorghum intercropped with cotton – the effects on heliothis egg parasitism and predator abundance in cotton. *Proceedings of the Twelfth Australian Cotton Conference*, Broadbeach, Queensland, 10-12 August, 2004.
- Scholz, B. and Parker, N. (2004). Evaluations of different release rates of *Trichogramma pretiosum* against *Helicoverpa armigera* eggs in sorghum and cotton. *Proceedings of the Twelfth Australian Cotton Conference*, Broadbeach, Queensland, 10-12 August, 2004.
- Scholz, B. and Parker, N. (2004). Lifespan, parasitism levels and progeny production of *Trichogramma pretiosum* reared on *Helicoverpa armigera* eggs in the laboratory. *Proceedings of the Twelfth Australian Cotton Conference*, Broadbeach, Queensland, 10-12 August, 2004.
- Parker, N., Scholz, B. and Mensah, R. (2004). An evaluation of the toxicity of two paraffin oils (Biopest® and Canopy®) on *Trichogramma pretiosum*. *Proceedings of the Twelfth Australian Cotton Conference*, Broadbeach, Queensland, 10-12 August, 2004.

## Presentations at Meetings by Brad Scholz (since July 2002)

1. **9-10 July 2002 – Emerald:** I gave a presentation on “Know your Beneficials” at Bill Dalton’s *Cotton IPM Short Course* workshop at the Emerald golf club.
2. **23 July 2002 – Goondiwindi:** I gave a presentation on “Know your Beneficials” at Bill Dalton’s *Cotton IPM Short Course* workshop at the Goondiwindi DPI complex.
3. **19 September 2002 - Dalby:** I spoke at the IPM Update, held at the Dalby RSL club. My talk was “*Trichogramma*: Small wasps that pack a big punch”.
4. **31 October 2002 – St. George:** I gave a presentation on “Know your Beneficials” at Mark Hickman’s *Cotton IPM Short Course* workshop at the St. George RSL club.
5. **18 November 2002 - Evanslea:** I spoke at the Evanslea area wide management meeting on our work with *Trichogramma*.
6. **20 November 2002 - Evanslea:** I spoke at the Cotton CRC Extension Technical Updates at Neil Nass’s farm (Evanslea).
7. **5 February 2003 – Branchview:** I spoke at a trial update day at the Matilda shed on the egg parasitism data that we found at the Branchview study site.
8. **5 February 2003 - Evanslea:** I spoke at the Evanslea area wide management meeting on our work at Neil Nass’ farm. This was a field walk and beneficial identification exercise in sorghum, cotton and lucerne.
9. **12 March 2003 – Condamine Plains:** I spoke at Harley Bligh’s with Matthew Holding on our Branchview egg parasitism results, and my concept for farmscapes.
10. **5 April 2003 – Kogan:** I spoke about my beneficial research, and identified some insects, at a landcare meeting at ‘Kia-Ora’, Kogan, at the invite of Sharon Lohse.
11. **25-26 June 2003 – Toowoomba:** I spoke about *Trichogramma* at the Farming Systems IPM Forum, held at the DPI conference centre in Toowoomba.
12. **15 July 2003 – Jimbour:** I attended an IPM meeting at St. John Kent’s, Jimbour. The meeting was organised by Austin McLennan and Melina Miles, and was attended by Dave Murray, St. John Kent, David Alexander and John Fuelling.
13. **23-24 July 2003 – Armidale:** I presented a paper at the Cotton CRC review titled “The role of natural enemies in dryland cotton IPM.”
14. **31 July 2003 – Evanslea:** I attended the Evanslea IPM group meeting and spoke briefly about our findings from the previous season, and proposed work under my new project.
15. **11 August 2003 – Jimbour:** I attended a meeting with St. John Kent to discuss trial plans at his farm for the 2003/04 season. Bernie Franzmann, Nat Parker and Lawry Smith also attended.
16. **14 August 2003 – St George:** I gave a presentation on “Know your Beneficials” at Mark Hickman’s *Cotton IPM Short Course* workshop at the St. George RSL club.
17. **27 August 2003 – Narrabri:** I gave a presentation called “*Trichogramma*: Small wasps that pack a big punch” at the CCA Cotton Production Seminar at the Narrabri Crossing Theatre.

## DAQ 125C - Technical Papers

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# **1. The nursery value of sorghum intercropped with cotton – the effects on heliothis egg parasitism and predator abundance in cotton.**

Brad Scholz and Nathaniel Parker.

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## **Introduction**

Sorghum is extremely attractive to ovipositing heliothis (*Helicoverpa armigera*) moths. Some cotton growers on the Darling Downs are intercropping cotton with sorghum because they believe that sorghum may act as a beneficial nursery, and that the beneficials may move into the adjacent cotton.

Here we report on a trial comparing the levels of heliothis egg parasitism and predator abundance in cotton adjacent to intercropped sorghum. The heliothis egg parasitoid *Trichogramma pretiosum* was released into half of the sorghum, and the levels of egg parasitism in the adjacent cotton were compared between the release and non-release sections of the field.

## **Methods**

The trial was conducted at Evanslea on the Darling Downs at Neil Nass's property on West Prairie Road. There were 48 row-pairs of single skip cotton (Sicot 80) planted either side of 80 rows of MR43 sorghum, with one metre row spacings. The trial was 880 m long, i.e. two 12.7 ha plantings of cotton either side of a 7 ha block of sorghum. The trial was planted during the last week of November 2002. The cotton was sprayed twice up until early February, viz. Costar<sup>®</sup> on 30 December 2002 and Tracer<sup>®</sup> on 14 January 2003.

### ***Heliothis Egg Parasitism***

A pre-release collection of heliothis eggs was completed on the 17<sup>th</sup> January 2003. Pre-flowering sorghum heads were cut and individually spun into a funnel that emptied into a small 30 mL plastic diet cup. The eggs from a single sorghum head were collected into a diet cup, and 22 heads were selected at random from the sorghum. The cups were returned to the laboratory, and the eggs were isolated individually in the cells of a plastic micro-titre tray. The egg colour was recorded, i.e. white or brown, and all eggs were held in a constant temperature room at ca. 25°C and 60% R.H. until they hatched or showed signs of parasitism (turned jet black). The proportion of brown eggs parasitised, and the mean number of eggs per sorghum head, were determined.

On the 17<sup>th</sup> January 180 cardboard capsules, each containing ca. 1,000 *T. pretiosum* wasps, were released in the northern half of the sorghum. The *Trichogramma* were released in a grid pattern, viz. 30 capsules per row; 14 m apart; in every 13<sup>th</sup> row commencing from row 7, i.e. rows 7, 20, 33, 46, 59 and 72.

*Heliothis* egg cards were used to assess the levels of egg parasitism because there were few natural eggs laid throughout the trial. Adult *H. armigera* moths were placed in oviposition chambers where they laid eggs onto paper towelling. Each card was made by stapling pieces of paper towelling containing approximately 20 *H. armigera* eggs to paper strips measuring 1.5 x 7 cm. The eggs were less than 24 hours old. Each card was stapled to the upper side of a leaf at the top of a plant. The egg cards were stapled on leaves in a grid pattern, viz. 20 cards per row in row-pairs 2, 5, 10, 25, and 45 of the cotton (on each side of the sorghum), and in rows 25 and 50 of the sorghum.

The egg cards were placed in approximately the same location each week, and were used weekly for three weeks - 17 January, 24 January and 31 January 2003. The egg cards were collected after 48 hours exposure in the field, and were allowed to develop in a constant temperature room at approximately 25°C and 60% R.H.. The proportion of viable egg cards containing parasitised eggs were recorded. Egg cards that contained no eggs after collection, or cards that were comprised entirely of collapsed or shrunken eggs, were discarded when calculating the levels of parasitism.

### ***Predator Abundance***

The numbers of predators in the cotton and inter-cropped sorghum were assessed weekly over four weeks from 7-28 January 2003. A yellow beat sheet (Scholz *et al.* 2001) was used to sample the cotton in row-pairs 2, 5, 10, 25 and 45 on either side of the sorghum, and in each half of the inter-cropped sorghum. Six beat samples were taken at each site in the cotton and sorghum. Predatory beetles, bugs, ants, lacewings and spiders were counted on the beat sheets.

## **Results and Discussion**

### ***Heliothis Egg Parasitism***

There were  $0.6 \pm 0.2$  eggs/head in the sorghum, and no eggs parasitised on the 17<sup>th</sup> January, i.e. egg pressure and egg parasitism were very low prior to the release of *Trichogramma*. There were negligible levels of *Heliothis* egg parasitism in the sorghum, and only low levels of parasitism in the cotton on the 17<sup>th</sup> and 24<sup>th</sup> January (Figure 1). The greatest levels of *Heliothis* egg parasitism were found in the cotton on the 31<sup>st</sup> January.

The level of egg parasitism in the cotton adjacent to the release sorghum (21%), was significantly higher than that in the cotton adjacent to the control sorghum (9%) on the 31<sup>st</sup>

January (Table 1). There were no significant differences in the levels of egg parasitism recorded on the other dates. The 31<sup>st</sup> January was approximately one generation after the wasps were released in the sorghum. This suggests that the *Trichogramma* were able to establish on natural eggs within the field, and that their abundance rose sufficiently over one generation to have a significant impact on parasitism levels in the adjacent cotton. There was low heliothis oviposition activity throughout this study, with egg densities peaking at 0.75 eggs/m (Table 2). No field collections of naturally laid eggs were completed.

The levels of egg card parasitism in the cotton varied across the field, with the highest levels (58%) recorded on the downwind, western side of the sorghum (Figure 1). This suggests that the sorghum may have been a nursery for the *Trichogramma*, and that their dispersal was affected greatly by prevailing winds.

### ***Predator Abundance***

Throughout the study there were more predators in the sorghum than in the adjacent cotton (Figure 2). The majority of predators counted were predatory beetles (57% in cotton and 73% in sorghum). Of these, the most frequently counted species were the red and blue beetle (*Dicranolaius bellulus*), and the white collared ladybeetle (*Hippodamia variegata*). These two beetles accounted for 94% of the adult beetles in cotton, and 91% of the adult beetles in sorghum. Ladybird larvae were only found in the sorghum.

There were significantly more predators on the downwind side of the sorghum than on the upwind side on three of the sampling dates (Table 3), suggesting that the sorghum may have had a role in supplementing the abundance of predators in the cotton. There were significant differences in the abundance of the two common beetle species, *D. bellulus* and *H. variegata*, between the downwind and upwind sections of the cotton. The sorghum appeared to be very attractive to these beetles, and there seems to be some flow-on benefits to nearby cotton.

Further research is needed to more fully understand the benefits of intercropping cotton with sorghum to enhance the abundance, and impact, of predators and parasitoids. This study was conducted during low heliothis pressure. Similar work should be completed under higher pest pressure. Ideally separate fields with and without sorghum intercropped should be compared.

## Key Findings

- Intercropping cotton with sorghum encouraged the establishment and population growth of *Trichogramma* and two species of predatory beetles in the cotton.
- Higher levels of heliothis egg parasitism were found in cotton adjacent to sorghum where *Trichogramma* were released, than in cotton adjacent to control sorghum. These differences were detected ca. one generation after the *Trichogramma* were released, suggesting that inoculative releases of wasps can supplement native populations, and enhance the overall impact of *Trichogramma* on heliothis.
- Predatory beetles were more abundant in sorghum than adjacent cotton. Red and blue beetles and the white collared ladybird were the most abundant predatory species found. Their abundance in cotton was greater downwind of intercropped sorghum than it was in upwind cotton, suggesting that there was some movement from the sorghum into the cotton.

## Acknowledgements

We thank Neil Nass for cooperating in this research, and the Cotton Research and Development Corporation for funding the research (project DAQ125C). Sue Maclean (DPI Toowoomba) supplied *Helicoverpa armigera* eggs. This assistance is gratefully acknowledged.

## References

Scholz, B., Cleary, A. and Lloyd, R. (2001). Sheet unbeatable for sampling predators in cotton. *Australian Cottongrower* 22(5): 14-17.

**TABLE 1**

The levels of heliothis egg card parasitism in conventional cotton intercropped with sorghum. *Trichogramma pretiosum* were released into half of the sorghum on 17 January 2003, and the levels of egg parasitism in the adjacent cotton were assessed in row-pairs 2, 5, 10, 25 and 45 on either side of the sorghum. The values represent the mean  $\pm$  standard error of parasitism levels assessed in ten row-pairs of cotton (20 egg cards per row-pair). Means for a given date followed by the same letter are not significantly different (Unpaired t-test, P = 0.05). Control = no *Trichogramma* released into sorghum; Release = *Trichogramma* released into sorghum.

<b>Date</b>	<b>Control Cotton</b>	<b>Release Cotton</b>
<b>17 January 2003</b>	2.5 $\pm$ 1.4 a	4.5 $\pm$ 2.0 a
<b>24 January 2003</b>	1.3 $\pm$ 0.9 a	1.3 $\pm$ 0.8 a
<b>31 January 2003</b>	8.8 $\pm$ 2.6 a	20.7 $\pm$ 4.8 b

**TABLE 2**

Mean heliothis egg densities per metre in cotton intercropped with sorghum at Evanslea.

<b>Date</b>	<b>White Eggs</b>	<b>Brown Eggs</b>	<b>Total Eggs</b>
<b>7 January 2003</b>	0	0.08	0.08
<b>13 January 2003</b>	0.25	0.25	0.50
<b>21 January 2003</b>	0.17	0.08	0.25
<b>28 January 2003</b>	0.25	0.50	0.75

**TABLE 3**

The total numbers of predators in cotton, downwind and upwind of intercropped sorghum. Values are the mean  $\pm$  standard error of 30 beat sheet samples. Means for a given date followed by the same letter are not significantly different (Un-paired t-test, P = 0.05).

<b>Date</b>	<b>Downwind (West)</b>	<b>Upwind (East)</b>
<b>7 January 2003</b>	1.00 $\pm$ 0.19 a	1.20 $\pm$ 0.18 a
<b>13 January 2003</b>	2.43 $\pm$ 0.35 a	1.43 $\pm$ 0.23 b
<b>21 January 2003</b>	2.17 $\pm$ 0.33 a	1.33 $\pm$ 0.18 b
<b>28 January 2003</b>	2.87 $\pm$ 0.37 a	1.96 $\pm$ 0.25 b

**TABLE 4**

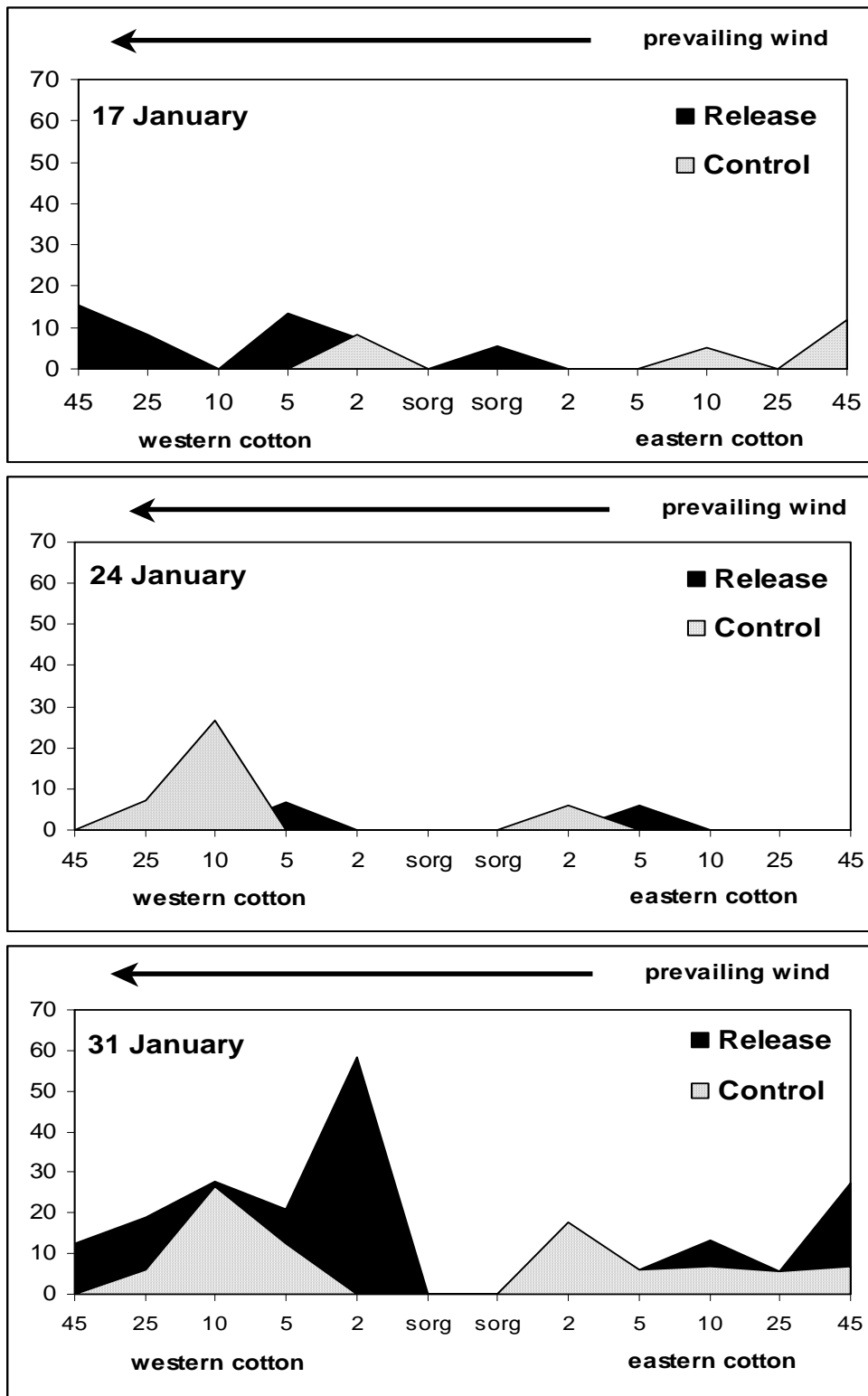
The numbers of white collared ladybeetles (*Hippodamia variegata*) in cotton, downwind and upwind of intercropped sorghum. Values are the mean  $\pm$  standard error of 30 beat sheet samples. Means for a given date followed by the same letter are not significantly different (Un-paired t-test, P = 0.05).

<b>Date</b>	<b>Downwind (West)</b>	<b>Upwind (East)</b>
<b>7 January 2003</b>	0.10 $\pm$ 0.07 a	0 a
<b>13 January 2003</b>	0.93 $\pm$ 0.22 a	0.17 $\pm$ 0.07 b
<b>21 January 2003</b>	0.33 $\pm$ 0.13 a	0.03 $\pm$ 0.03 b
<b>28 January 2003</b>	0.50 $\pm$ 0.16 a	0.38 $\pm$ 0.16 a

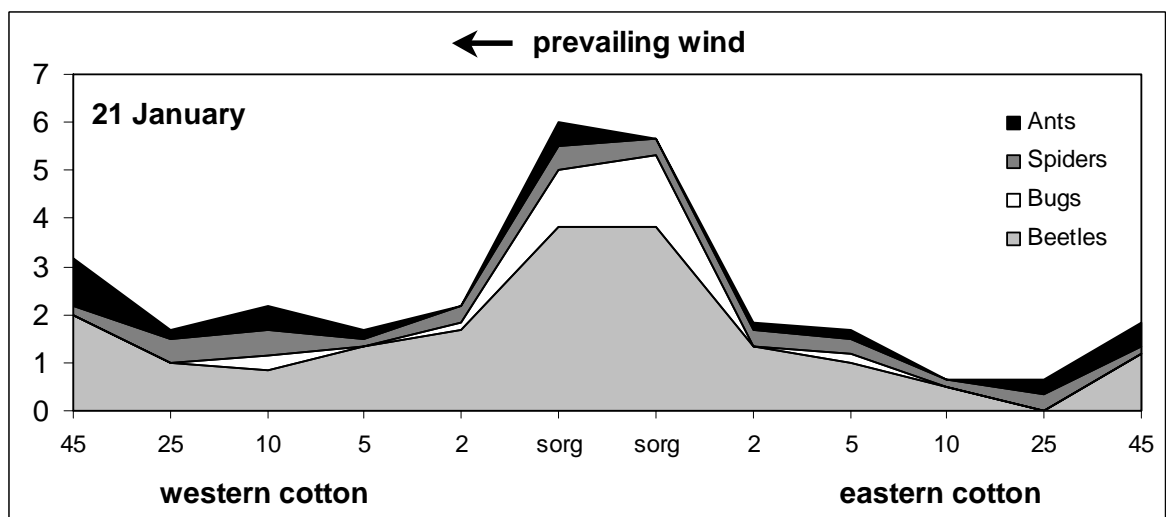
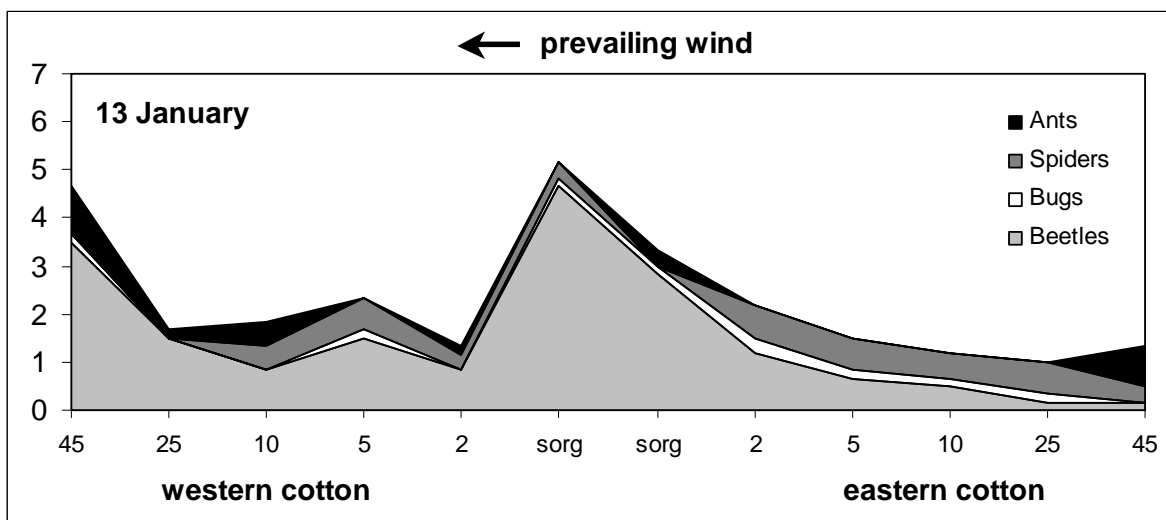
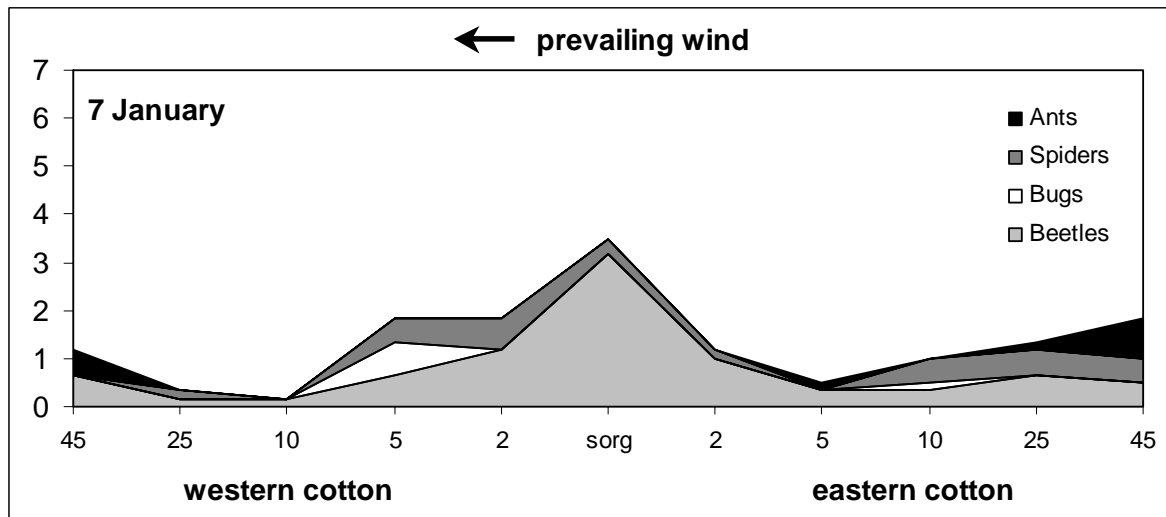
**TABLE 5**

The numbers of red and blue beetles (*Dicranolaius bellulus*) in cotton, downwind and upwind of intercropped sorghum. Values are the mean  $\pm$  standard error of 30 beat sheet samples. Means for a given date followed by the same letter are not significantly different (Un-paired t-test, P = 0.05).

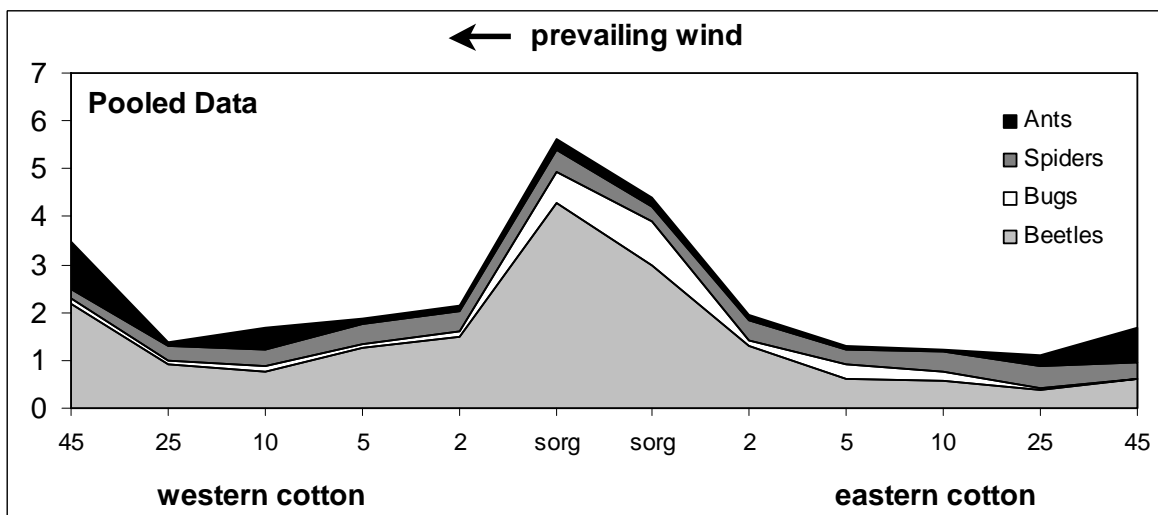
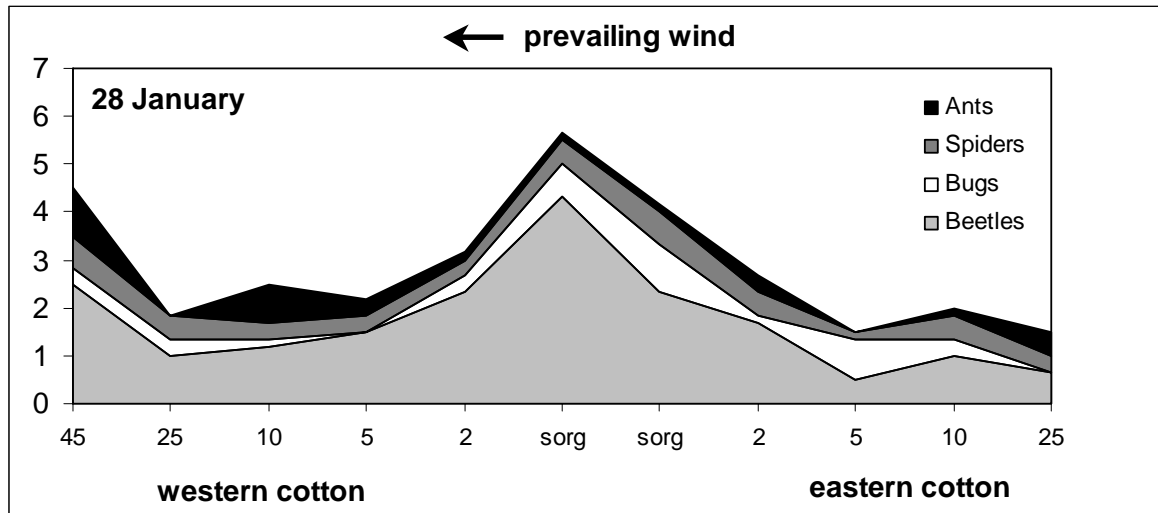
<b>Date</b>	<b>Downwind (West)</b>	<b>Upwind (East)</b>
<b>7 January 2003</b>	0.37 $\pm$ 0.10 a	0.53 $\pm$ 0.13 a
<b>13 January 2003</b>	0.63 $\pm$ 0.16 a	0.33 $\pm$ 0.10 a
<b>21 January 2003</b>	1.00 $\pm$ 0.18 a	0.67 $\pm$ 0.16 a
<b>28 January 2003</b>	1.17 $\pm$ 0.24 a	0.50 $\pm$ 0.17 b



**Figure 1:** The levels of heliothis egg parasitism in conventional cotton inter-cropped with sorghum. The values are the % of egg cards parasitised at weekly intervals following the release of *T. pretiosum* on 17 January 2003 into the northern half of the sorghum. The egg cards were placed in the cotton at regular intervals on either side of the sorghum (row-pairs 2, 5, 10, 25 and 45), and in sorghum rows 25 and 50. The release rate was 60,000 wasps/ha. The levels of egg parasitism increased approximately one generation after the release (on the 31<sup>st</sup> January) on the down wind side of the sorghum.



**Figure 2:** The numbers of predators per metre in conventional cotton inter-cropped with sorghum. The values are the means of six beat sheet samples. The beat samples were taken in the cotton at regular intervals on either side of the sorghum (row-pairs 2, 5, 10, 25 and 45), and at random in each half of the sorghum. Row-pair 45 was approximately 135 m from the edge of the sorghum.



**Figure 2 (cont.):** The numbers of predators per metre in conventional cotton intercropped with sorghum. The values are the means of six beat sheet samples. The beat samples were taken in the cotton at regular intervals on either side of the sorghum (row-pairs 2, 5, 10, 25 and 45), and at random in each half of the sorghum. Row-pair 45 was approximately 135 m from the edge of the sorghum. Pooled data are the overall means for all four sampling dates.

## APPENDIX 1

The numbers of predators per metre in conventional cotton inter-cropped with sorghum. The values are the means of six beat sheet samples. The beat samples were taken in the cotton at regular intervals on either side of the sorghum (row-pairs 2, 5, 10, 25 and 45), and at random in each half of the sorghum.

DATE	Crop	Location	Row Pair No.	Beetles	Bugs	Spiders	Lacewings	Ants	TOTAL
7-Jan-03	Cotton	west	<b>45</b>	0.667	0	0	0	0.5	1.167
7-Jan-03	Cotton	west	<b>25</b>	0.167	0	0.167	0	0	0.333
7-Jan-03	Cotton	west	<b>10</b>	0.167	0	0	0	0	0.167
7-Jan-03	Cotton	west	<b>5</b>	0.667	0.667	0.5	0.167	0	1.5
7-Jan-03	Cotton	west	<b>2</b>	1.167	0	0.667	0	0	1.833
<b>7-Jan-03</b>	<b>Sorghum</b>			<b>3.167</b>	<b>0</b>	<b>0.333</b>	<b>0</b>	<b>0</b>	<b>3.5</b>
7-Jan-03	Cotton	east	<b>2</b>	1	0	0.167	0	0	1.167
7-Jan-03	Cotton	east	<b>5</b>	0.333	0	0	0.167	0.167	0.667
7-Jan-03	Cotton	east	<b>10</b>	0.333	0.167	0.5	0	0	1
7-Jan-03	Cotton	east	<b>25</b>	0.667	0	0.5	0	0.167	1.333
7-Jan-03	Cotton	east	<b>45</b>	0.5	0	0.5	0	0.833	1.833
13-Jan-03	Cotton	west	<b>45</b>	3.5	0.167	0	0	1	4.667
13-Jan-03	Cotton	west	<b>25</b>	1.5	0	0	0.333	0.167	2
13-Jan-03	Cotton	west	<b>10</b>	0.833	0	0.5	0	0.5	1.833
13-Jan-03	Cotton	west	<b>5</b>	1.5	0.167	0.667	0	0	2.333
13-Jan-03	Cotton	west	<b>2</b>	0.833	0	0.333	0	0.167	1.333
<b>13-Jan-03</b>	<b>Sorghum</b>			<b>4.667</b>	<b>0.167</b>	<b>0.333</b>	<b>0.333</b>	<b>0</b>	<b>5.5</b>
<b>13-Jan-03</b>	<b>Sorghum</b>			<b>2.833</b>	<b>0.167</b>	<b>0</b>	<b>0.333</b>	<b>0.333</b>	<b>3.667</b>
13-Jan-03	Cotton	east	<b>2</b>	1.167	0.333	0.667	0	0	2.167
13-Jan-03	Cotton	east	<b>5</b>	0.667	0.167	0.667	0	0	1.5
13-Jan-03	Cotton	east	<b>10</b>	0.5	0.167	0.5	0	0	1.167
13-Jan-03	Cotton	east	<b>25</b>	0.167	0.167	0.667	0	0	1
13-Jan-03	Cotton	east	<b>45</b>	0.167	0	0.333	0	0.833	1.333
21-Jan-03	Cotton	west	<b>45</b>	2	0	0.167	0	1	3.167
21-Jan-03	Cotton	west	<b>25</b>	1	0	0.5	0	0.167	1.667
21-Jan-03	Cotton	west	<b>10</b>	0.833	0.333	0.5	0	0.5	2.167
21-Jan-03	Cotton	west	<b>5</b>	1.333	0	0.167	0	0.167	1.667
21-Jan-03	Cotton	west	<b>2</b>	1.667	0.167	0.333	0	0	2.167
<b>21-Jan-03</b>	<b>Sorghum</b>			<b>3.833</b>	<b>1.167</b>	<b>0.5</b>	<b>0.167</b>	<b>0.5</b>	<b>6.167</b>
<b>21-Jan-03</b>	<b>Sorghum</b>			<b>3.833</b>	<b>1.5</b>	<b>0.333</b>	<b>0</b>	<b>0</b>	<b>5.667</b>
21-Jan-03	Cotton	east	<b>2</b>	1.333	0	0.333	0	0.167	1.833
21-Jan-03	Cotton	east	<b>5</b>	1	0.167	0.333	0	0.167	1.667
21-Jan-03	Cotton	east	<b>10</b>	0.5	0	0.167	0	0	0.667
21-Jan-03	Cotton	east	<b>25</b>	0	0	0.333	0	0.333	0.667
21-Jan-03	Cotton	east	<b>45</b>	1.167	0	0.167	0	0.5	1.833

### APPENDIX 1 (cont.)

The numbers of predators per metre in conventional cotton inter-cropped with sorghum. The values are the means of six beat sheet samples. The beat samples were taken in the cotton at regular intervals on either side of the sorghum (row-pairs 2, 5, 10, 25 and 45), and at random in each half of the sorghum.

DATE	Crop	Location	Row Pair No.	Beetles	Bugs	Spiders	Lacewings	Ants	TOTAL	
28-Jan-03	Cotton	west	<b>45</b>	2.5	0.333	0.667	0.167	1	4.667	
28-Jan-03	Cotton	west	<b>25</b>	1	0.333	0.5	0	0	1.833	
28-Jan-03	Cotton	west	<b>10</b>	1.167	0.167	0.333	0	0.833	2.5	
28-Jan-03	Cotton	west	<b>5</b>	1.5	0	0.333	0	0.333	2.167	
28-Jan-03	Cotton	west	<b>2</b>	2.333	0.333	0.333	0	0.167	3.167	
28-Jan-03	<b>Sorghum</b>			4.333	0.667	0.5	0	0.167	5.667	
28-Jan-03	<b>Sorghum</b>			2.333	1	0.667	0	0.167	4.167	
28-Jan-03	Cotton	east	<b>2</b>	1.667	0.167	0.5	0	0.333	2.667	
28-Jan-03	Cotton	east	<b>5</b>	0.5	0.833	0.167	0.167	0	1.667	
28-Jan-03	Cotton	east	<b>10</b>	1	0.333	0.5	0	0.167	2	
28-Jan-03	Cotton	east	<b>25</b>	0.667	0	0.333	0	0.5	1.5	
28-Jan-03	Cotton	east	<b>45</b>	not sampled due to flood irrigation						
Pooled	Cotton	west	<b>45</b>	2.167	0.125	0.208	0.042	0.975	3.417	
Pooled	Cotton	west	<b>25</b>	0.917	0.083	0.292	0.083	0.083	1.458	
Pooled	Cotton	west	<b>10</b>	0.75	0.125	0.333	0	0.458	1.667	
Pooled	Cotton	west	<b>5</b>	1.25	0.083	0.417	0.042	0.125	1.917	
Pooled	Cotton	west	<b>2</b>	1.5	0.125	0.417	0	0.083	2.125	
Pooled	<b>Sorghum</b>			4.278	0.667	0.444	0.167	0.222	5.778	
Pooled	<b>Sorghum</b>			3	0.889	0.333	0.111	0.167	4.5	
Pooled	Cotton	east	<b>2</b>	1.292	0.125	0.417	0	0.125	1.958	
Pooled	Cotton	east	<b>5</b>	0.625	0.292	0.292	0.083	0.083	1.375	
Pooled	Cotton	east	<b>10</b>	0.583	0.167	0.417	0	0.042	1.208	
Pooled	Cotton	east	<b>25</b>	0.375	0.042	0.458	0	0.25	1.125	
Pooled	Cotton	east	<b>45</b>	0.611	0	0.333	0	0.722	1.667	

## APPENDIX 2

The mean numbers of predators per metre in conventional cotton inter-cropped with sorghum. The values are the means of 60 beat sheet samples in the cotton and 12 beat sheet samples in the sorghum on each date.

Predator	Mean Number of Predators per Metre								Overall Means (Pooled Dates)		Total Counts (Pooled Dates)	
	7-Jan-03		14-Jan-03		21-Jan-03		28-Jan-03		Cotton	Sorgh	Cotton	Sorgh
<b>Predatory Bugs:</b>												
Big-Eyed Bug Nymph	0	0	0.033	0	0.017	0	0.074	0	0.03	0	7	0
Big-Eyed Bug Adult	0.033	0	0.017	0	0.017	0	0.019	0	0.021	0	5	0
Brown Smudge Bug Nymph	0	0	0	0.083	0	0.333	0.019	0	0.004	0.119	1	5
Brown Smudge Bug Adult	0	0	0.017	0	0	0	0.037	0	0.013	0	3	0
Damsel Bug Nymph	0	0	0	0	0	0	0.056	0	0.013	0	3	0
Damsel Bug Adult	0	0	0	0	0	0	0	0	0	0	0	0
Glossy Shield Bug Nymph	0	0	0	0	0.017	0	0	0	0.004	0	1	0
Glossy Shield Bug Adult	0	0	0	0	0	0	0	0	0	0	0	0
Pirate Bug Nymph	0	0	0	0	0	0	0	0.333	0	0.095	0	4
Pirate Bug Adult	0	0	0	0.083	0	1	0.056	0.5	0.013	0.452	3	19
Predatory Shield Bug Nymph	0	0	0.05	0	0.017	0	0.019	0	0.021	0	5	0
Predatory Shield Bug Adult	0	0	0	0	0	0	0	0	0	0	0	0
<b>Total Bugs</b>	<b>0.033</b>	<b>0</b>	<b>0.117</b>	<b>0.167</b>	<b>0.067</b>	<b>1.333</b>	<b>0.278</b>	<b>0.833</b>	<b>0.12</b>	<b>0.667</b>	<b>28</b>	<b>28</b>
<b>Predatory Beetles:</b>												
Red & Blue Beetle	0.45	0.5	0.483	0.75	0.833	1.167	0.87	0.5	0.654	0.762	153	32
Spotted Ladybird	0	0	0	0	0	0	0	0.083	0	0.024	0	1
Striped Ladybird	0	0	0	0	0	0	0	0	0	0	0	0
Three Banded Ladybird	0	0	0	0	0	0	0	0	0	0	0	0
Transverse Ladybird	0.05	0.333	0.033	0.083	0.067	0.417	0.037	0	0.047	0.19	11	8
Two-Spotted Ladybird	0.017	0	0.017	0	0	0	0	0.167	0.009	0.048	2	2
Variable Ladybird	0	0	0	0	0	0	0.019	0	0.004	0	1	0
White Collared Ladybird	0.05	1.167	0.55	2.75	0.183	1.583	0.444	2	0.303	1.976	71	83
Ladybird Larvae (all)	0	1.167	0	0.167	0	0.667	0	0.583	0	0.571	0	24
<b>Total Beetles</b>	<b>0.567</b>	<b>3.167</b>	<b>1.083</b>	<b>3.75</b>	<b>1.083</b>	<b>3.833</b>	<b>1.37</b>	<b>3.333</b>	<b>1.017</b>	<b>3.571</b>	<b>238</b>	<b>150</b>
<b>Spiders:</b>												
Flower	0.083	0	0.133	0	0.083	0.083	0.093	0.083	0.098	0.048	23	2
Jumping	0.017	0	0.067	0	0.117	0.083	0.074	0.167	0.068	0.071	16	3
Lynx	0.05	0	0.067	0	0	0.083	0.019	0	0.034	0.024	8	1
Swith Ground	0.017	0	0	0	0	0	0.019	0	0.009	0	2	0
Tangle Web	0.017	0	0.033	0	0.033	0	0.093	0.25	0.043	0.071	10	3
Yellow Night Stalker	0.083	0.167	0.067	0	0.067	0.167	0.037	0	0.064	0.071	15	3
Other Spiders	0.033	0.167	0.067	0.167	0.067	0.167	0.074	0.083	0.043	0.095	10	4
<b>Total Spiders</b>	<b>0.3</b>	<b>0.333</b>	<b>0.433</b>	<b>0.167</b>	<b>0.3</b>	<b>0.417</b>	<b>0.407</b>	<b>0.583</b>	<b>0.359</b>	<b>0.381</b>	<b>84</b>	<b>16</b>
<b>Lacewings:</b>												
Brown Lacewing Larva	0	0	0	0	0	0.083	0	0	0	0.024	0	1
Brown Lacewing Adult	0.017	0	0	0.083	0	0	0	0	0.004	0.024	1	1
Green Lacewing Larva	0.017	0	0.017	0.25	0	0	0.037	0	0.017	0.071	4	3
Green Lacewing Adult	0	0	0.017	0	0	0	0	0	0.004	0	1	0
<b>Total Lacewings</b>	<b>0.033</b>	<b>0</b>	<b>0.033</b>	<b>0.333</b>	<b>0</b>	<b>0.083</b>	<b>0.037</b>	<b>0</b>	<b>0.026</b>	<b>0.119</b>	<b>6</b>	<b>5</b>
<b>Ants:</b>												
Small Black	0.1	0	0.267	0.167	0.283	0.25	0.278	0.083	0.231	0.143	54	6
Large Black	0.067	0	0	0	0.017	0	0.093	0.083	0.043	0.024	10	1
Other Ants	0	0	0	0	0	0	0	0	0	0	0	0
<b>Total Ants</b>	<b>0.167</b>	<b>0</b>	<b>0.267</b>	<b>0.167</b>	<b>0.3</b>	<b>0.25</b>	<b>0.037</b>	<b>0.026</b>	<b>0.274</b>	<b>0.167</b>	<b>64</b>	<b>7</b>
<b>TOTAL PREDATORS</b>	<b>1.1</b>	<b>3.5</b>	<b>1.933</b>	<b>4.583</b>	<b>1.75</b>	<b>5.917</b>	<b>2.463</b>	<b>4.917</b>	<b>1.795</b>	<b>4.905</b>	<b>420</b>	<b>206</b>

## 2. Evaluations of different releases rates of *Trichogramma pretiosum* against *Helicoverpa armigera* eggs in sorghum and cotton.

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### Introduction

There is increasing interest in utilising *Trichogramma* egg parasitoids against heliothis (*Helicoverpa* spp.) in cotton, and crops that may act as sources of parasitoids for cotton – such as sorghum. Very little has been published on the effect of different release rates of *Trichogramma* wasps against heliothis.

Here we report on field trials evaluating different release rates of *Trichogramma pretiosum* against *Helicoverpa armigera* in sorghum and cotton at Evanslea on the eastern Darling Downs.

### Methods

*Trichogramma pretiosum* wasps were purchased from Bugs for Bugs (Mundubbera, Qld.) in cardboard capsules. Each capsule contained ca. 1,000 wasps. Trials comparing different release rates were conducted in sorghum and cotton (two trials in each crop). A standard plot size (60 m x 40 rows) and standard release protocol were used for all trials. All plots were separated by buffer crop of at least 40 m.

The treatments were: 1) **control** – no wasps released, 2) **low** – 8 capsules per plot, ca. 33K wasps/ha, 3) **medium** – 16 capsules per plot, ca. 67K wasps/ha, and 4) **high** – 32 capsules per plot, ca. 133K wasps/ha. Sorghum trial 2 was a randomised block design, and all other trials were latin square designs. The release capsules were stapled to the upper leaf surface of plants in rows 5, 15, 25 and 35 of each plot. There were 2, 4 and 8 capsules per row in the low, medium and high release rate plots respectively. The distance between cards decreased as the release rate rose, viz. 20, 16 and 7 m between cards within a row for the low, medium and high release rate plots respectively.

To sample the sorghum trials pre-flowering heads were cut and spun in a funnel that emptied into a 30 mL plastic cup. The eggs from a single sorghum head were collected in a cup, and ten heads were selected at random from each plot. The cups were returned to the laboratory, and the eggs were isolated individually in the cells of plastic microtitre trays. Egg colour was recorded, i.e. white, brown, black-head or black parasitised.

All eggs were held in a constant temperature room until they hatched, or egg parasitoids completed their development and emerged. The proportion of brown eggs parasitised was calculated for each plot, and the mean number of heliothis eggs per sorghum head was determined.

Heliothis egg cards were used to assess the levels of egg parasitism in cotton because there were few natural eggs laid throughout the trials. Adult *H. armigera* moths were placed in oviposition chambers where they laid eggs onto paper towelling. Each card was made by stapling pieces of paper towelling containing approximately 20 *H. armigera* eggs to paper strips measuring 1.5 x 7 cm. The eggs were less than 24 hours old. Each card was stapled to the upper side of a leaf at the top of a plant. The egg cards were stapled on leaves in a grid pattern, viz. 25 cards per row in plot rows 4, 12, 20, 28, and 36 (every eighth row), 10 m apart within each row.

### ***Sorghum Trial 1***

The first sorghum trial was conducted at Gary Rasmussen's. Buster sorghum was sown on 5 October 2002. The *Trichogramma* were released on 17 December 2002, and naturally laid *H. armigera* eggs were collected from pre-flowering sorghum heads on 20 December and 23 December.

### ***Sorghum Trial 2***

The second sorghum trial was conducted at Andrew Speed's. The sorghum (86G87) was planted in two strips on the 30<sup>th</sup> September 2002. The overall size of the planting was too small to conduct a trial with four treatments. Three treatments were tested in sorghum trial 2, viz. control, medium and high release rates. The *Trichogramma* were released on 20 December 2002, and naturally laid *H. armigera* eggs were collected from pre-flowering sorghum heads on 24 December.

### ***Cotton Trial 1***

The first cotton trial was conducted at Neil Nass's in Sicot 71 conventional cotton that had been sprayed once with Costar<sup>®</sup>, on 30 December 2002, at the time of the trial. The *Trichogramma* were released on 10 February 2003, and heliothis egg cards were stapled in the crop on 13 February and collected on 15 February.

### ***Cotton Trial 2***

The second cotton trial was conducted at Andrew Speed's in Siokra V-17 conventional cotton that had been spray three times at the time of the trial. The last spray (Affirm<sup>®</sup>) was applied on the 29<sup>th</sup> January 2003. *Trichogramma* were released on 17 February, and heliothis egg cards were stapled in the crop on 19 February and collected on 21 February.

## Results and Discussion

### *Sorghum Trials*

The levels of natural *H. armigera* egg parasitism were high during both of the sorghum trials (66-73%, Figures 1 and 2). There was moderate-high egg pressure during the sorghum trials, i.e. 5.7 and 6.2 eggs per sorghum head on Dec 20 and Dec 23 (Trial 1), and 2.1 eggs per head on Dec 24 (Trial 2).

There was no significant increase in the levels of egg parasitism following the release of *Trichogramma* at any rate during the sorghum trials. The high background (control) levels of egg parasitism indicates that *Trichogramma* can build up rapidly early season in sorghum, and has implications for managing parasitoids in adjacent, nearby and subsequent crops. However, releasing *Trichogramma* in this situation may not be the best use of funds because: 1) the released wasps did not significantly increase egg mortality, and 2) other options, e.g. heliothis virus (such as Gemstar® and Vivus®), may be a better use of funds. Virus would target hatching larvae, while conserving natural populations of *Trichogramma* that have an enormous propensity to build up in sorghum. This would be cheaper than using *Trichogramma* at the standard (medium) rate. The approximate costs of *Trichogramma* are \$25, \$50 and \$100 per hectare for the low, medium and high rates evaluated in this trial (without application costs). By contrast the cost of an application of Gemstar® in sorghum is \$28/ha (without application costs). The costs are comparable if low *Trichogramma* release rates are used, however an application of virus is easier to manage than a release of *Trichogramma*. The findings suggest that the use of *Trichogramma* as inundative biocontrol agents in crops that have high natural levels of egg parasitoid activity is difficult to justify in terms of a marked increase in heliothis mortality, ease of application and economic viability.

Releases into crops that have no, or low, levels of natural egg parasitoid activity may be useful in ‘kick starting’ or boosting natural activity as an inoculative or supplemental technique. However additional research is needed to clarify this, i.e. trials in crops that have low levels of background egg parasitism. It is important to realise that heliothis egg mortality on the Darling Downs due to natural populations of *Trichogramma* rises rapidly. In Trial 1 the pre-release level of egg parasitism was 35% on 17 December. This rose over 30% to 66% parasitism on the 20<sup>th</sup> December in the control plots. The most effective way to use *Trichogramma* may be to conserve and enhance natural populations. We need to determine if supplemental releases of *Trichogramma* will add significantly to the levels of egg parasitism achieved by conservation approaches alone. Perhaps natural populations should simply be allowed to build-up by using selective insecticides and other *Trichogramma* conservation practices. This would be easier than releasing wasps.

### ***Cotton Trials***

The background levels of egg parasitism in the cotton trials were much lower than those in the sorghum trials (4-11%, Figure 3). There was low egg pressure during the cotton trials, and it was not possible to collect field laid eggs.

During Trial 1 there were significant differences in the levels of egg parasitism achieved by releasing *T. pretiosum*, however the maximum level of egg parasitism was only 31%. This would not be sufficient to manage heliothis in most situations and additional control practices would probably have to be employed. The level of egg parasitism in the low release treatment (20%) was only marginally less than that in the medium release treatment (21%), suggesting that the low release rate may be worth pursuing as a more economically feasible option.

In Trial 2 there was no significant difference in the levels of egg parasitism recorded between treatments (Figure 3). Again, the level of egg parasitism in the low release treatment (18%) was only marginally less than that in the medium release treatment (20%).

The findings suggest that inundative releases do not have a high immediate impact. However there may be flow on benefits by using low release rates as inoculative or supplemental practices. Additional research is needed to clarify these options, while bearing in mind that high natural populations of egg parasitoids develop in cotton that is not heavily sprayed, e.g. *B.t.* transgenic crops (Scholz *et al.* 2002), or conventional crops that are sprayed with selective insecticides.

### ***General***

The use of inundative releases of *Trichogramma* wasps, as a substitute for applications of insecticides is difficult to justify based on the findings reported here. Only small increases in the levels of heliothis egg parasitism were recorded in the low (6-16%), and medium rate trials (8-21%), in both sorghum and cotton. The high release rates increased egg parasitism by 16-27% over the controls, and would be difficult to justify in economic terms (four times the cost of low releases).

Low release rates may be the most cost effective method of utilising *Trichogramma*, as we found little net benefit, in terms of increased in levels of egg parasitism, by using medium/high release rates compared to low rates (Table 1). To date there has not been enough research directed at comparing different release rates of *Trichogramma*.

*Trichogramma* have traditionally been thought of as inundative biocontrol agents, however they are probably not best suited in this role against heliothis. There are other ways that *Trichogramma* can be utilised in pest management programs, viz:

- Incorporation in IPM programs;
- Conservation and enhancement of existing (natural) populations of wasps; and
- Other release strategies, e.g. inoculative or supplemental releases.

These strategies need to be explored. The value of releasing wasps to boost natural populations early season, or to seed *Trichogramma* nursery crops, needs to be investigated in detail before we can make sound recommendations about releasing *Trichogramma*. In addition, further rate trials need to be completed under a range of pest densities. The impact of releases may be greater under higher densities of pests than were encountered during the trials reported here.

## Key Findings

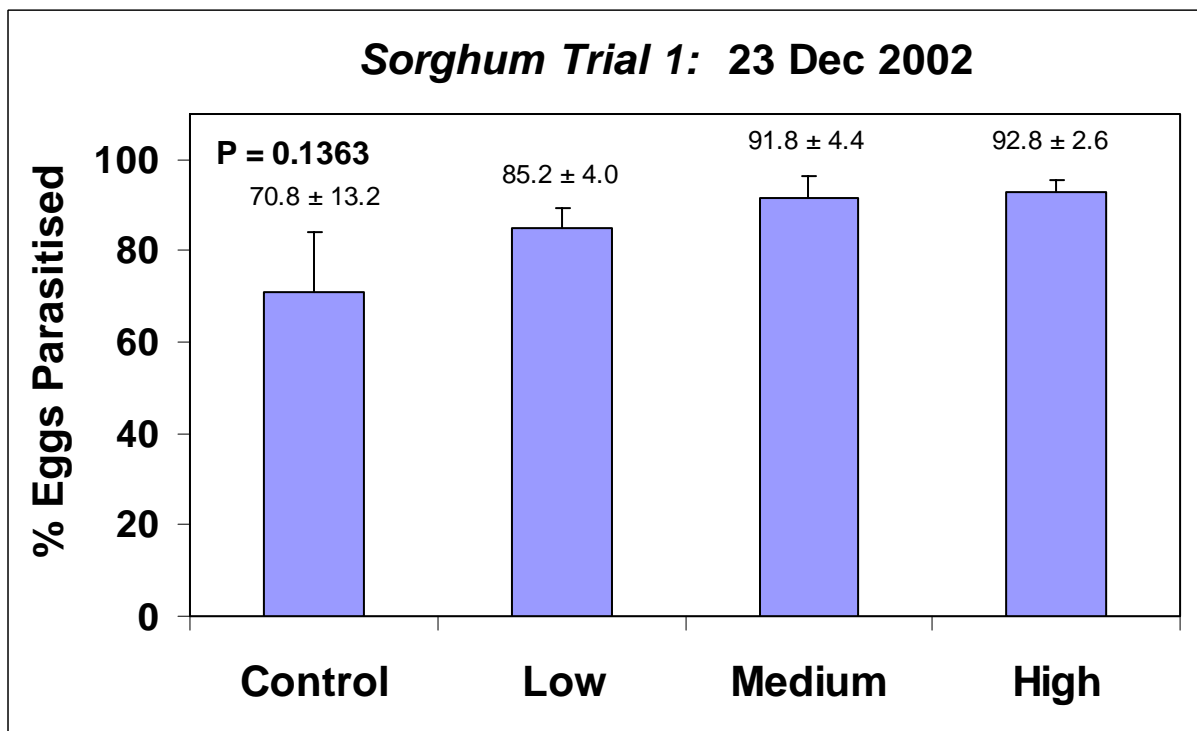
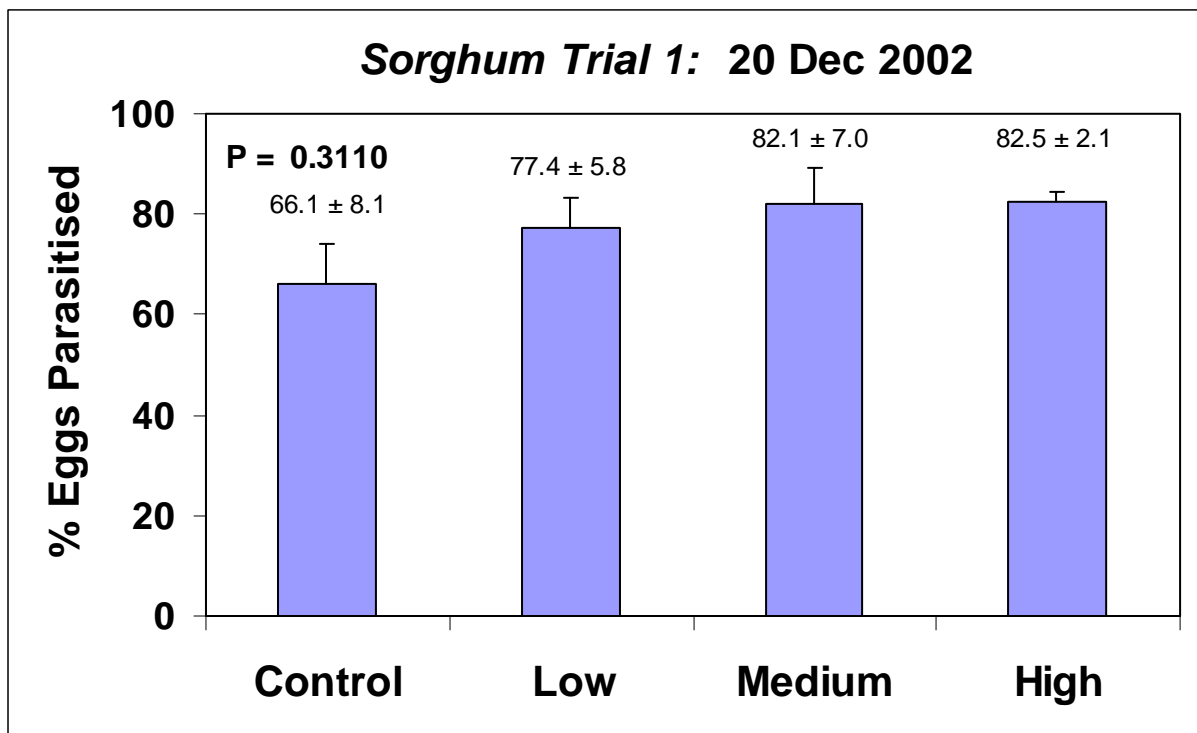
- Releasing *Trichogramma pretiosum* increased the levels of heliothis egg parasitism in sorghum and cotton by 6-27%. Such increases are difficult to justify as an inundative tactic, but may have merit as an inoculative or supplementative approach.
- The levels of egg parasitism achieved with low level release rates (ca. 30,000 wasps per hectare) were comparable with medium release rates (ca. 60,000 wasps/ha), and are worthy of additional investigation because of the associated cost saving.
- High background levels of egg parasitism (66-70%) were found in sorghum in December. The activity of natural populations of egg parasitoids should be assessed before purchasing and releasing *Trichogramma*. The benefit obtained from releasing wasps may be small, and the impact of the natural populations may be sufficient to manage heliothis in some circumstances. The use of selective insecticides may represent a better investment should subsequent small populations of heliothis larvae develop and require management.

## Acknowledgements

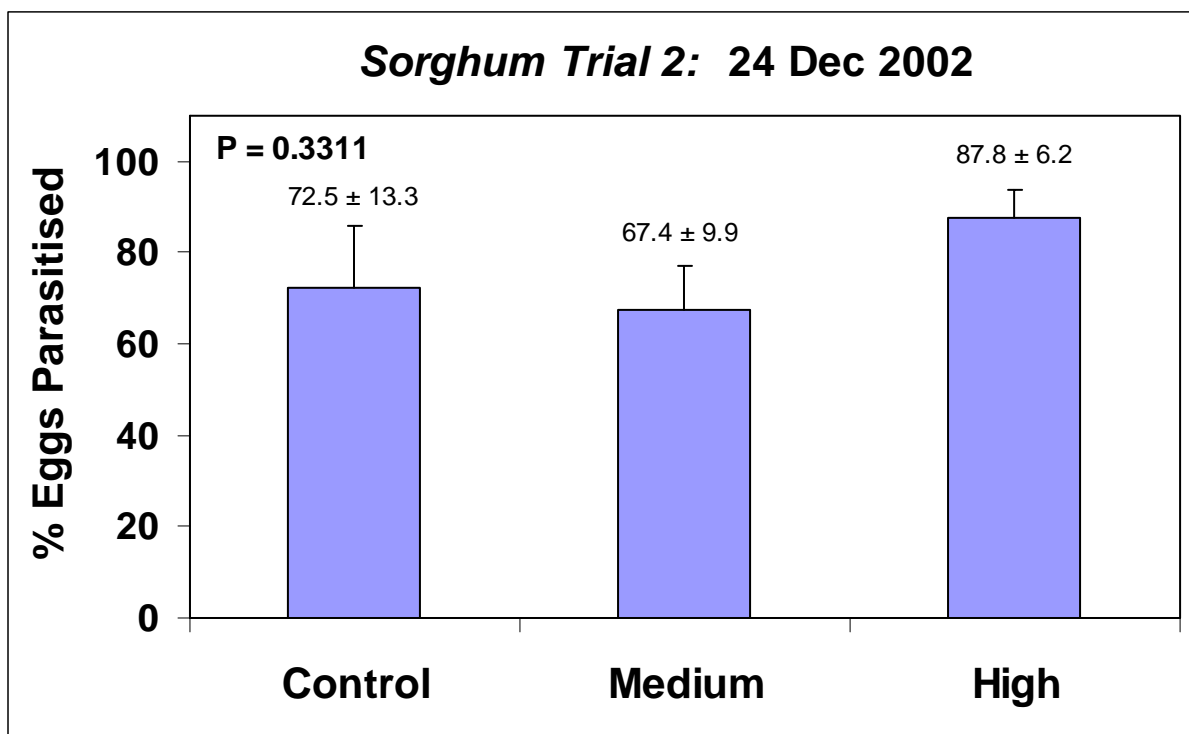
We thank Gary Rasmussen, Neil Nass and Andrew Speed for cooperating in this research, and the Cotton Research and Development Corporation for funding the research (project DAQ125C). Sue Maclean (DPI Toowoomba) supplied *Helicoverpa armigera* eggs. This assistance is gratefully acknowledged.

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- Scholz, B., Parker, N. and Lloyd, R. (2002). An evaluation of unsprayed INGARD strips as nurseries for beneficials in dryland cotton on the Darling Downs. *Proceedings of the Eleventh Australian Cotton Conference*, Brisbane, Queensland, 13-15 August, 2002. pp. 297-306.



**Figure 1:** The levels of heliothis (*H. armigera*) egg parasitism following the release of different rates of *Trichogramma pretiosum* into sorghum at Rasmussen's, Evanslea. Values are the mean  $\pm$  standard error of four replicates, and were arcsine transformed for analyses (ANOVA). No significant differences were found between the means.

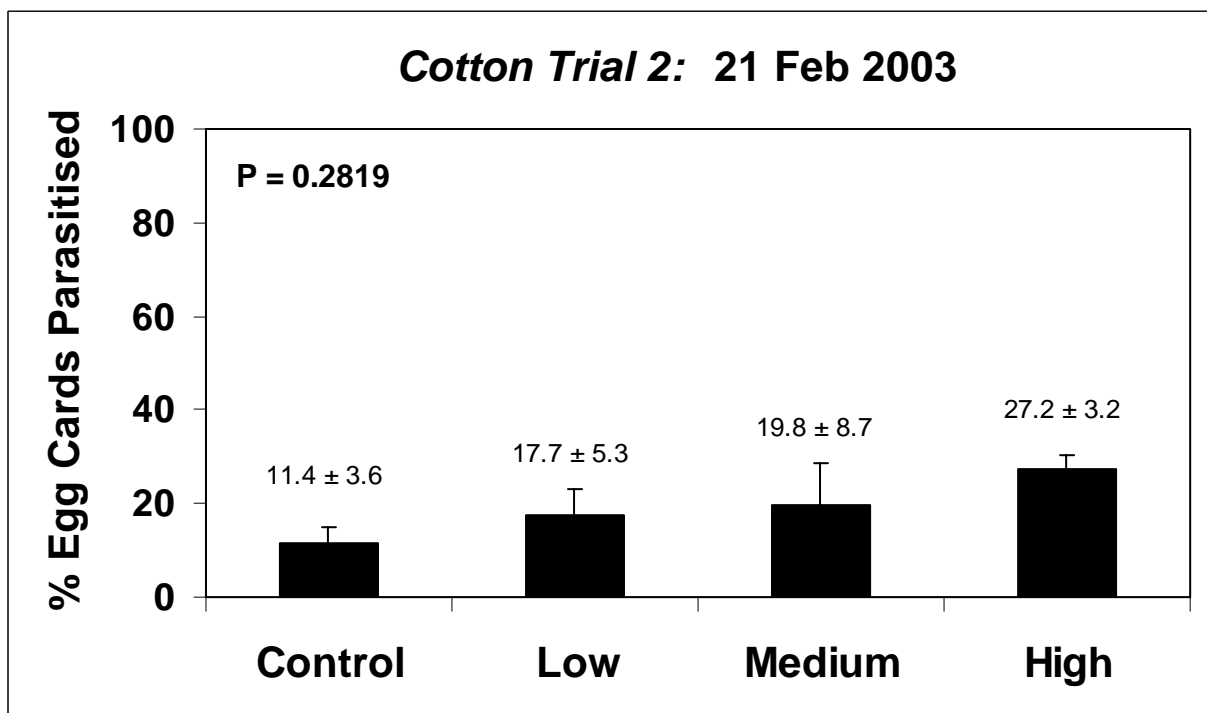
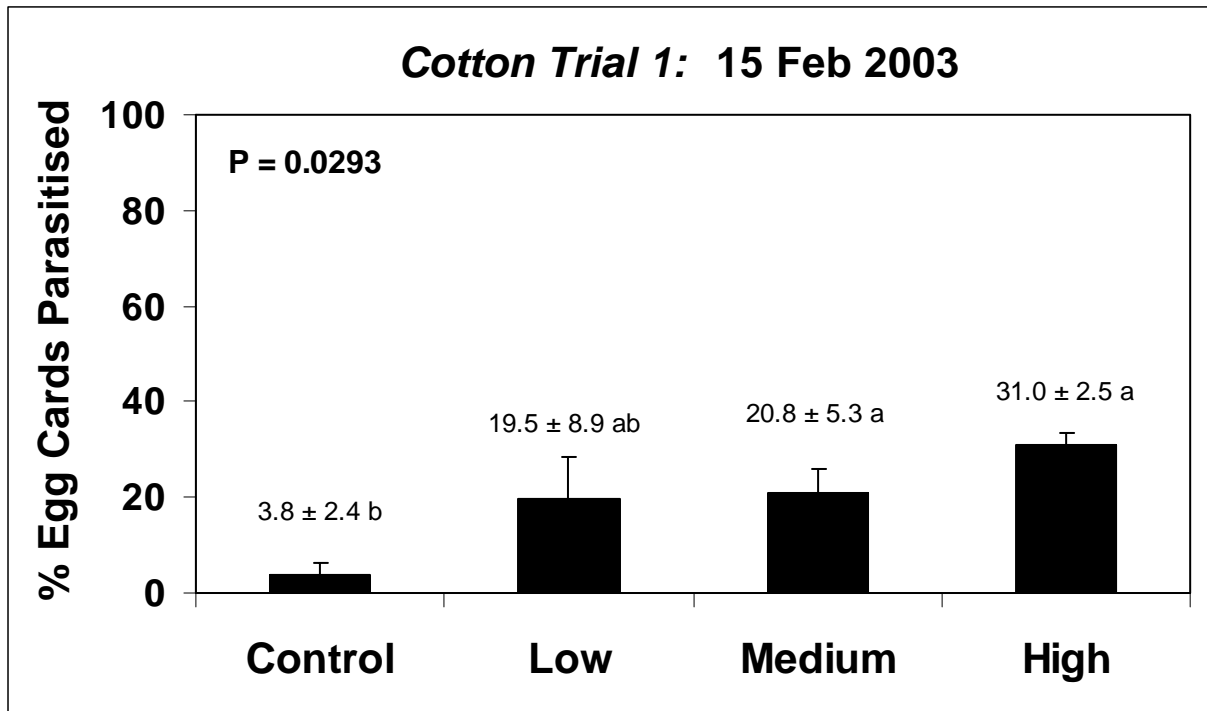


**Figure 2:** The levels of heliothis (*H. armigera*) egg parasitism following the release of different rates of *Trichogramma pretiosum* into sorghum at Speed's, Evanslea. Values are the mean  $\pm$  standard error of four replicates, and were arcsine transformed for analyses (ANOVA). No significant differences were found between the means.

**TABLE 1**

The benefits of increasing release rates. The values represent the increase in levels of heliothis egg parasitism (multiplication factor) found between different release rates of *T. pretiosum*. **Example:** In *Sorghum Trial 1* the increase in the parasitism from control to medium was 1.42 times that of control to low, i.e. the net benefit (in terms of increased levels of egg parasitism) of using medium release rates was 1.42 times that of using low release rates. Low = 33K/ha, Medium = 67K/ha, High = 133K/ha. Values represent total wasps (males and females) released per hectare.

Trial Number	Low to Medium	Low to High	Medium to High
<i>Sorghum Trial 1</i>	1.42	1.45	1.03
<i>Sorghum Trial 2</i>	1.46	1.53	1.05
<i>Cotton Trial 1</i>	1.08	1.73	1.31
<i>Cotton Trial 2</i>	1.33	2.51	1.88
<b>Mean Difference</b>	<b>1.32</b>	<b>1.81</b>	<b>1.32</b>



**Figure 3:** The levels of heliothis (*H. armigera*) egg parasitism on egg cards following the release of different rates of *Trichogramma pretiosum* into conventional cotton. Values are the mean  $\pm$  standard error of four replicates, and were arcsine transformed for analyses. Means followed by the same letter are not significantly different ( $P=0.05$ , ANOVA, Fisher's LSD comparison).

### **3. Natural levels of heliothis egg parasitism in irrigated cotton on the Darling Downs.**

Brad Scholz and Nathaniel Parker.

Queensland Department of Primary Industries & Australian Cotton CRC, PO Box 102, Toowoomba 4350.

#### **Introduction**

Egg parasitoids of heliothis are becoming increasingly important on the Darling Downs. In recent years we have documented the impact of *Trichogramma pretiosum* in dryland cotton on the Darling Downs (Scholz *et al.* 2002), but have collected few data from irrigated cotton.

Here we report on assessments of natural levels of heliothis egg parasitism in irrigated cotton on the Darling Downs.

#### **Methods**

The research was conducted at Phil Jauncey's property "Branchview", near Brookstead on the Darling Downs. The levels of heliothis egg parasitism were assessed in several cotton blocks on the farm at various times throughout the season (Table 1). Brown heliothis eggs were collected on leaves and placed in bry-nylon cloth bags. The bags were held in an esky for transfer to the laboratory. The eggs were individually transferred to the 6 mm diameter wells of plastic micro-titre trays (96 wells per tray) using a fine paint brush dipped in water. The trays were covered with sticky tape and held in a constant temperature room at approximately 25°C and 60% R.H. for the egg parasitoids to develop. The egg shell of parasitised eggs turn a distinctive jet black colour after four days at 25°C, and adults wasps emerge after another six days (ten day cycle from egg to adult at 25°C).

The numbers of heliothis eggs per metre of crop were counted in the G4 Standard and G4 Soft blocks. The yield in these blocks was also estimated by hand harvesting 6 x 5 m lengths of crop in each block.

**TABLE 1:** The cotton varieties planted at Branchview.

Block Number	Variety	Acreage (ha)	Planting Date
D3	Conventional Sicot 71	44	24 October 2002
D4	INGARD <sup>®</sup> Sicot 289RRi	40	8 October 2002
G2	Conventional Sicot 80	36	25 October 2002
G4 Soft IPM	Conventional Sicot 80	25	19-22 October 2002
G4 Standard IPM	Conventional Sicot 80	25	18 October 2002

**TABLE 2:** Insecticide applications in each cotton block at Branchview.

Block Number	Spray No.	Spray Date	Product
<b>D3</b>	1	13 Dec 2002	Tracer, Gemstar, Aminofeed
	2	31 Dec 2002	Tracer, Gemstar, Aminofeed
	3	15 Jan 2003	Tracer, Gemstar, Aminofeed
	4	21 Jan 2003	Steward, Ovasyn
	5	1 Feb 2003	Affirm
	6	11 Feb 2003	Steward, Gemstar, Aminofeed, Dimethoate
<b>D4 (north)</b>	1	18 Feb 2003	Dimethoate, Gemstar
<b>G2</b>	1	13 Dec 2002	Tracer, Gemstar, Aminofeed
	2	23 Dec 2002	Gemstar, Aminofeed
	3	31 Dec 2002	Tracer, Gemstar, Aminofeed
	4	15 Jan 2003	Tracer, Gemstar, Aminofeed
	5	21 Jan 2003	Steward, Ovasyn
	6	1 Feb 2003	Affirm
	7	11 Feb 2003	Steward, Gemstar, Aminofeed
<b>G4 Soft IPM</b>	1	31 Dec 2002	Tracer, Gemstar, Aminofeed
	2	17 Jan 2003	Tracer, Gemstar, Aminofeed
	3	1 Feb 2003	Steward, Ovasyn
	4	24 Feb 2003	Gemstar, Aminofeed, Dimethoate
<b>G4 Standard IPM</b>	1	6 Dec 2002	Prodigy
	2	14 Dec 2002	Tracer, Gemstar, Aminofeed
	3	31 Dec 2002	Tracer, Gemstar, Aminofeed
	4	15 Jan 2003	Tracer, Gemstar, Aminofeed
	5	6 Feb 2003	Steward, Ovasyn

## Results and Discussion

The levels of natural *Helicoverpa* egg parasitism rose progressively throughout the season on the Branchview farm, and peaked at ca. 95% in February (Figure 1). Some of the cotton blocks had 60 eggs/m (Matthew Holding, pers. comm.) and 96% egg parasitism. Parasitised eggs were collected during December, which is earlier than we detected *Trichogramma* in dryland cotton trials at Jimbour. At Jimbour egg parasitism was first detected in mid-January and rose to high levels (90+%) from late January onwards.

There was a slight drop in the levels of egg parasitism on the 29<sup>th</sup> January and 7<sup>th</sup> February (Figure 1). This may have been due to insecticide drift from neighbouring farms, as the spray schedule at Branchview was reasonable soft (Table 2). The selective spray regimes utilised at Branchview undoubtedly contributed to the conservation of local populations of *Trichogramma*. The contribution of off-habitats to the population of *Trichogramma* was not studied. However, there was sorghum planted on an adjacent farm that may have contributed to the build up of *Trichogramma* in the Branchview cotton.

At Branchview the levels of brown egg parasitism were much higher than those found in white eggs (Table 3). This supports the current recommendations for assessing egg parasitism, i.e. collect brown eggs because collections based on white eggs are likely to under-estimate the actual levels of egg parasitism.

Almost all of the egg parasitoids collected were *Trichogramma pretiosum* (99.3%). This supports previous work indicating that this species has become widespread and abundant in agricultural crops since it was introduced on the Darling Downs in 1995 (Scholz *et al.* 1996). The other species of egg parasitoids collected were *Trichogramma carverae*, *Trichogrammatoidea bactrae* and a species of *Telenomus* – these three species accounted for 0.7% of parasitised eggs (there was only 1 parasitised egg collected for each of these species).

The heliothis egg densities rarely exceed 5 eggs/m in the G4 Standard and G4 Soft blocks (Figure 2). The levels of egg parasitism in both blocks were similar on most sampling dates (Figure 3). The G4 Standard cotton was sprayed once more than the G4 Soft (5 vs. 4 sprays). However, there was no significant difference between the yields found in the G4 Standard ( $5.6 \pm 0.2$  b/ha) and G4 Soft ( $5.9 \pm 0.3$  b/ha) cotton ( $P=0.4844$ , ANOVA). This suggests that at least one of the insecticide sprays in the G4 Standard block may have not been necessary. The third spray on the G4 Soft block (Steward<sup>®</sup>/Ovasyn<sup>®</sup>) was accidental, so perhaps a two spray saving would have been possible. The farm consultant is keen to incorporate *Trichogramma* into spray decisions next season in order to pursue additional spray savings.

**TABLE 3**

The fate of heliothis eggs collected from irrigated cotton at Branchview 2002-2003.

	<b>White Eggs</b>	<b>Brown Eggs</b>	<b>White + Brown Eggs</b>
<b>No. Eggs Collected</b>	374	738	1112
<b>No. Eggs Hatched</b>	235	272	507
<b>No. Eggs Collapsed</b>	48	102	150
<b>No. Eggs Parasitised</b>	91	364	455
<b>% Egg Hatch</b>	62.8	36.9	45.6
<b>% Eggs Collapsed</b>	12.8	13.8	13.5
<b>% Viable Egg Parasitism</b>	27.9	57.2	47.3

## Key Findings

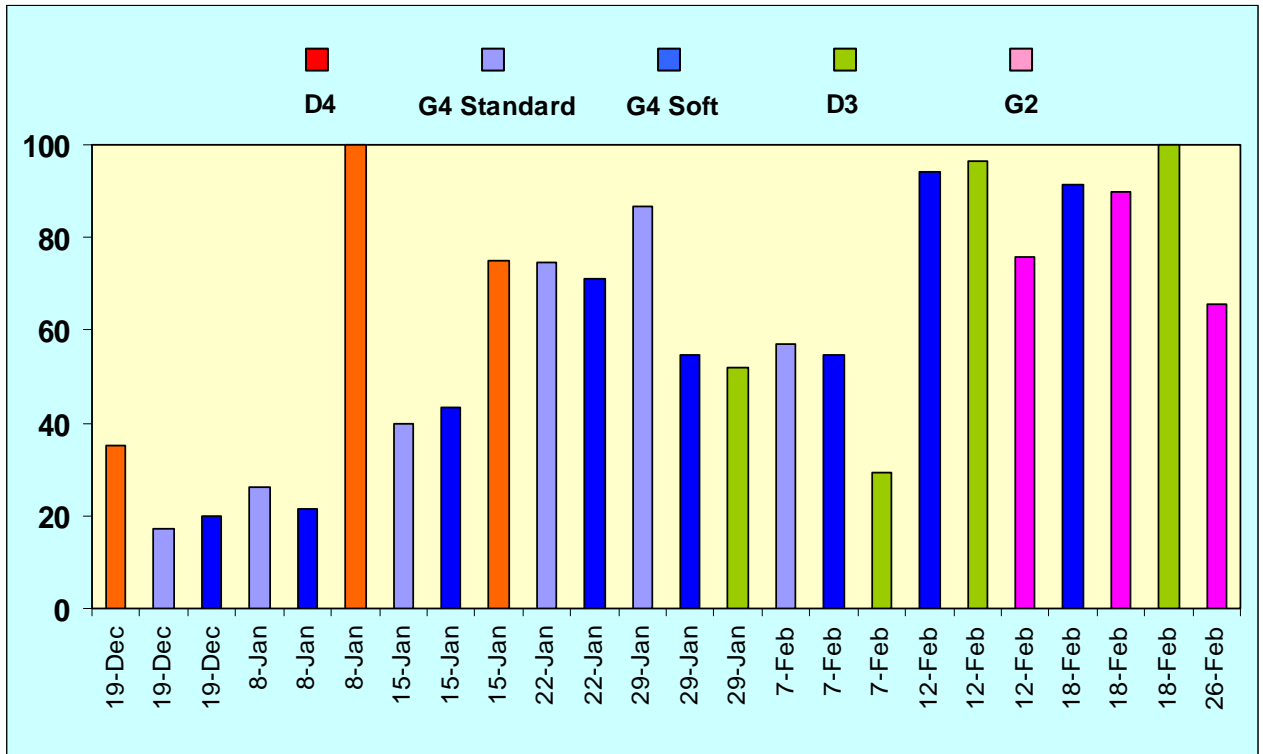
- The levels of heliothis egg parasitism rose from 20% in December to 96% in February in irrigated conventional cotton at Branchview on the Darling Downs. A selective insecticide regime was used to manage pests, with the conventional cotton receiving 4-7 sprays for the season.
- The high levels of egg parasitism in January and February reduced the need to spray for heliothis. Adjacent blocks of Sicot 80 cotton, G4 Standard and G4 Soft, received 5 and 4 sprays respectively, with no significant difference in yields (5.6 and 5.9 b/ha respectively).
- The majority of egg parasitoids were *Trichogramma pretiosum* (99%), introduced to the Darling Downs in 1995. This species now seems widespread and abundant in agricultural environments.

## Acknowledgements

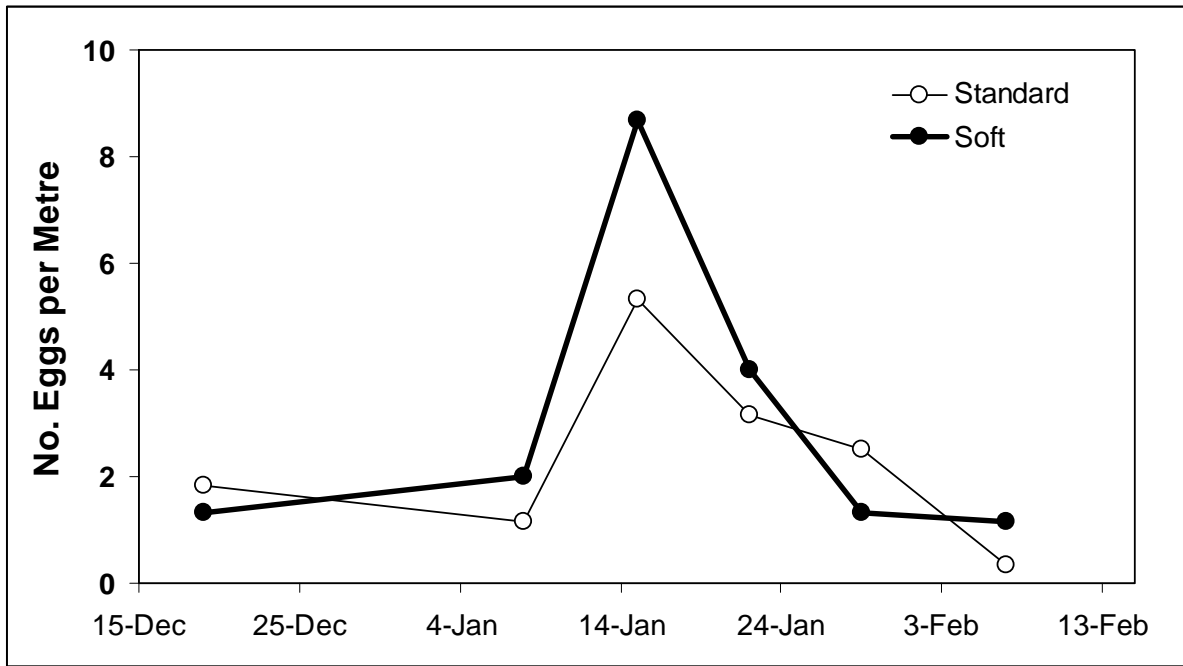
We thank Phil Jauncey, Matthew Holding and Antony McConville for cooperating in this research, and the Cotton Research and Development Corporation for funding the research (project DAQ125C). This assistance is gratefully acknowledged.

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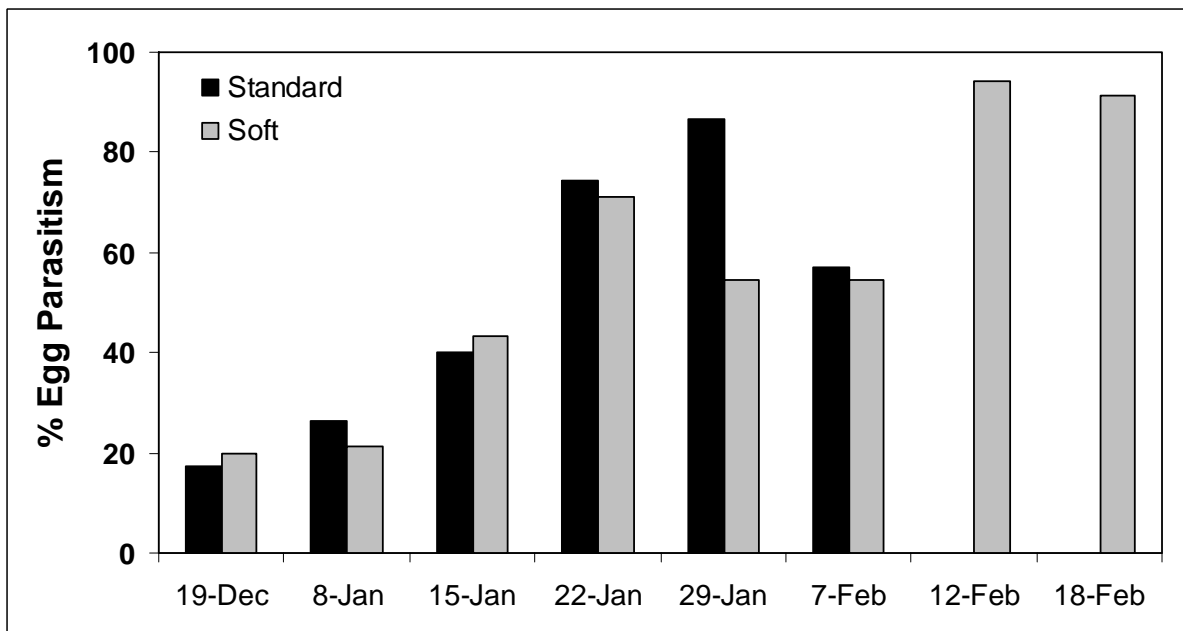
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**Figure 1:** The levels (%) of *Helicoverpa* egg parasitism in different blocks of irrigated cotton at Branchview on the Darling Downs. Block D4 was INGARD<sup>®</sup> cotton. All other blocks were conventional cottons (see Table 1). The insecticides applied to each block are listed in Table 2.



**Figure 2:** The numbers of *Helicoverpa* spp. eggs found in conventional cotton at Branchview (blocks G4 Standard and G4 Soft). The spray details for each block are provided in Table 2. G4 Standard was sprayed 5 times, and G4 Soft was sprayed 4 times.



**Figure 3:** The levels (%) of *Helicoverpa* egg parasitism in conventional irrigated cotton at Branchview on the Darling Downs (blocks G4 Standard and G4 Soft). The spray details for each block are provided in Table 2. G4 Standard was sprayed 5 times, and G4 Soft was sprayed 4 times.

## APPENDIX 1

The levels of *Helicoverpa* egg parasitism recorded in irrigated cotton at Branchview.

<b>Block</b>	<b>Variety</b>	<b>Date</b>	<b>No. Brown Eggs Collected</b>	<b>% Egg Parasitism</b>
<b>D3</b>	Sicot 71	29-Jan-03	74	52.1
		7-Feb-03	68	29.4
		18-Feb-03	4	96.3
<b>D4</b>	Sicot 289 RRi	19-Dec-02	37	35.3
		8-Jan-03	4	100
		15-Jan-03	6	75.0
<b>G2</b>	Sicot 80	12-Feb-03	36	75.8
		18-Feb-03	22	90.0
		26-Feb-03	34	65.5
<b>G4 Soft</b>	Sicot 80	19-Dec-02	17	20.0
		8-Jan-03	14	21.4
		15-Jan-03	77	43.5
		22-Jan-03	44	71.1
		29-Jan-03	13	55.6
		7-Feb-03	18	55.6
		12-Feb-03	40	94.3
		18-Feb-03	23	91.3
<b>G4 Standard</b>	Sicot 80	19-Dec-02	30	17.2
		8-Jan-03	22	26.3
		15-Jan-03	46	40
		22-Jan-03	50	74.4
		29-Jan-03	16	86.7
		7-Feb-03	13	57.1

## **4. A survey of egg parasitoids in sorghum on the Darling Downs.**

Nat Parker and Brad Scholz.

Queensland Department of Primary Industries and Australian CRC, PO Box 102, Toowoomba 4350.

### **Introduction**

Sorghum is one of the major crops grown on the Darling Downs during summer, with 365,000 ha grown in 2001/02 (ABS data). Ovipositing heliothis moths (*Helicoverpa armigera*) are attracted to sorghum, particularly at the pre-flowering stage of development. The introduction of midge resistant sorghums has had flow-on benefits for heliothis management, i.e. sorghum is now rarely sprayed with broad-spectrum insecticides and is a safe environment for beneficial arthropods. High densities of heliothis eggs are often found in sorghum, and it is most likely an important nursery crop for *Trichogramma* egg parasitoids.

Here we report surveys of heliothis egg parasitism in sorghum across the Darling Downs during 2003.

### **Methods**

Heliothis eggs were collected from fully emerged, pre-flowering sorghum heads at various locations on the Darling Downs. Randomly selected pre-flowering heads were cut using secateurs, and spun into a plastic funnel that emptied into a small 30 ml plastic diet cup. The number of heads sampled in a field varied according to egg density, and ranged from 20-75 heads.

The plastic cups were stored in an esky, and returned to the laboratory for sorting and collecting eggs. Only brown eggs were used to assess the levels of heliothis egg parasitism within each field. The eggs were individually transferred to the 6 mm diameter wells of a plastic microtitre tray (96 wells per tray) using a fine paint brush dipped in water. The trays were covered with sticky tape and stored in a constant temperature room at 25<sup>0</sup>C and 60% R.H. until the egg parasitoids had emerged.

## Results and Discussion

Eighteen collections were made during the survey. The levels of egg parasitism were high in most fields, with 87.1% of all eggs collected parasitised. On fifteen occasions parasitism was over 80% and on six occasions it was over 90% (Figure 1). On one occasion, at Warwick, a parasitism level of 20% was found (Figure 1, 6<sup>th</sup> March). The sorghum from this location was interspersed with weeds that had a strong mint odour. This may have adversely affected the searching behaviour of the *Trichogramma*, and reduced the level of egg parasitism.

There were no *Trichogramma* releases made in the sorghum that was sampled, i.e. the parasitism levels achieved were due to the natural populations of *Trichogramma* that had built up during summer.

The introduced species *Trichogramma pretiosum* was the most common egg parasitoid collected, accounting for 98.6% of all parasitised eggs. Two other species were collected, i.e. *Trichogrammatoidea bactrae* (1.3%) and *Trichogramma (Trichogramma) carverae* (0.1%), both of which are native species. This is a marked change from collections done in sorghum during 1985-88 when 92.1% of the species recovered from the Darling Downs were *T. bactrae* (Scholz 1990).

Clearly, *T. pretiosum* has become widespread and abundant on the Darling Downs. *T. pretiosum* was also recovered from Goondiwindi, and identified in parasitised eggs received from Wee Waa and Gunnedah. This species is available commercially from Bugs for Bugs and is being used by cotton growers in various districts, including Emerald, Goondiwindi, St. George and Wee Waa.

It is likely that *T. pretiosum* will become established in other regions, and every effort should be made to monitor and assess its impact on heliothis eggs. A species that can kill 90% of heliothis eggs is a significant natural enemy, and insecticidal intervention may not always be necessary when it is present. It is, however, important to be aware of the levels of egg parasitism so that unnecessary sprays are avoided. Spray decisions should not be based on egg densities alone if *Trichogramma* are suspected to be present in an area, because the eggs may not hatch. If egg collections are not possible, then the development of neonate larvae should be monitored closely, i.e. when *Trichogramma* are active you find eggs but very few larvae.

High levels of heliothis egg parasitism are associated with sorghum and future research should be directed at evaluating the benefits, if any, of growing sorghum and cotton in mixed farming systems. Sorghum is an important nursery of *Trichogramma*, and these wasps probably move into nearby cotton. Consequently, the mosaic pattern of sorghum and cotton may be important in any given region. That is, large cotton fields may not get as much benefit from sorghum because the fields may be too big, or too distant, for *Trichogramma* to invade and colonise.

## Key findings

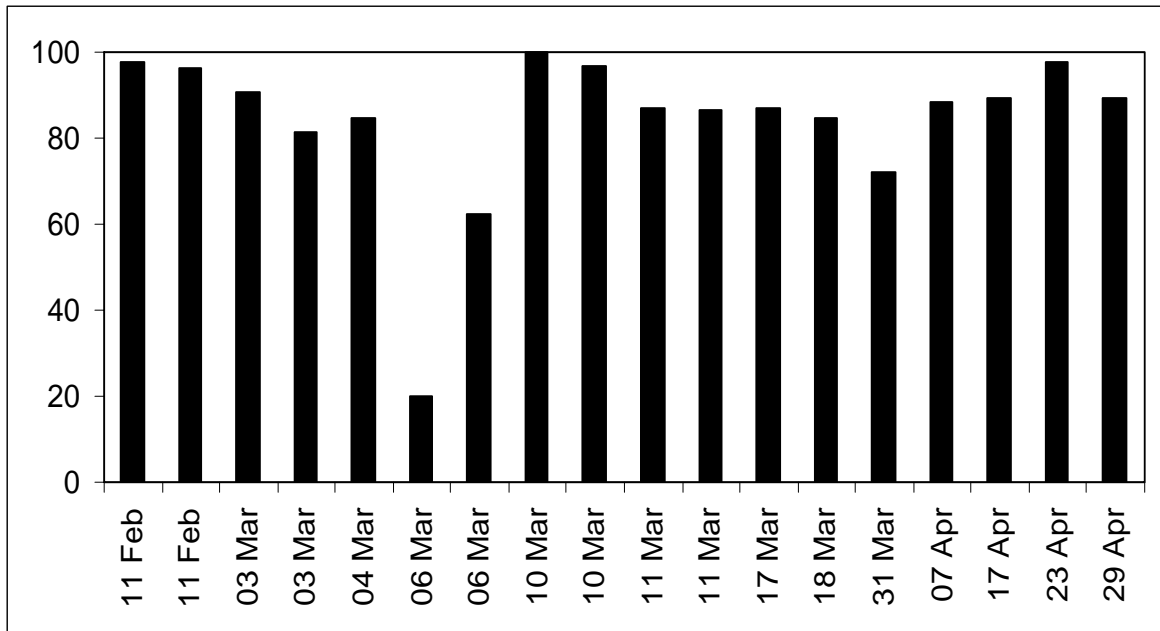
- *Trichogramma* parasitism levels were high (over 80%) in sorghum across the Darling Downs from the beginning of February to the end of April.
- The main egg parasitoid of *Helicoverpa armigera* in sorghum on the Darling Downs was *Trichogramma pretiosum*, accounting for 99% of all eggs parasitised. This species is spreading and should be monitored in other cotton growing districts.

## Acknowledgements

We thank the Cotton Research and Development Corporation for funding the research (project DAQ125C). Mark Hickman and Julie O'Hallaran supplied egg collections from NSW. This assistance is gratefully acknowledged.

## References

Scholz, B.C.G. (1990). Pre-release evaluation studies of egg parasitoids for the management of *Heliothis* in Australian cotton. *Master of Agricultural Science Thesis*, University of Queensland. 203 pages.



**Figure 1.** The percentage of heliothis (*Helicoverpa armigera*) eggs parasitised from various sorghum crops on the Darling Downs during 2003. All of the sorghum sampled was on farms where no *Trichogramma* releases had been carried out.

## APPENDIX 1

The levels of heliothis (*Helicoverpa armigera*) egg parasitism from various sorghum crops on the Darling Downs during 2003. The data are for brown eggs.

Date	Location	GPS	No. Eggs Collected	% Eggs Parasitised
11 Feb	Warra	26 <sup>0</sup> 59.31, 151 <sup>0</sup> 00.52	96	97.6
11 Feb	Dalby	27 <sup>0</sup> 13.25, 151 <sup>0</sup> 19.04	96	96.4
03 Mar	Brookstead	27 <sup>0</sup> 45.38, 151 <sup>0</sup> 27.60	60	90.9
03 Mar	Millmerran	27 <sup>0</sup> 51.24, 151 <sup>0</sup> 19.53	30	81.5
04 Mar	Goondiwindi	28 <sup>0</sup> 37.25, 150 <sup>0</sup> 23.06	45	84.6
06 Mar	Warwick	28 <sup>0</sup> 10.76, 152 <sup>0</sup> 02.79	29	20.0
06 Mar	Hermitage	28 <sup>0</sup> 12.20, 152 <sup>0</sup> 05.99	31	62.5
10 Mar	Kingsthorpe	27 <sup>0</sup> 23.49, 151 <sup>0</sup> 44.69	43	100.0
10 Mar	Evanslea	27 <sup>0</sup> 27.04, 151 <sup>0</sup> 33.54	41	96.7
11 Mar	Kingsthorpe	27 <sup>0</sup> 28.79, 151 <sup>0</sup> 45.68	62	87.0
11 Mar	St Ruth	27 <sup>0</sup> 23.47, 151 <sup>0</sup> 19.20	30	86.4
17 Mar	Evanslea	27 <sup>0</sup> 27.15, 151 <sup>0</sup> 33.82	43	87.1
18 Mar	St Ruth	27 <sup>0</sup> 23.68, 151 <sup>0</sup> 25.42	48	84.8
31 Mar	St Ruth	27 <sup>0</sup> 23.59, 151 <sup>0</sup> 21.99	48	72.2
07 Apr	St Ruth	27 <sup>0</sup> 23.59, 151 <sup>0</sup> 21.99	60	88.2
17 Apr	Evanslea	27 <sup>0</sup> 28.38, 151 <sup>0</sup> 31.91	132	89.1
23 Apr	Evanslea	27 <sup>0</sup> 28.38, 151 <sup>0</sup> 31.91	60	97.9
29 Apr	Evanslea	27 <sup>0</sup> 28.38, 151 <sup>0</sup> 31.91	60	89.1
	<b>TOTALS</b>		<b>1014</b>	<b>87.1</b>

## **5. Lifespan, parasitism levels and progeny production of *Trichogramma pretiosum* reared on *Helicoverpa armigera* eggs in the laboratory.**

Brad Scholz and Nathaniel Parker.

Queensland Department of Primary Industries & Australian Cotton CRC, PO Box 102, Toowoomba 4350.

### **Introduction**

*Trichogramma pretiosum* is a key natural enemy of heliothis (*Helicoverpa* spp.) on the Darling Downs. The data presented here describe some of the biological parameters of *T. pretiosum*, and may be useful in future population models that predict the impact of this valuable egg parasitoid on heliothis.

Here we report on the lifespan, parasitism levels and progeny production of *T. pretiosum* reared on *Helicoverpa armigera* in the laboratory.

### **Methods**

The trials were conducted in a constant temperature room at approximately  $25 \pm 1^\circ\text{C}$  and  $60 \pm 10\%$  R.H.. The strain of *T. pretiosum* was originally collected from Kununurra (Western Australia) and had been reared in the laboratory for 198 generations on eggs of *H. armigera*.

Newly emerged wasps were isolated as pairs (one ♂ and one ♀) in a clear gelatin capsule (Parke-Davis No. 100) for 0.5 hour to allow for mating. The females were then removed and isolated individually in small glass vials (25 x 50 mm) capped with gauze lids for ventilation. The lids were provisioned with a smear of honey as food for the females. Fresh laboratory reared *H. armigera* eggs (< 24 hours old) were added to each vial. Each female wasp was relocated to a new glass vial with fresh *H. armigera* eggs after 2, 4 and 6 hours on the first day, and daily thereafter until death. There were 24 replicates at the start of the trial, and the number of replicates declined as the females died. The maximum number of replicates (24) were alive for six days. Cumulative data were plotted for days 1-10, as there were less than 10 females alive after the tenth day.

All vials were held in the constant temperature room at  $25^\circ\text{C}$  and 60% R.H. until the parasitoids had emerged. The numbers of eggs parasitised and progeny produced were counted, and all progeny were sexed. Larvae that hatched from unparasitised eggs were removed daily to prevent them from feeding on parasitised eggs.

## Results and Discussion

Female *T. pretiosum* lived for  $10.1 \pm 0.6$  days in the laboratory. During this time they parasitised  $58.3 \pm 4.4$  *H. armigera* eggs and produced  $50.6 \pm 3.8$  female offspring and  $20.7 \pm 3.8$  male offspring. Females comprised 72.6% of all progeny.

Pak and Oatman (1982) found that *T. pretiosum* had a lifespan of 10.2 days at 25°C. They conducted their work on cabbage looper, *Trichoplusia ni*, eggs. *T. pretiosum* parasitised 51.3 *T. ni* eggs and produced 50.0 female offspring, with females comprising 77.9% of progeny. These figures are similar to those reported here for *H. armigera*.

The daily levels of egg parasitism and progeny production are shown in Figure 1. Most eggs were parasitised on day 1, and 44% of these were parasitised within the first 2 hours (Figure 1). The levels of egg parasitism generally remained over 5 eggs/day up until day 8, while the production of female progeny declined steadily over time. By contrast, male progeny production peaked on day 1, dropped markedly and then rose steadily (Figure 1), presumably because the sperm supply within the females were gradually declining (*Trichogramma* eggs typically have to be fertilised to produce female offspring). The sex ratio of progeny was female biased for 11 days, and peaked on day 2 (Figure 2).

Strong correlations were found between the numbers of eggs parasitised and progeny production over time (Figure 3), and may be useful in models that predict the impact of *Trichogramma* on heliothis mortality.

## Key Findings

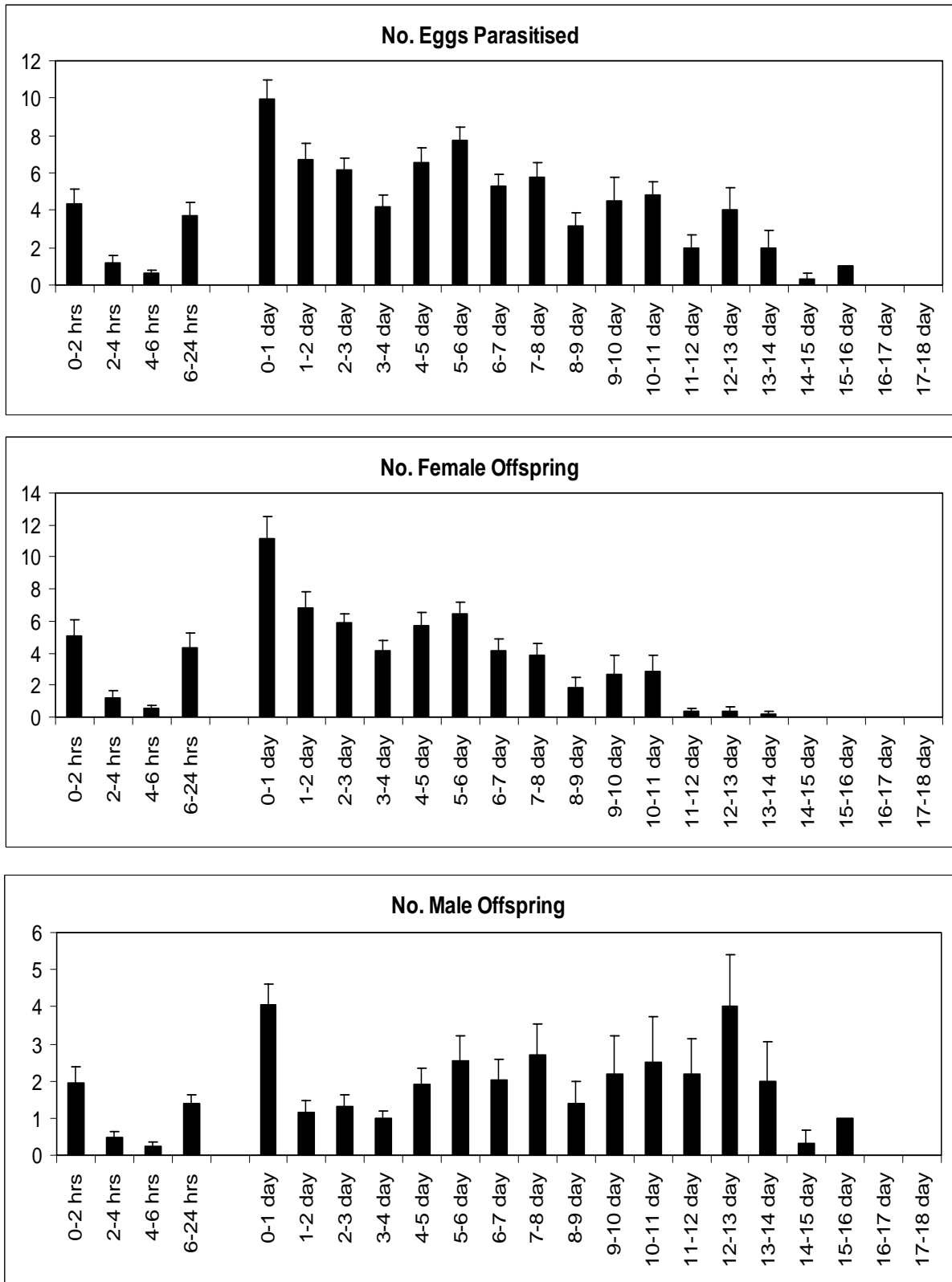
- *Trichogramma pretiosum* parasitises 58 *Helicoverpa armigera* eggs per lifetime in the laboratory. Approximately 10 eggs were parasitised on the first day.
- *T. pretiosum* progeny production is female biased, with over 70% of offspring produced being females. *T. pretiosum* females produced 51 female offspring in the laboratory. If a female always produces 51 female offspring, then one female can theoretically produce 6.7 million female offspring in four generations (approx. 40 days in summer). Only female wasps attack eggs.
- *T. pretiosum* females live for ca. 10 days in the laboratory.

## **Acknowledgements**

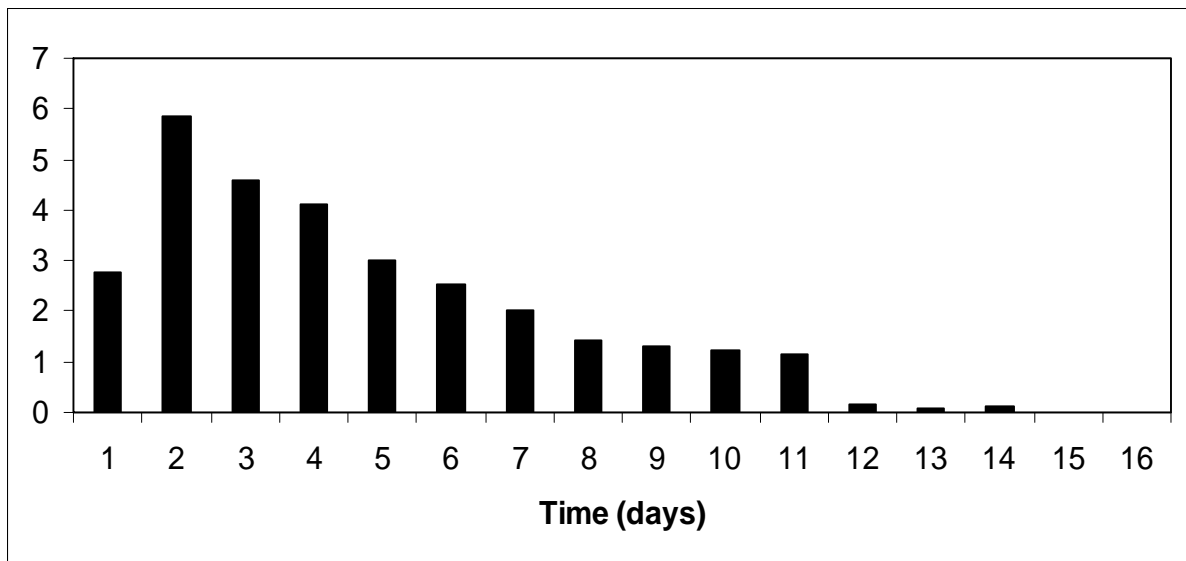
We thank Sue Maclean (QDPI Toowoomba) for supplying *H. armigera* eggs, and the Cotton Research and Development Corporation for funding the research (project DAQ 125C). This assistance is gratefully acknowledged.

## **References**

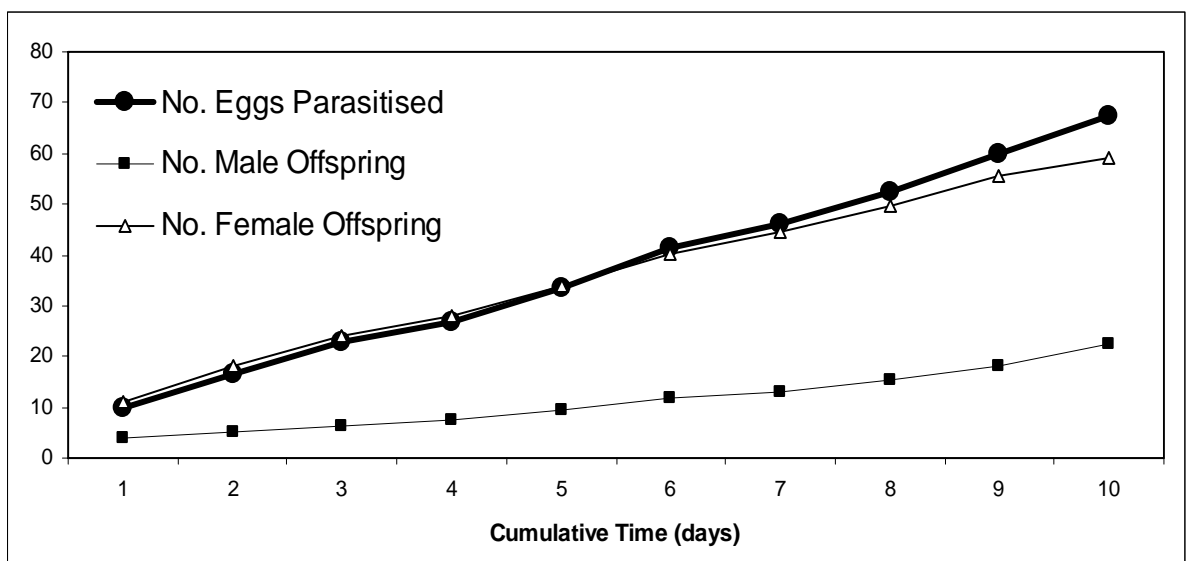
Pak, G.A. and Oatman, E.R. (1982). Comparative life table, behavior and competition studies of *Trichogramma brevicapillum* and *T. pretiosum*. *Entomologia Experimentalis et Applicata* 32: 68-79.



**Figure 1:** Laboratory biological parameters for *Trichogramma pretiosum* reared on eggs of *Helicoverpa armigera* at 25°C and 60% RH. Data represent the mean  $\pm$  standard error of a starting study group of 24 females. Day 1 assessments were completed 2, 4, 6 and 24 hours after the females were first given access to eggs. Thereafter the assessments were completed daily.



**Figure 2:** Daily sex ratio (females : males) of *Trichogramma pretiosum* reared on eggs of *Helicoverpa armigera* at 25°C and 60% R.H..



**Figure 3:** Cumulative parasitism levels and progeny production for *Trichogramma pretiosum* reared on eggs of *Helicoverpa armigera* at 25°C and 60% R.H.. The following linear regressions were fitted to the data:

No. Eggs Parasitised =	6.72 x Time (days)	$r^2 = 0.990$
No. Male Offspring =	2.05 x Time (days)	$r^2 = 0.959$
No. Female Offspring =	6.35 x Time (days)	$r^2 = 0.952$

## APPENDIX 1

Some basic lifetime biological parameters for *Trichogramma pretiosum* parasitising eggs of *Helicoverpa armigera* in the laboratory at 25°C and 60% RH. Data are the mean  $\pm$  standard error of  $n$  samples.

Time	$n$	No. Eggs Parasitised	No. Male Offspring	No. Female Offspring	% Females
0-2 hrs	24	4.4 $\pm$ 0.8	2.0 $\pm$ 0.4	5.1 $\pm$ 1.0	72.9 $\pm$ 4.7
2-4 hrs	24	1.2 $\pm$ 0.4	0.5 $\pm$ 0.2	1.2 $\pm$ 0.5	69.9 $\pm$ 9.4
4-6 hrs	24	0.6 $\pm$ 0.2	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	66.0 $\pm$ 12.9
6-24 hrs	24	3.7 $\pm$ 0.7	1.4 $\pm$ 0.3	4.3 $\pm$ 0.9	75.6 $\pm$ 3.3
0-1 day	24	9.9 $\pm$ 1.1	4.0 $\pm$ 0.6	11.2 $\pm$ 1.3	70.6 $\pm$ 4.0
1-2 day	24	6.7 $\pm$ 0.9	1.2 $\pm$ 0.3	6.8 $\pm$ 1.0	85.8 $\pm$ 3.5
2-3 day	24	6.1 $\pm$ 0.7	1.3 $\pm$ 0.3	5.9 $\pm$ 0.6	85.6 $\pm$ 3.1
3-4 day	24	4.2 $\pm$ 0.6	1.0 $\pm$ 0.2	4.1 $\pm$ 0.6	74.4 $\pm$ 5.4
4-5 day	24	6.6 $\pm$ 0.7	1.9 $\pm$ 0.4	5.8 $\pm$ 0.7	74.3 $\pm$ 4.5
5-6 day	24	7.7 $\pm$ 0.7	2.5 $\pm$ 0.7	6.4 $\pm$ 0.8	73.7 $\pm$ 5.6
6-7 day	23	5.3 $\pm$ 0.7	2.0 $\pm$ 0.5	4.1 $\pm$ 0.8	66.8 $\pm$ 7.7
7-8 day	20	5.8 $\pm$ 0.8	2.7 $\pm$ 0.8	3.9 $\pm$ 0.8	63.7 $\pm$ 9.5
8-9 day	16	3.1 $\pm$ 0.8	1.4 $\pm$ 0.6	1.8 $\pm$ 0.7	61.7 $\pm$ 13.1
9-10 day	10	4.5 $\pm$ 1.3	2.2 $\pm$ 1.0	2.7 $\pm$ 1.1	51.7 $\pm$ 18.6
10-11 day	6	4.8 $\pm$ 0.7	2.5 $\pm$ 1.2	2.8 $\pm$ 1.0	53.9 $\pm$ 19.3
11-12 day	6	2.0 $\pm$ 0.7	2.2 $\pm$ 1.0	0.3 $\pm$ 0.2	26.7 $\pm$ 19.4
12-13 day	6	4.0 $\pm$ 1.2	4.0 $\pm$ 1.4	0.3 $\pm$ 0.3	13.3 $\pm$ 13.3
13-14 day	5	2.0 $\pm$ 0.9	2.0 $\pm$ 1.0	0.2 $\pm$ 0.2	16.7 $\pm$ 16.7
14-15 day	3	0.3 $\pm$ 0.3	0.3 $\pm$ 0.3	0 $\pm$ 0	0
15-16 day	1	1.0	1.0	0	0
16-17 day	1	0	0	0	
17-18 day	1	0	0	0	
Lifetime	24	58.3 $\pm$ 4.4	20.7 $\pm$ 3.8	50.6 $\pm$ 3.8	72.6 $\pm$ 3.3
Mean Lifespan (days)	24	10.1 $\pm$ 0.6			

## 6. An evaluation of the toxicity of two paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on *Trichogramma pretiosum*.

Nat Parker<sup>A</sup>, Brad Scholz<sup>A</sup> and Robert Mensah<sup>B</sup>.

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<sup>B</sup>NSW Agriculture, Australian Cotton Research Institute, Locked Bag 1000, Narrabri 2390.

### Introduction

*Trichogramma pretiosum* Riley is an important parasitoid of heliothis (*Helicoverpa* spp.) eggs on the Darling Downs. In the past heliothis have been managed using broad-spectrum insecticides such as pyrethroids and organophosphates. These insecticides usually cause high mortality of *Trichogramma* and other beneficial insects. Increasingly farmers and consultants are looking for soft chemistry to control pests without killing the beneficial arthropods in the farming system. Paraffin oils are now being explored as an option to control heliothis in cotton without causing high mortality of beneficial arthropods. *Trichogramma* are particularly sensitive to chemical insecticides and can act as bioindicators of the toxicity of insecticidal products. If an insecticide does not impact on *Trichogramma* it is likely it will be conducive to most of the beneficial fauna in the farm ecosystem.

This report documents the impact of two paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on *Trichogramma pretiosum* during larval, pupal and adult stages of development.

### Methods

#### *Egg card bioassay*

The effects of dipping parasitised heliothis eggs in paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on *Trichogramma pretiosum* larvae was studied during different stages of immature *Trichogramma* development. *Helicoverpa armigera* eggs laid onto sheets of paper towelling (Teakle and Jensen 1985) were parasitised by *T. pretiosum* for two hours in the laboratory. The sheet of parasitised eggs was cut into 1 x 3 cm strips and each strip was stapled onto a piece of standard white paper, measuring ca. 1.5 x 7 cm, to make handling and dipping the eggs easier.

Ten randomly selected egg cards were dipped into each treatment (Table 1) for one second on days 1, 2, 4, 5 and 9 after oviposition. These developmental stages corresponded approximately to *Trichogramma* eggs (d1), larvae (d2), pre-pupae (d4), early pupae (d5) and late pupae (d9). Dipped egg cards were hung on a string-using fold back clips to dry at ambient temperature in the treatment room. Once dry, each egg card was placed in a small glass vial and held in a constant temperature room at 25°C and 60% R.H. until all healthy parasitoids had emerged.

**Table 1.** Treatment details.

<b>Treatment</b>	<b>Insecticide Formulation</b>	<b>Active constituent</b>	<b>Oils (% volume)</b>
Water	Control	Nil	Nil
Canopy	Paraffin Oil	815 g/l	2%
Biopest	Paraffin Oil	792 g/l	2%

#### ***Dipped surface residue bioassays***

The effect of paraffin oil residues on the survival of adult *Trichogramma pretiosum* was studied in ventilated glass bioassay chambers measuring 15 cm long and 4 cm in diameter (Scholz 1994). Whatmans filter paper was saturated in a solution of each treatment (Table 1). The filter paper was then hung on a string with fold back clips and allowed to dry. The treated filter paper was used to line the inside of the glass bioassay tubes.

The same technique was also used to test residues on cotton leaves.

Approximately 20 newly emerged *T. pretiosum* wasps were placed into each bioassay tube and exposed to the treated filter paper/cotton leaves for four hours. After exposure the numbers of dead and live wasps were recorded.

#### ***Direct contact bioassays (adults)***

The contact impact of paraffin oils on adult *T. pretiosum* was evaluated by spraying wasps held in fine gauze cages (Scholz and Zalucki 2000). The cages were 5 x 10 cm cylinders constructed from very fine *Trichogramma* proof stainless steel mesh (47 strands/cm; 0.125 mm aperture; 0.08 mm diameter wire), with a removable lid at each end. A 4 cm hole was cut in each lid and covered with the same wasp proof stainless steel mesh.

Approximately 20 newly emerged *T. pretiosum* wasps were transferred to each cage. The cages were hung on string then sprayed with each treatment from a distance of 30cm using a hand held atomiser.

The cages were left to dry for 20 minutes before the numbers of dead and live wasps were counted. Water sensitive paper was placed in some cages to monitor the amount of spray penetrating the walls of the cages. Good spray penetration was achieved.

### ***Boom-sprayed bioassays***

To better simulate a field application of insecticide additional experiments were carried out using a hand held boom sprayer.

Biopest<sup>®</sup>, Canopy<sup>®</sup> and water were applied through a boom spray onto parasitised heliothis eggs to see if the treatments had an effect on *Trichogramma* emergence. Two day old parasitised egg cards were prepared, as previously described, and temporarily stuck to a cement path with sticky tape over a length of ten metres.

The path was sprayed with a 2 m wide hand held boom sprayer. DG Tee Jet 110015 nozzles were used giving a flow rate of 101L/ha at 3 bar and a speed of 7 km hr. The boom was held 0.5 metres above the cement and applied over the egg cards. A 2% oil concentration was used for the Biopest<sup>®</sup> and Canopy<sup>®</sup> treatments. Once dry, each egg card was placed in a small glass vial and held in a constant temperature room at 25°C and 60% R.H. until all healthy parasitoids had emerged.

The boom sprayer was also used to treat cotton leaves for a bioassay. Three series of potted cotton plants were placed on a 10m strip of grass, and sprayed with the different treatments (Table 1). The treated leaves were allowed to dry, and ten leaves were randomly selected and placed into ventilated glass bioassay chambers (as described above). Approximately 20 newly emerged *T. pretiosum* wasps were placed into each bioassay tube and exposed to the treated leaves for four hours. After exposure the numbers of dead and live wasps were recorded.

The impact of the direct contact of boom-spray droplets on *T. pretiosum* adults was also assessed during the same application. Eight fine gauze cages containing ca. 20 adult *Trichogramma* were hung on sticks randomly over each 10 m strip and sprayed at the same time as the leaves. The cages were left to dry for 20 minutes before the numbers of dead and live wasps were counted.

## **Results**

### ***The effect of treatments on immature development***

*Trichogramma* emergence was significantly reduced following dipping parasitised *Helicoverpa armigera* eggs into solutions of Canopy<sup>®</sup> and Biopest<sup>®</sup> at all stages of immature development. Emergence from eggs treated with Canopy<sup>®</sup> was significantly higher than Biopest<sup>®</sup> one and five days after *Trichogramma* oviposition. At two, four and nine days after *Trichogramma* oviposition there was no significant difference in emergence of eggs treated with Canopy<sup>®</sup> and Biopest<sup>®</sup> (Figure 1).

There was no significant difference in the emergence of parasitoids when parasitised heliothis eggs were sprayed by boom (Figure 2).

#### ***The effect of surface residues on adult survival***

The mortality of *Trichogramma* adults exposed to oil on dipped filter paper and dipped cotton leaves was significantly higher than that in the control (Figure 3). There was no significant difference in mortality between treatments when *Trichogramma* adults were exposed to cotton leaves treated by boom spray (Figure 4).

#### ***The effect of direct contact on adult survival***

There was no significant difference between mortality of *Trichogramma* adults directly sprayed by treatments in cages (Figure 5).

## **Discussion**

The results highlighted differences between the dipping and spraying assessments used to assess the impact of paraffin oils on developing *Trichogramma*, and the impact of oil residues on adult wasp survival.

Dipping parasitised eggs and leaves had a greater impact on parasitoid survival than spraying (Figure 1 vs. 2; Figure 3 vs. 4). This suggests that the oils can ‘smother’ development when coverage is complete (Figures 1 and 3). Survival was not adversely affected when coverage was incomplete, i.e. following a spray (Figures 2 and 4).

The survival of adult *Trichogramma* on dipped cotton leaves decreased from 94% in the control to 50% and 58% on the Canopy<sup>®</sup> and Biopest<sup>®</sup> treated leaves respectively (Figure 3). The paraffin oils did not dry well on cotton leaves. Small pools of oil remained on some leaves. These pools trapped *Trichogramma* that came into contact with them, i.e. they drowned. Consequently some of the mortality recorded was a result of this drowning, rather than the action of a residual toxin.

The findings suggest that sprayed paraffin oils are reasonably safe on *Trichogramma* at the rates and volumes likely to be used in cotton. However, paraffin oils can cause considerable mortality of developing *Trichogramma* when parasitised eggs are immersed in oil solutions. This may be an issue in high volume spray applications, e.g. horticulture where 500-800 L/ha spray volumes are used.

## Key Findings

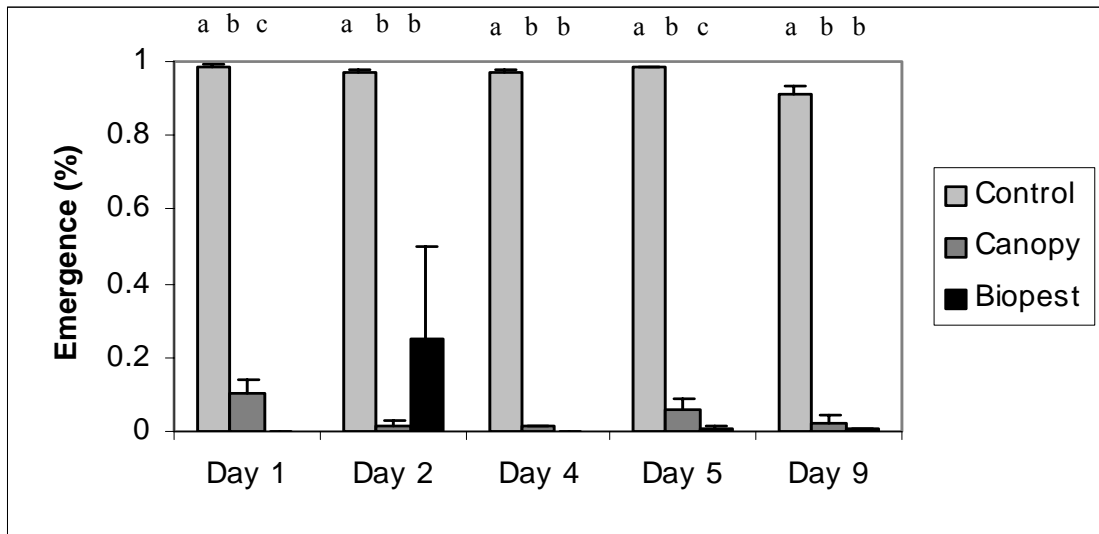
- The development of immature *Trichogramma* in parasitised eggs was adversely affected by dipping parasitised eggs in paraffin oils. However, survival was not significantly affected by spraying parasitised eggs with paraffin oils. This suggests that coverage is an important factor when considering the impact of paraffin oils on immature *Trichogramma*.
- The survival of adult *Trichogramma* on filter paper and cotton leaves dipped in paraffin oils was significantly lower than that in water alone, but was not significantly different when the wasps were exposed to sprayed cotton leaves. The assessment technique – dipping vs. spraying – gave contrasting results.
- The survival of adult *Trichogramma* in sprayed wire cages was not significantly affected by paraffin oils.
- Paraffin oils are likely to be reasonably safe on *Trichogramma pretiosum* at the recommended cotton rates and volumes.

## Acknowledgements

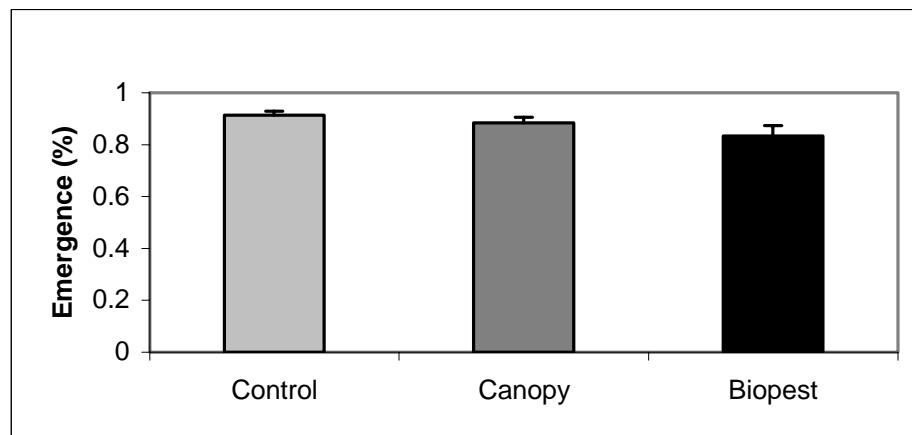
We thank the Cotton Research and Development Corporation for funding the research (project DAQ125C) and Sue Maclean (DPI Toowoomba) for supplying *Helicoverpa armigera* eggs. This assistance is gratefully acknowledged.

## References

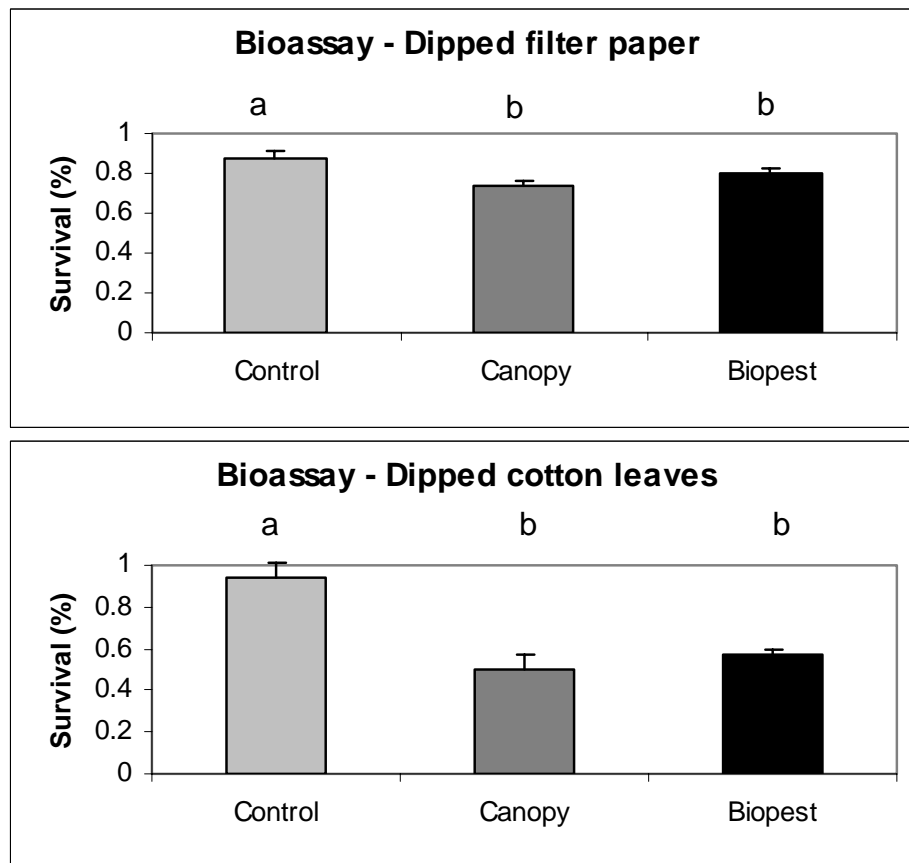
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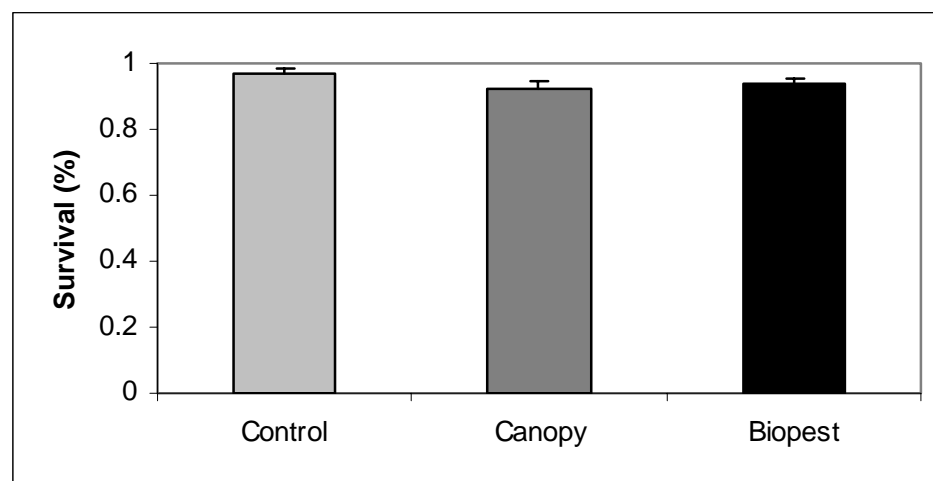
**Figure 1:** The emergence of *Trichogramma pretiosum* wasps after **dipping** parasitised *H. armigera* eggs in paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>). The age of the parasitised eggs ranged from 1 to 9 days. Values are the mean  $\pm$  standard error of 10 replicates, and were arcsine transformed for analyses. Means for a given day followed by the same letter are not significantly different ( $P=0.05$ , ANOVA, Fisher's LSD comparison).



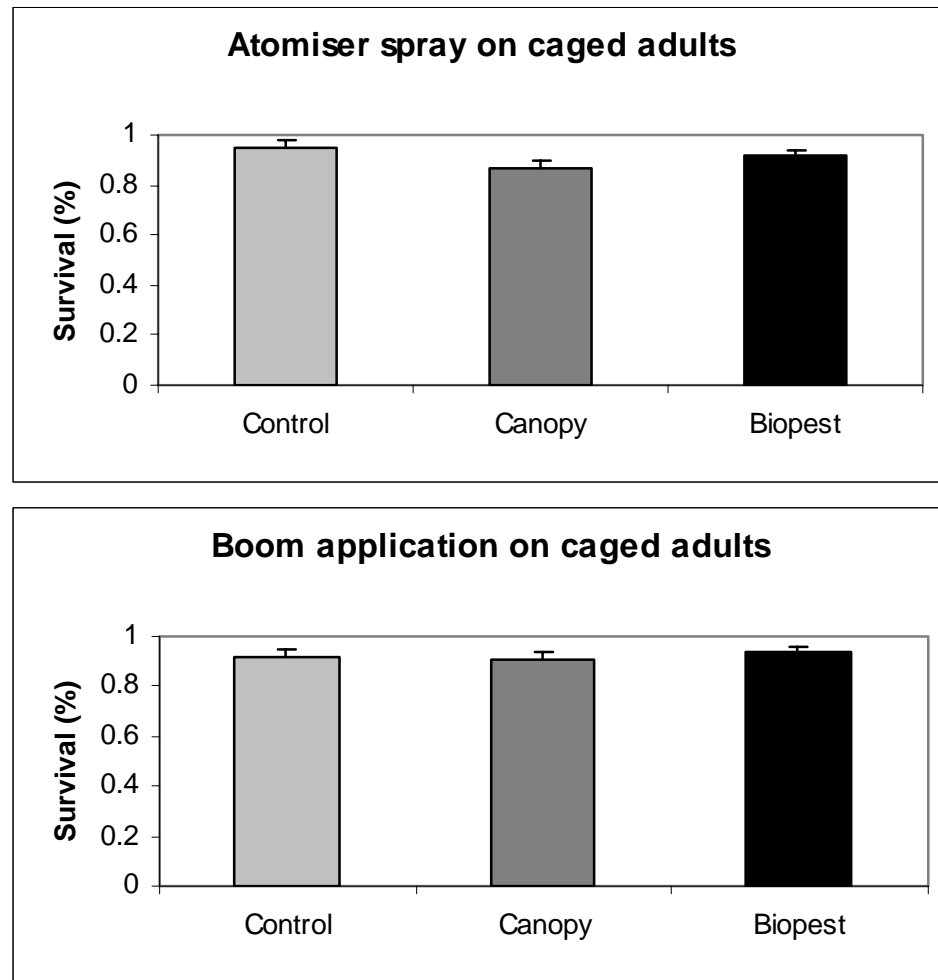
**Figure 2:** The emergence of *Trichogramma pretiosum* wasps after **spraying** 2-day old parasitised *H. armigera* eggs with paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>). Values are the mean  $\pm$  standard error of 10 replicates, and were arcsine transformed for analyses. There was no significant difference in survival ( $P=0.05$ , ANOVA).



**Figure 3:** The survival of adult *Trichogramma pretiosum* wasps after 4 hours exposure to paraffin oil residues on **dipped** filter paper or **dipped** cotton leaves. Values are the mean  $\pm$  standard error of 10 replicates, and were arcsine transformed for analyses. Means followed by the same letter are not significantly different ( $P=0.05$ , ANOVA, Fisher's LSD comparison).



**Figure 4:** The survival of adult *Trichogramma pretiosum* wasps after 4 hours exposure to paraffin oil residues on **sprayed** cotton leaves. Values are the mean  $\pm$  standard error of 10 replicates, and were arcsine transformed for analyses. There was no significant difference in survival ( $P=0.05$ , ANOVA).



**Figure 5:** The survival of adult *Trichogramma pretiosum* wasps after exposure to paraffin oil residues in **sprayed** wire cages. Values are the mean  $\pm$  standard error of 8 replicates, and were arcsine transformed for analyses. There was no significant difference in survival for either mode of spraying ( $P=0.05$ , ANOVA).

## APPENDICES

**Appendix 1:** The impact of **dipping** parasitised heliothis eggs in paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on the emergence of *Trichogramma pretiosum*. Data are mean  $\pm$  standard error of ten replicates and were arcsine transformed for analyses. Column means followed by the same letter are not significantly different (P=0.05, ANOVA, Fisher's LSD comparison).

% <i>Trichogramma</i> Emergence										
Dipping Day*	Day 1		Day 2		Day 4		Day 5		Day 9	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	0.986 a	0.005	0.97 a	0.011	0.97 a	0.008	0.982 a	0.005	0.911 a	0.026
Canopy	0.103 b	0.034	0.015 b	0.011	0.012 b	0.005	0.062 b	0.024	0.024 b	0.017
Biopest	0 c	0	0.25 b	0.25	0 b	0	0.011 c	0.005	0.006 b	0.005

\* Dipping day = number of days after *Trichogramma* oviposition that egg cards were dipped in treatments.

**Appendix 2:** The impact of **boom-spraying** 2-day old parasitised heliothis eggs with paraffin oils (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on the emergence of *Trichogramma pretiosum*. Data are mean  $\pm$  standard error of ten replicates and were arcsine transformed for analyses. There was no significant difference in the emergence between treatments (P=0.05, ANOVA).

Treatment	% Emergence	
	Mean	SE
Control	0.913	0.016
Canopy	0.885	0.021
Biopest	0.834	0.039

**Appendix 3:** The impact of paraffin oil residues (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on the percentage survival of *Trichogramma pretiosum* after 4 hours exposure to **dipped** filter paper or cotton leaves. Data are mean  $\pm$  standard error of ten replicates and were arcsine transformed for analyses. Column means followed by the same letter are not significantly different (P=0.05, ANOVA, Fisher's LSD comparison).

Treatment	Filter Paper		Cotton Leaves	
	Mean	SE	Mean	SE
Control	0.88 a	0.028	0.938 a	0.014
Canopy	0.733 b	0.034	0.501 b	0.071
Biopest	0.805 b	0.021	0.576 b	0.074

**Appendix 4:** The impact of paraffin oil residues (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on the survival of *Trichogramma pretiosum* after 4 hours exposure to **sprayed** cotton leaves. Data are mean ± standard error of ten replicates and were arcsine transformed for analyses. There was no significant difference in the emergence between treatments (P=0.05, ANOVA).

Treatment	% Survival	
	Mean	SE
Control	0.972	0.013
Canopy	0.924	0.024
Biopest	0.935	0.015

**Appendix 5:** The direct contact impact of paraffin oil **sprays** (Biopest<sup>®</sup> and Canopy<sup>®</sup>) on the percentage survival of adult *Trichogramma pretiosum* in wire cages. Data are mean ± standard error of eight replicates and were arcsine transformed for analyses. There was no significant difference in the emergence between treatments for either method of spraying (P=0.05, ANOVA).

Treatment	Atomiser		Boom Spray	
	Mean	SE	Mean	SE
Control	0.949	0.017	0.919	0.029
Canopy	0.866	0.049	0.907	0.030
Biopest	0.914	0.029	0.933	0.027

## 7. Validating molecular diagnostics for assessing levels of *Helicoverpa* spp. egg parasitism.

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### Introduction

*Trichogramma pretiosum* is an important egg parasitoid of heliothis (*Helicoverpa* spp.) on the Darling Downs and in some other cotton growing regions. Sometimes the levels of egg parasitism are in excess of 90%, and as such represent an important mortality factor.

Assessments of the levels of egg parasitism are important so that spray decisions can be made that take into account the egg mortality caused by egg parasitoids. When parasitism is high sprays may not be necessary.

To monitor the levels of egg parasitism brown eggs are collected, transferred to the wells of microtitre trays, and held until egg hatch and parasitism can be evaluated. This method relies on the natural development of heliothis and *Trichogramma*. Normal (unparasitised) brown heliothis eggs hatch approximately two days after collection, and parasitised brown eggs turn jet black approximately three days after collection and storage at 25°C. Earlier detection of the levels of egg parasitism (and hence egg mortality) would greatly assist consultants in making pest management decisions.

The Centre for Insect Diagnostics (CID), University of Queensland, has been developing a DNA diagnostic test for assessing the levels of heliothis egg parasitism. The test is currently specific for *Trichogramma pretiosum*. It is also possible to identify eggs to species, i.e. *H. armigera* or *H. punctigera*. These tests can be completed in four hours and have the potential to more rapidly assess egg parasitism levels than conventional developmental methods. If parasitism levels were known quickly, consultants could make spray decisions incorporating the egg mortality due to *T. pretiosum*.

Here we compare the levels of egg parasitism in paired egg collections from cotton and sorghum on the Darling Downs. For each paired collection a sample of eggs was sent to the CID for DNA diagnostic analyses of egg parasitism, and a matching sample was held at the DPI Toowoomba Entomology laboratories for standard developmental assessments of egg parasitism.

## Methods

Heliothis eggs were collected from various sorghum and cotton crops on the Darling Downs from February to April 2003.

### *Sorghum Sampling*

Pre-flowering heads were cut and spun in a funnel that emptied into a 30 mL plastic cup. The eggs from a five sorghum heads were collected in a cup, and 25-50 heads were selected at random from each site. The cups were returned to the laboratory, and the eggs were isolated individually in the cells of plastic microtitre trays using a fine paint brush dipped in water.

### *Cotton Sampling*

Leaves and squares with eggs laid onto them were picked from plants and placed into a bry-nylon cloth bag. The leaves were returned to the laboratory, and the eggs were isolated individually in the cells of plastic microtitre trays using a fine paint brush dipped in water.

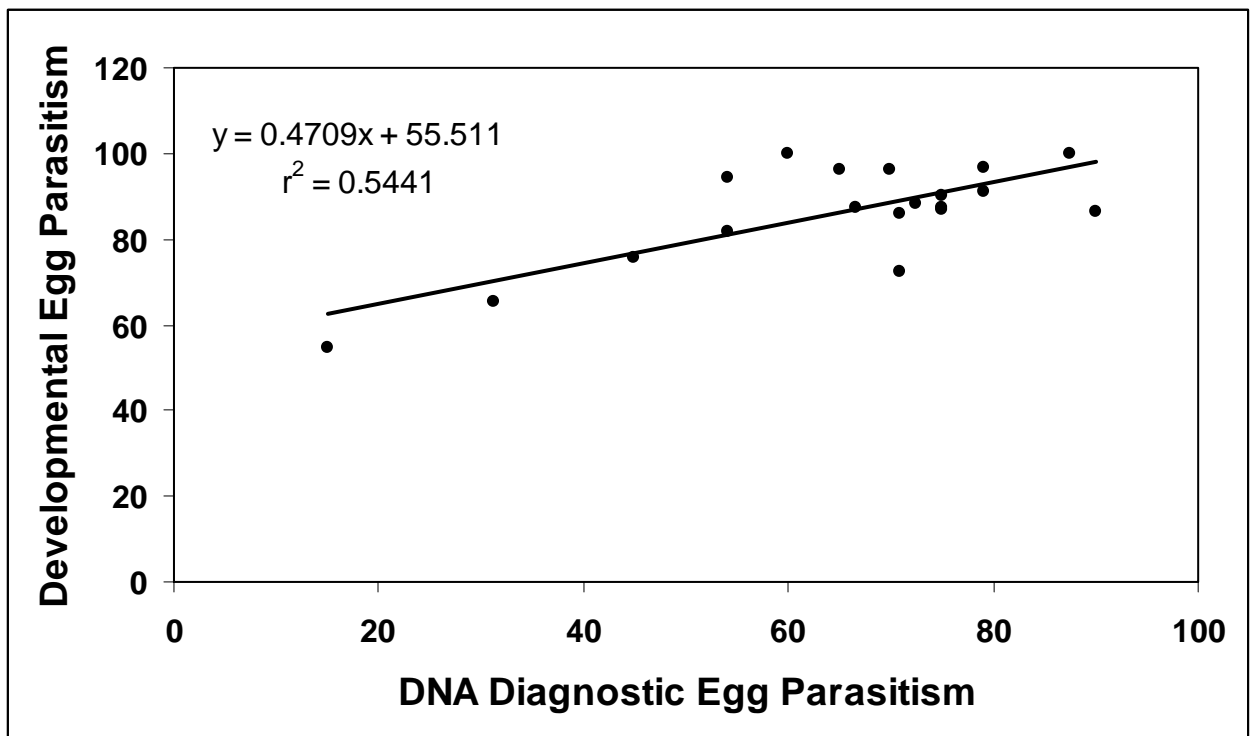
The eggs were sorted according to colour, viz. white and brown, and transferred to separate rows within each microtitre tray. Two sub-samples were taken from each collection. One sub-sample (12-30 eggs) was sent to the CID for DNA diagnostic testing of the levels of egg parasitism, and to identify eggs to species. The partner sub-sample was held in the Toowoomba DPI Entomology laboratory at 25°C and 60% R.H. to determine the levels of egg parasitism after standard development. A total of 19 paired samples were assessed. The levels of brown egg parasitism were used to compare the diagnostic and developmental methods.

## Results and Discussion

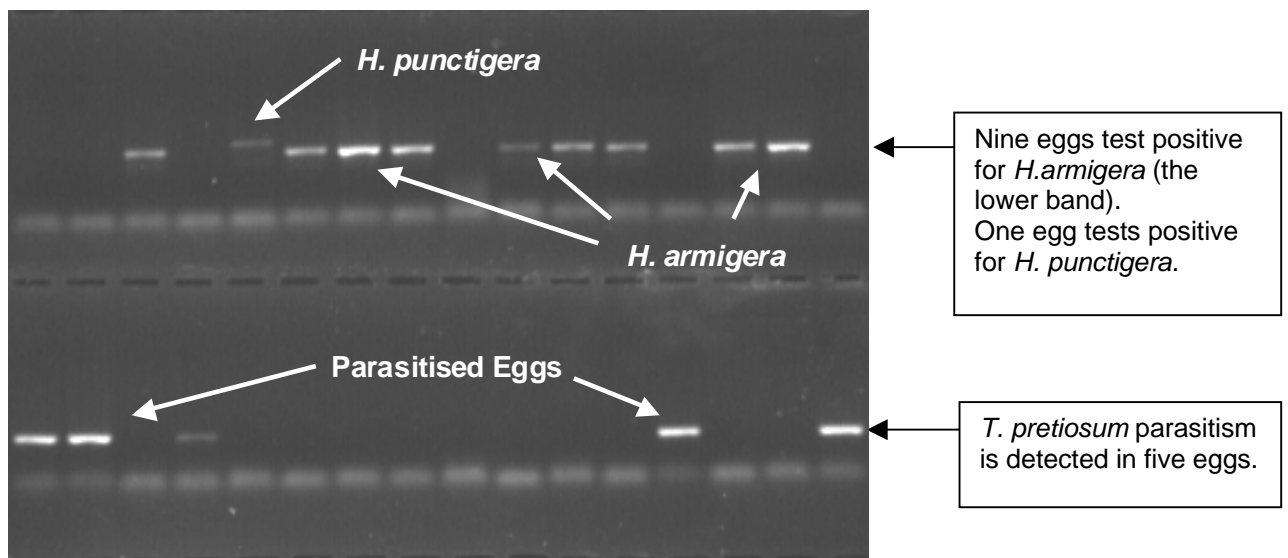
*Trichogramma pretiosum* parasitism was readily detected using the CID PCR method, and a correlation was found between the two techniques (Figure 1). The DNA test consistently underestimated the levels of parasitism. The average DNA estimate of parasitism was 74% that of the developmental standard.

## Key Findings

- A DNA test for assessing heliothis egg parasitism was correlated with standard developmental assessments of egg parasitism. The test is specific for *Trichogramma pretiosum*, and does not detect parasitism by other species of egg parasitoids.
- The DNA test has potential to rapidly estimate the levels of egg parasitism without having to wait for developmental changes, provided eggs can be collected and delivered for testing quickly.



**Figure 1:** The relationship between the levels of heliothis (*Helicoverpa* spp.) egg parasitism detected using a DNA diagnostic test compared with standard developmental assessments of egg parasitism. Naturally laid brown eggs were collected from cotton and sorghum, and the samples were split for the comparisons.



**Figure 2:** Agarose electrophoresis gel showing results for 16 *Helicoverpa* spp. eggs. Five eggs tested positive to parasitism by *Trichogramma pretiosum*. Unparasitised eggs can be identified to species – in this case nine were *H. armigera*, and one was *H. punctigera*.

## APPENDIX 1

The levels of brown egg parasitism found by DNA diagnostic and standard developmental methods for *Helicoverpa* spp. egg collections from sorghum and cotton on the Darling Downs. The DNA test detected egg parasitism by *T. pretiosum*.

Site	Collection Date	Crop	DNA Diagnostic Egg Parasitism (%)	Development Egg Parasitism (%)
Branchview G4 Soft	7-Feb-03	Cotton	15.0	54.6
Warra	11-Feb-03	Sorghum	54.2	94.2
Dalby	11-Feb-03	Sorghum	70.0	96.4
Branchview G2	12-Feb-03	Cotton	45.0	75.8
Branchview D3	12-Feb-03	Cotton	65.0	96.3
Kingsthorpe Control	13-Feb-03	Cotton	75.0	87.5
Kingsthorpe Release	13-Feb-03	Cotton	60.0	100.0
Branchview G2	18-Feb-03	Cotton	75.0	90.0
Branchview G2	26-Feb-03	Cotton	31.3	65.5
Brookstead	3-Mar-03	Sorghum	79.2	90.9
Millmerran	3-Mar-03	Sorghum	54.2	81.5
Evanslea - Neil Nass	10-Mar-03	Sorghum	79.2	96.8
Kingsthorpe - Doug Gordon	10-Mar-03	Sorghum	87.5	100.0
Kingsthorpe - Dalby Hwy	11-Mar-03	Sorghum	75.0	87.0
St Ruth - Jondaryan	11-Mar-03	Sorghum	90.0	86.4
Evanslea - Neil Nass	17-Mar-03	Sorghum	66.7	87.5
St Ruth - Jondaryan	18-Mar-03	Sorghum	70.8	86.1
St Ruth - Jondaryan	31-Mar-03	Sorghum	70.8	72.2
St Ruth - Jondaryan	7-Apr-03	Sorghum	72.5	88.2

## 8. TECHNICAL APPENDIX 1

### Mounting *Trichogramma* on microscope slides

*Notes prepared after talks with Gary Platner, University of California, Riverside.  
A detailed description of techniques can be found in Platner et al. (1999).*

Specimens of *Trichogramma* need to be mounted on microscope slides for identification. The following technique is used at the University of California (Riverside).

**Preparation:** Specimens that have been stored for a long time (over 2 years) in alcohol may become distended. A sharp minuten pin should be used to pierce the thorax and abdomen from side to side. Live specimens should be killed in 70% alcohol, and treated the same as alcohol preserved specimens.

**KOH Treatment:** The specimen has to be cleared in potassium hydroxide (KOH). Transfer the specimen from the 70% alcohol into a watchglass containing 10% KOH. Place the watchglass in a sealed container with a dish of water. This will prevent the KOH from evaporating overnight. Soak the specimen in KOH overnight at room temperature. After soaking, check the amount of clearing. Heavily sclerotised specimens may require additional clearing.

**Alcohol Treatment:** After clearing, remove the KOH and replace with 10% alcohol (to which a small amount of dishwashing detergent has been added). Specimens can be left in 10% alcohol for 24 hours if necessary. Each specimen is then transferred through a series of alcohol baths.

Firstly transfer to a depression plate containing 20% alcohol. A minuten pin shaped to form a small loop is useful for transferring specimens. Carefully position the specimen between two 5 mm coverslips and gently flatten the specimen dorso-ventrally so that a desired mounting position is attained. The cuticle will remain soft until the specimen is transferred to higher alcohol concentrations.

Remove from the 20% alcohol, and transfer to 50% alcohol. Repeat this process using 70%, 95%, 95% (two times at 95%) and 100% alcohol. Allow 30 minutes in each concentration.

**Mounting:** After the final alcohol bath, transfer the specimen to clove oil for 30 minutes. The specimen is now ready for mounting in Canada Balsam. *Trichogramma* are usually mounted whole, with one specimen per slide. The genitalia should be on the lower surface.

Dip a small probe into Canada Balsam and then quickly into some xylene (to stop the Balsam from becoming too tacky). Add a drop to a clean microscope slide, and position the specimen using a small minuten pin. Add a drop of Balsam to a 10 mm coverslip and gently lower the coverslip onto the specimen. A drop of xylene can be added to the slide on the edge of the coverslip to help draw the balsam to the edge of the coverslip.

The mounted specimens are then placed in an oven at 40-45°C for 3 days to dry. Label all slides.

#### Reference

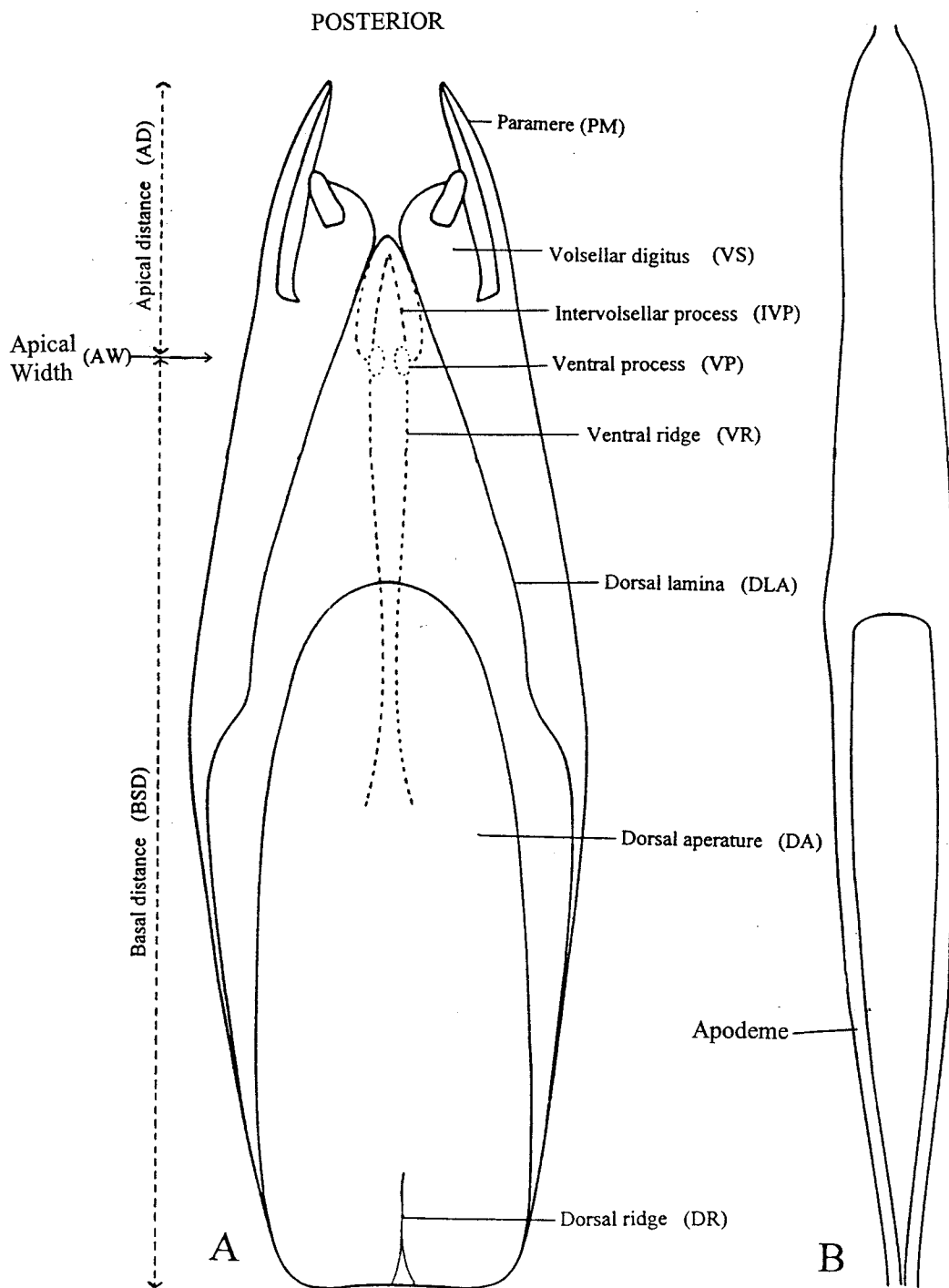
Platner, G.R., Velten, R.K., Planoutene, M. and Pinto, J.D. (1999). Slide-mounting techniques for *Trichogramma* (Trichogrammatidae) and other minute parasitic hymenoptera. *Entomological News* 110: 56-64.

## 9. TECHNICAL APPENDIX 2

### **Guide to identifying Australian *Trichogramma* species.**

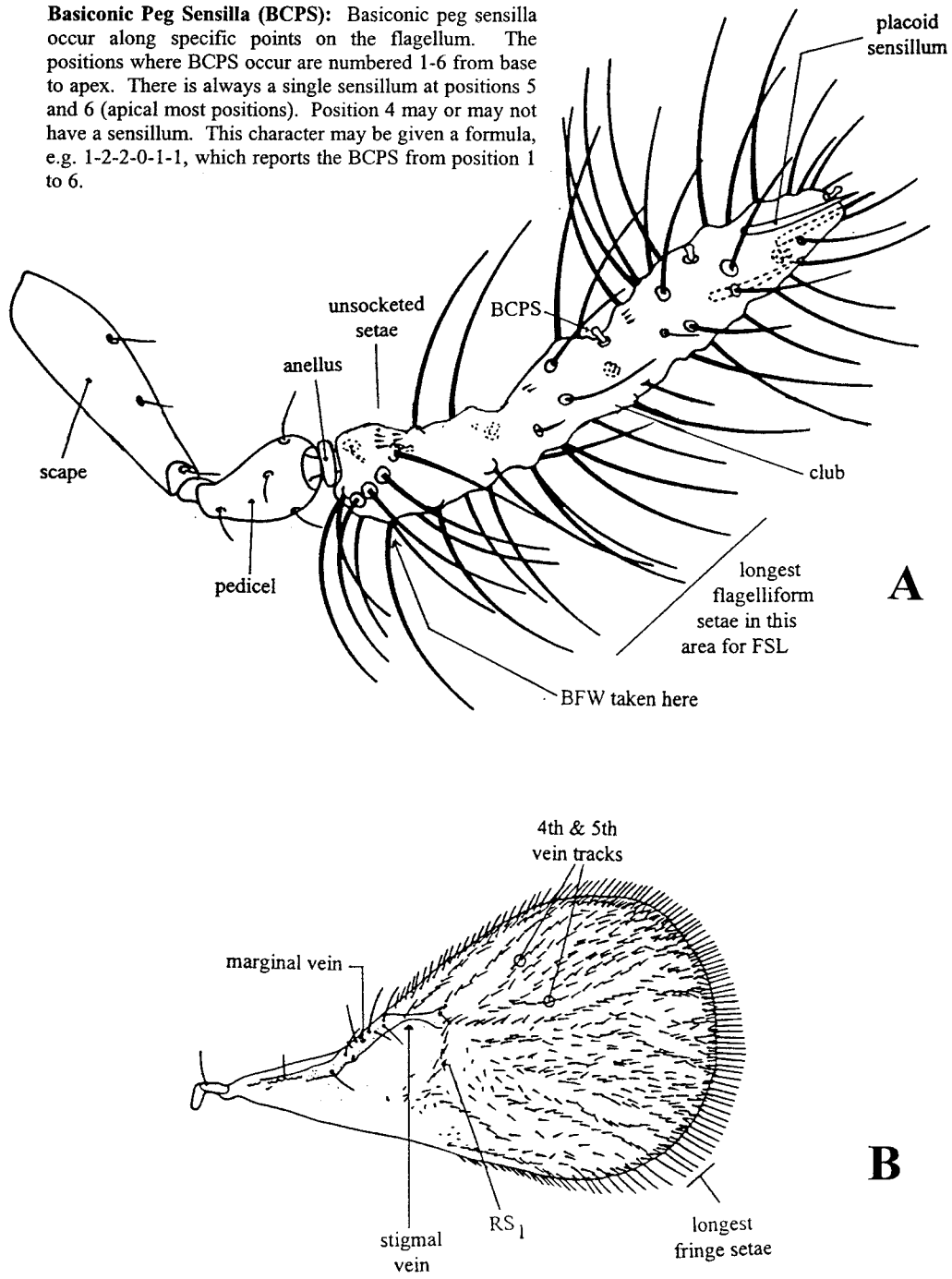
The following key to identifying Australian species of *Trichogramma* is a guide only. The key was prepared by Prof. John Pinto, University of California, Riverside. This key was not intended for publication as a revision of the Australian fauna is required. The key was part of a *Trichogramma* identification workshop presented by Prof. Pinto, and held at the University of Queensland, St. Lucia on 20-21 September 1995.

The photographic images in the key were digitised by Nat Parker (DPI Toowoomba) from mounted specimens provided by John Pinto.



**Figure 1:** Generalised male *Trichogramma* genitalia. (A) Genital capsule. (B) Aedeagus.

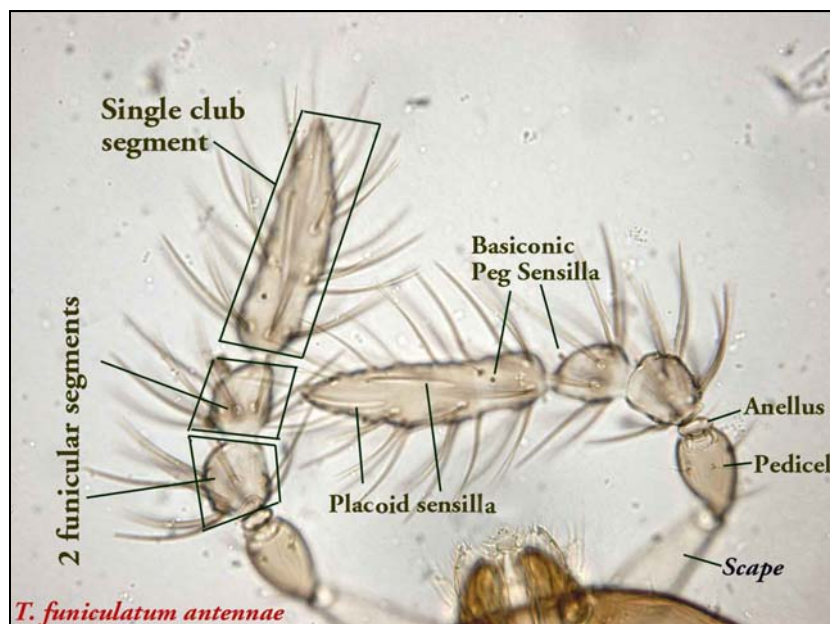
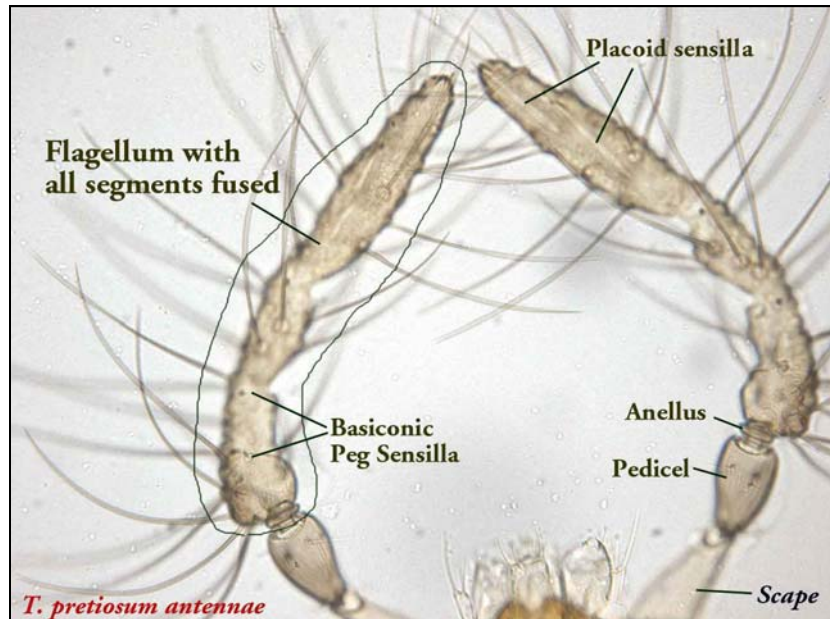
**Basiconic Peg Sensilla (BCPS):** Basiconic peg sensilla occur along specific points on the flagellum. The positions where BCPS occur are numbered 1-6 from base to apex. There is always a single sensillum at positions 5 and 6 (apical most positions). Position 4 may or may not have a sensillum. This character may be given a formula, e.g. 1-2-2-0-1-1, which reports the BCPS from position 1 to 6.



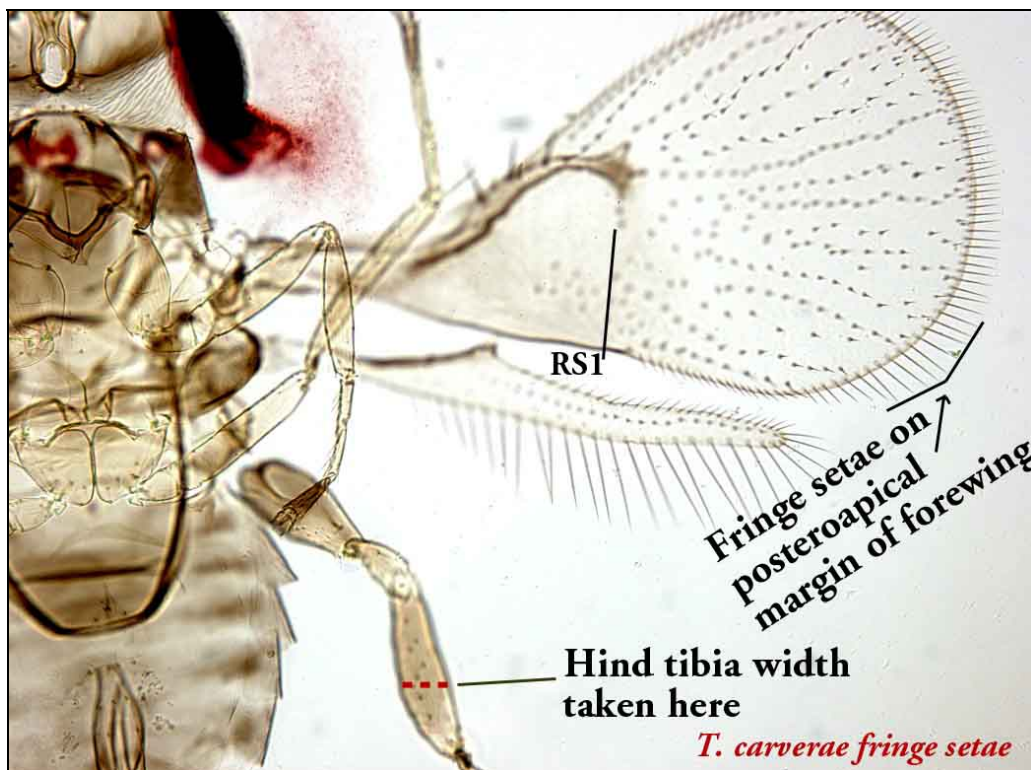
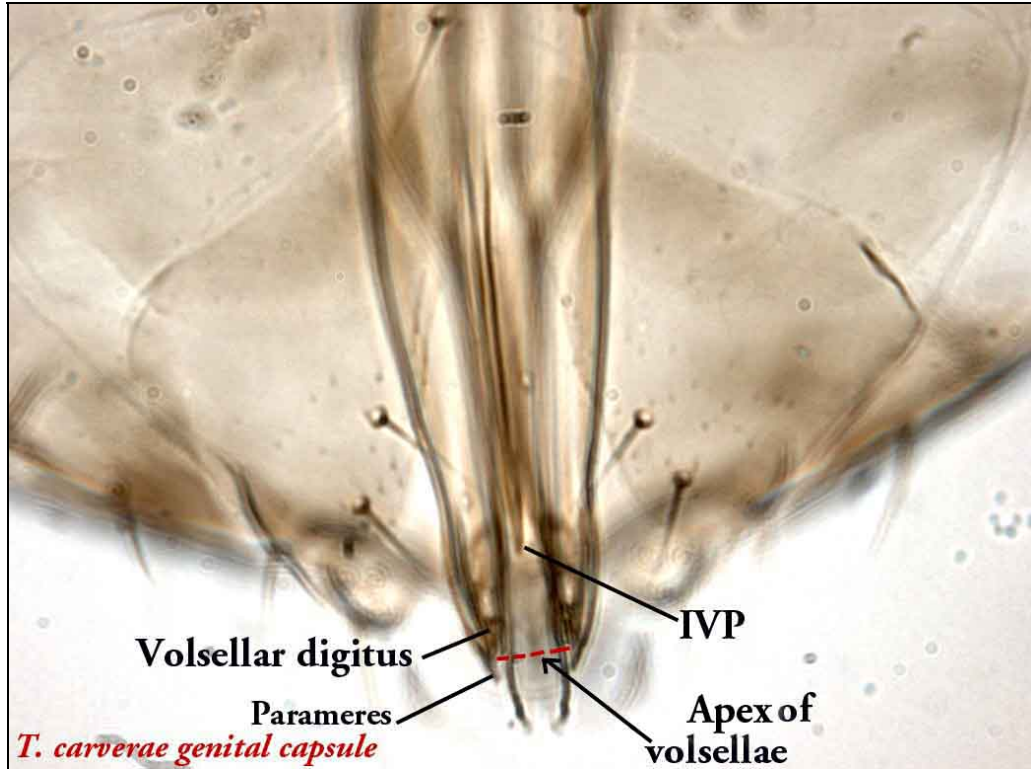
**Figure 2:** *Trichogramma* characters. (A) male antenna. (B) Forewing.

## Key to males of Australian *Trichogramma*

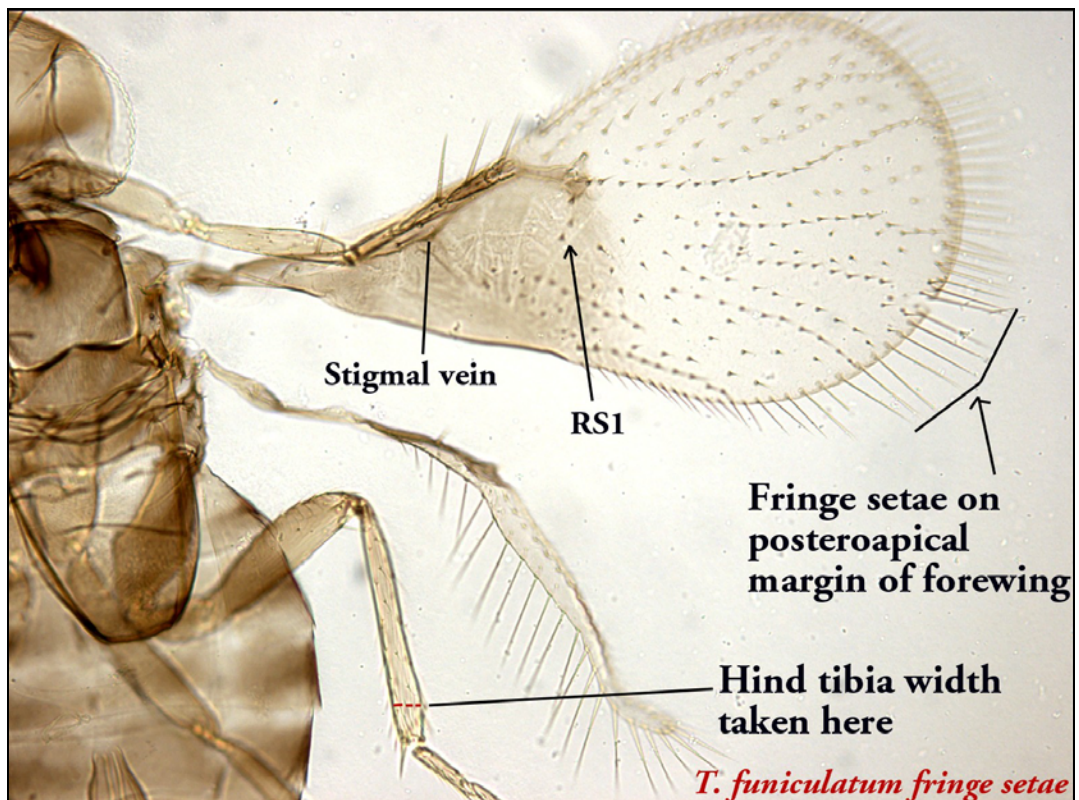
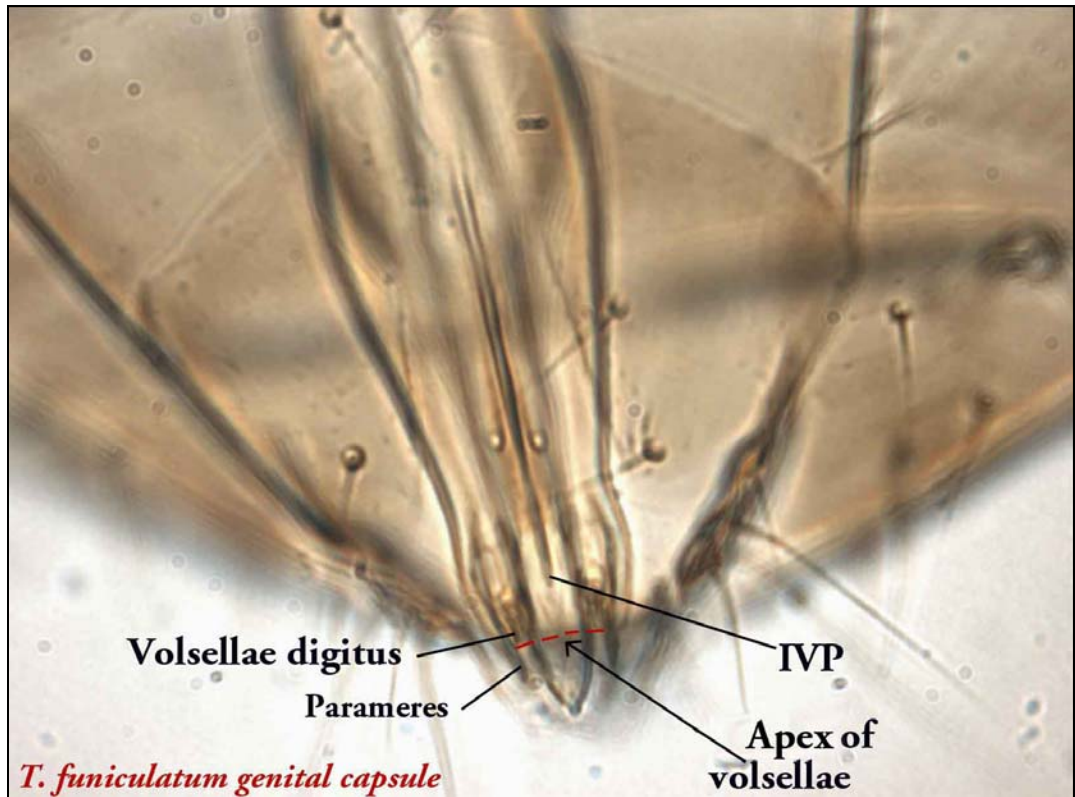
1. Flagellum of antenna with 2 funicular and 3 club segments.....*T. primaevum*  
 Flagellum of antenna with 2 funicular segments and a single club segment,  
 or with all segments fused to form a single elongate segment.....2
2. Flagellum of antenna with 2 funicular segments and  
 a single club segment.....3...(*T. Trichogrammanza*)  
 Flagellum of antenna with all segments fused  
 to form a single elongate segment.....6...(*T. Trichogramma*)



3. Intervolsellar process (IVP) of genitalia short, length distinctly less than half that of volsellae (VS); fringe setae on posteroapical margin of forewing short, longest setae usually less than 2X width of hind tibia..... 4

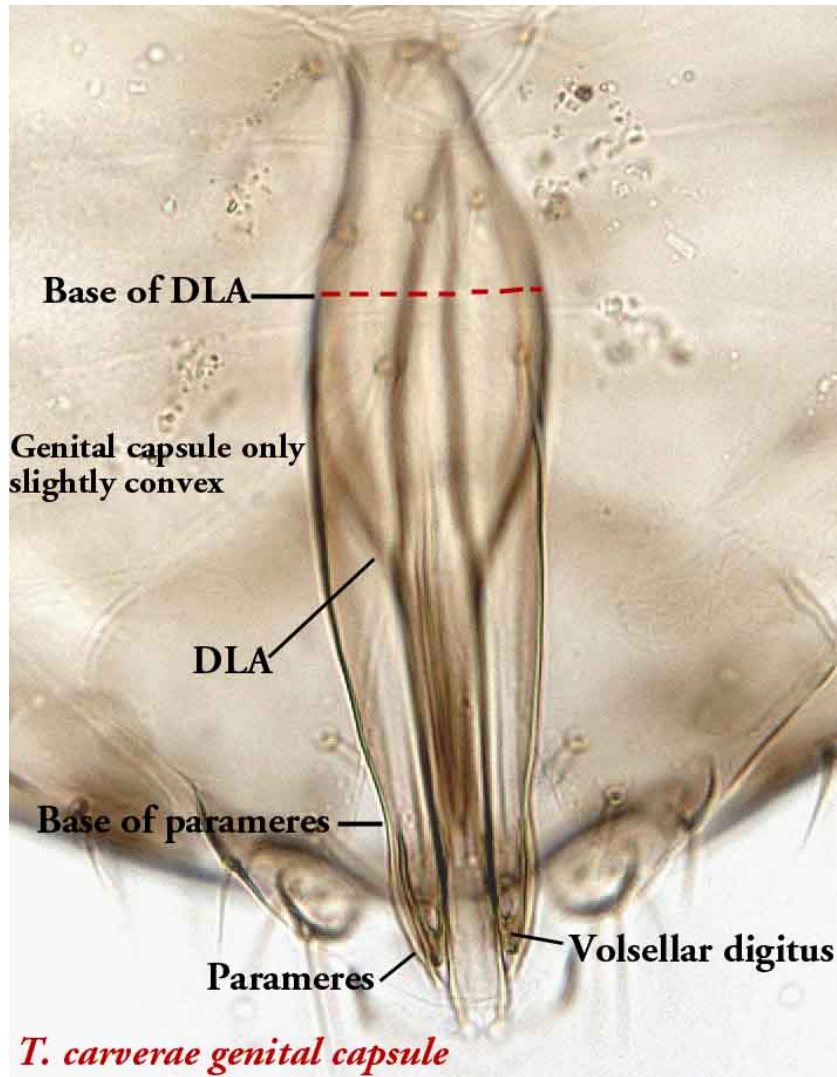


Intervolsellar process of genitalia elongate, length at least half that of volsellae; fringe setae on posteroapical margin of forewing elongate, longest setae 2X width of hind tibia or greater.....5

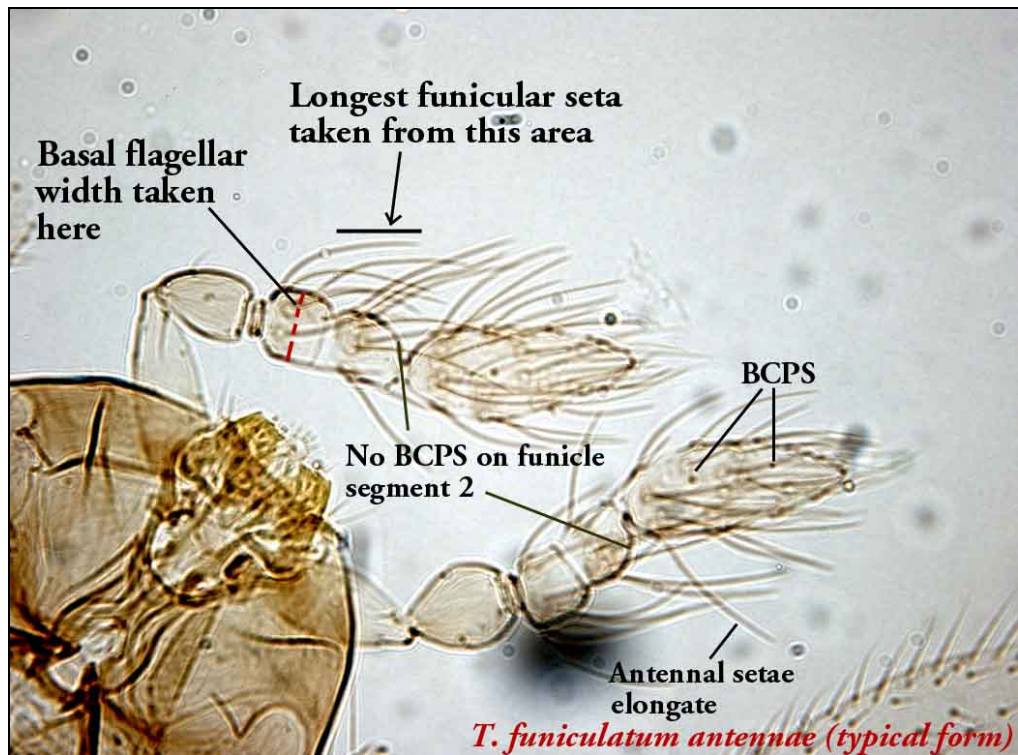


4. Genital capsule (GC) with sides distinctly convex and narrowing abruptly near base of parameres (PM); genital capsule widest posterior to base of dorsal lamina (DLA); uniformly dark brown.....*tenebrosum*

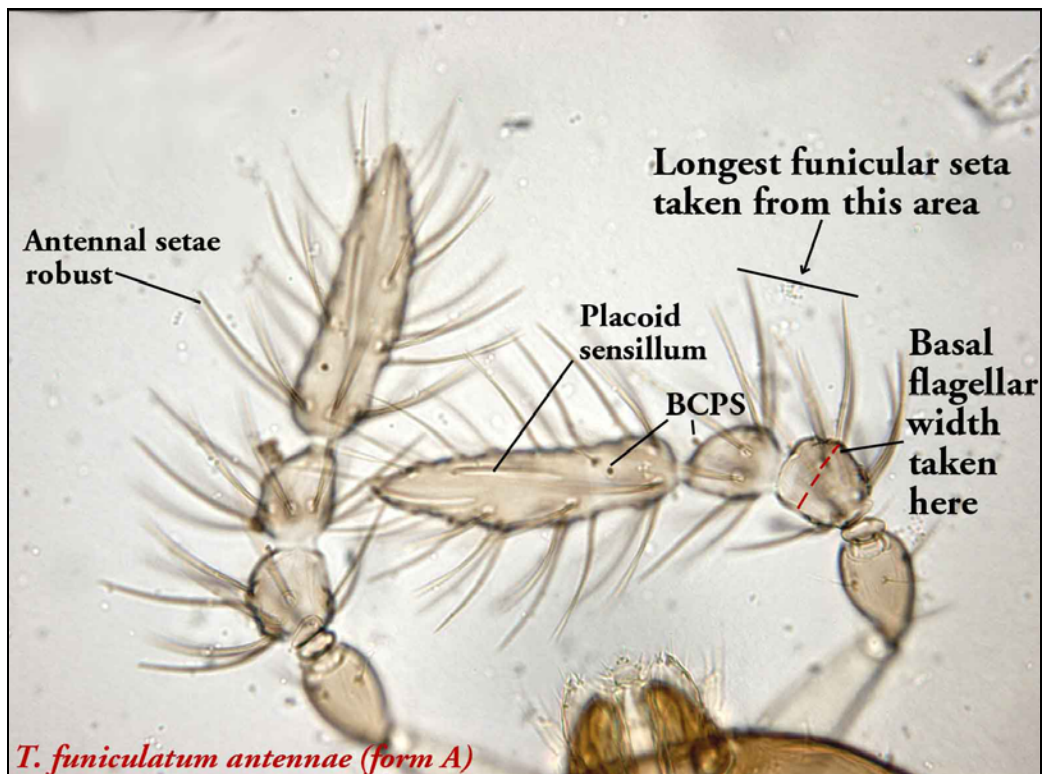
Genital capsule with sides broadly convex and narrowing more gradually near base of parameres; genital capsule widest at base of dorsal lamina; colour yellow and brown.....*carverae*



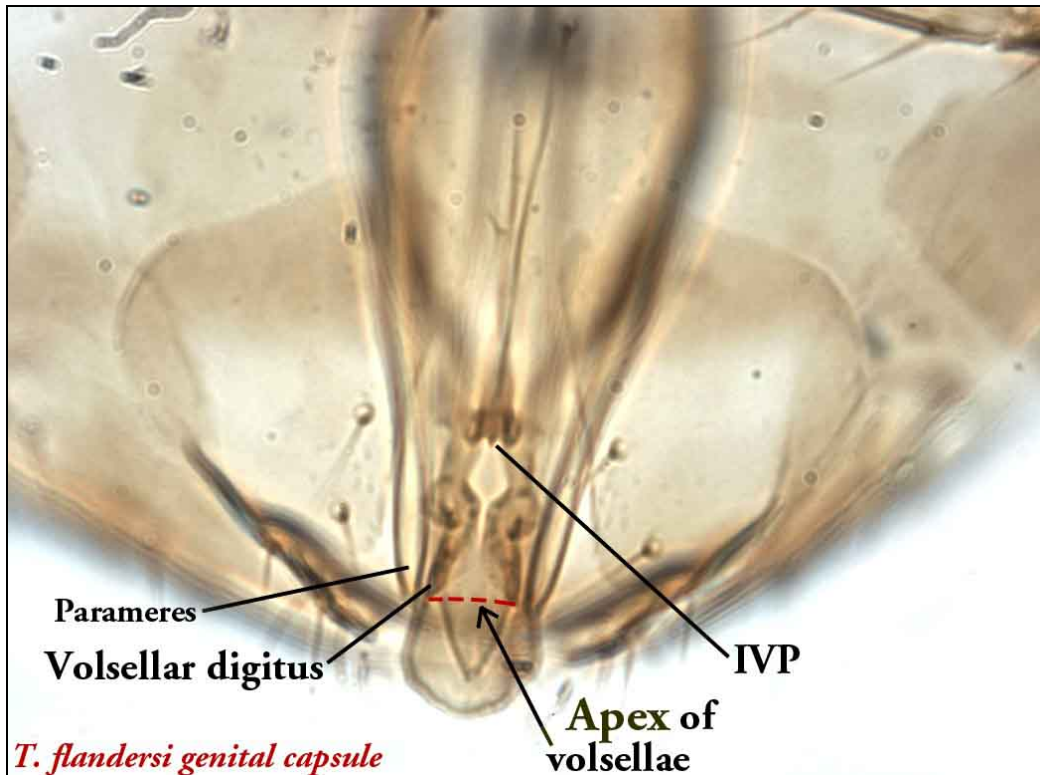
5. Funicle segment II without a basiconic capitate peg sensillum (BCPS) at apex; antennal setae elongate, length of longest funicular seta greater than 2X basal flagellar width (BFW), setae gradually tapering to apex.....*funiculatum* (typical form)



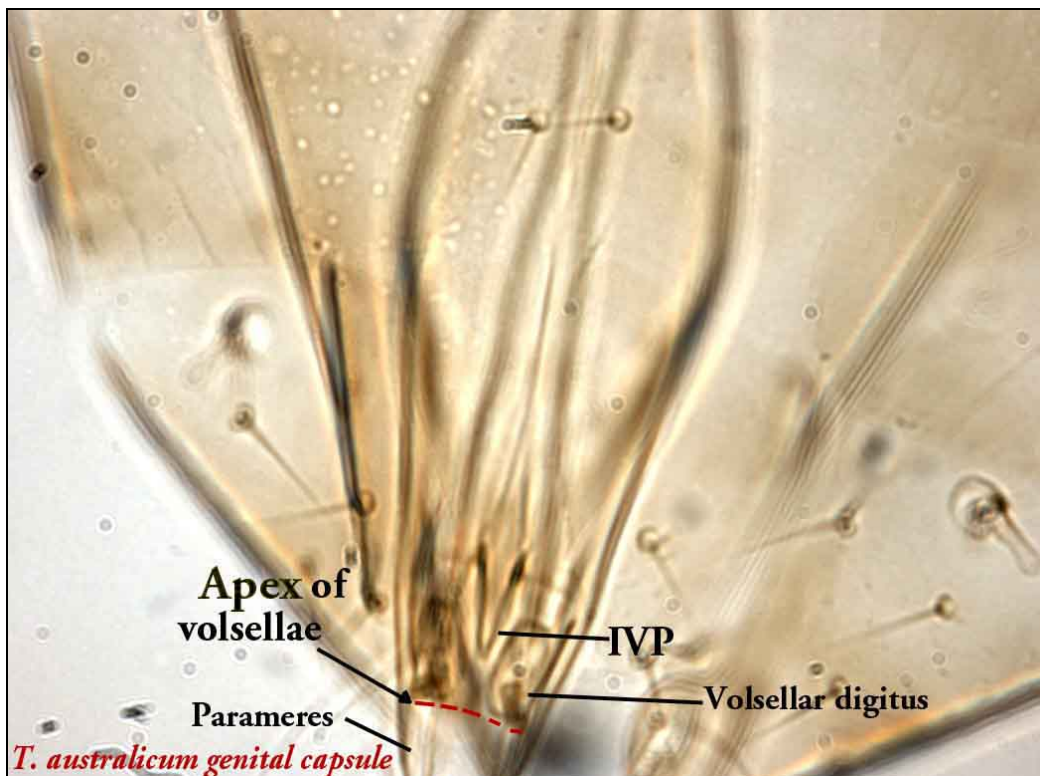
- Funicle segment II with a basiconic capitate peg sensillum at apex; antennal setae short, length of longest funicular seta less than 2X basal flagellar width, setae robust, usually tapering abruptly to apex.....*funiculatum* (Form A)



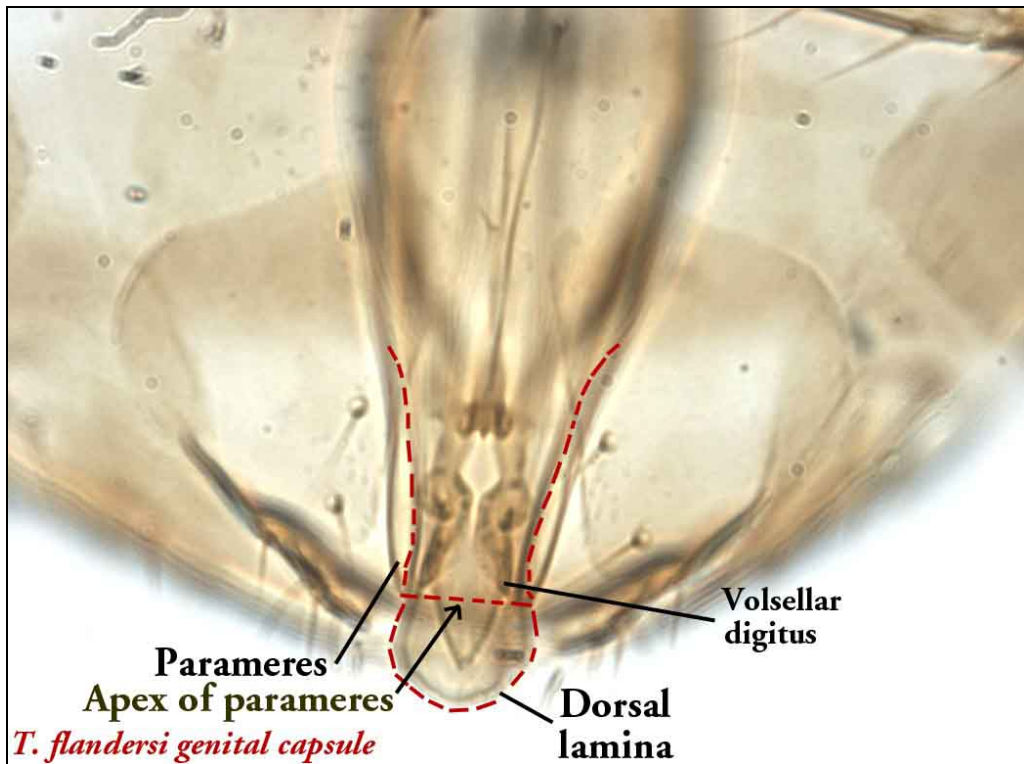
6. Intervolsellar process (IVP) minute, inconspicuous, its length only about 0.1 volsellar (VS) length.....7



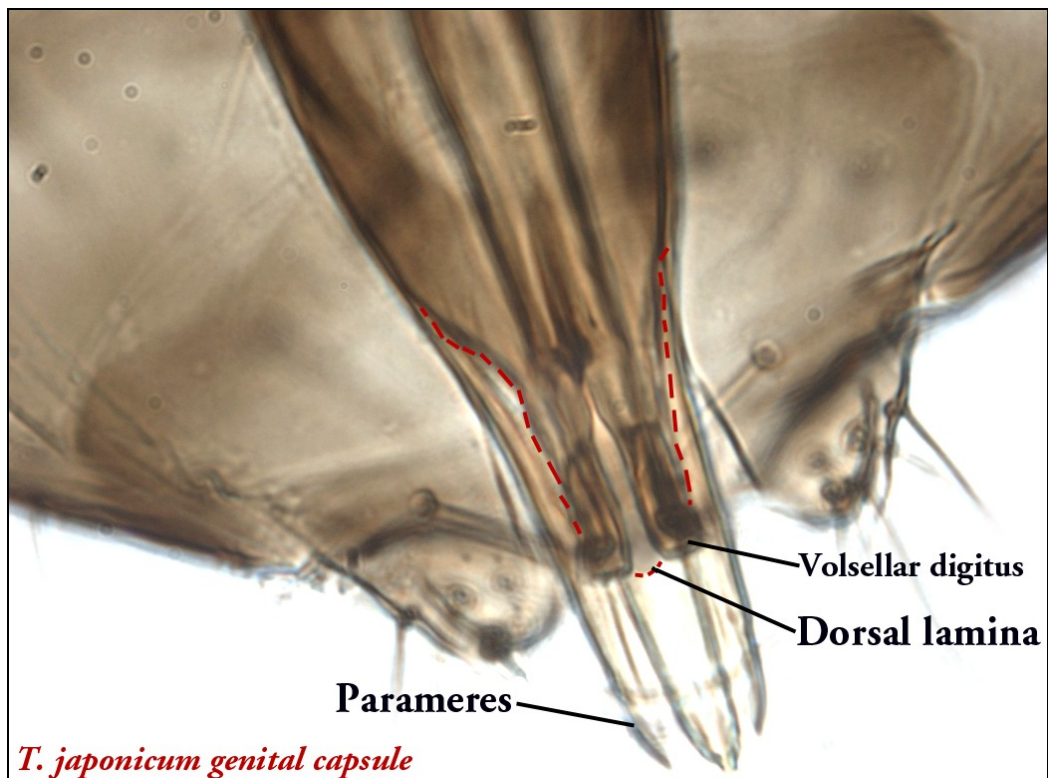
- Intervolsellar process distinct, its length at least 0.5 volsellar length .....9



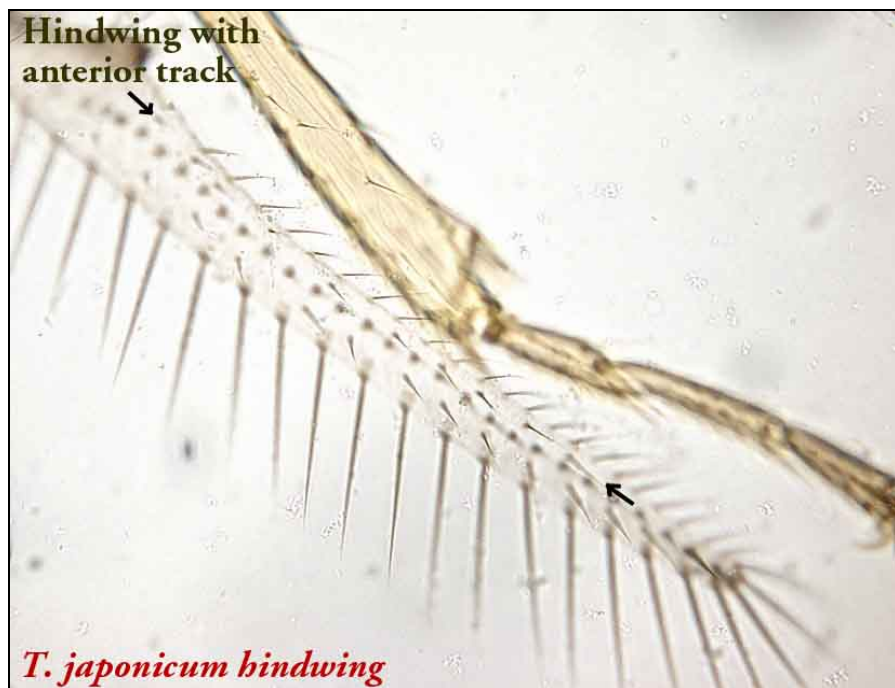
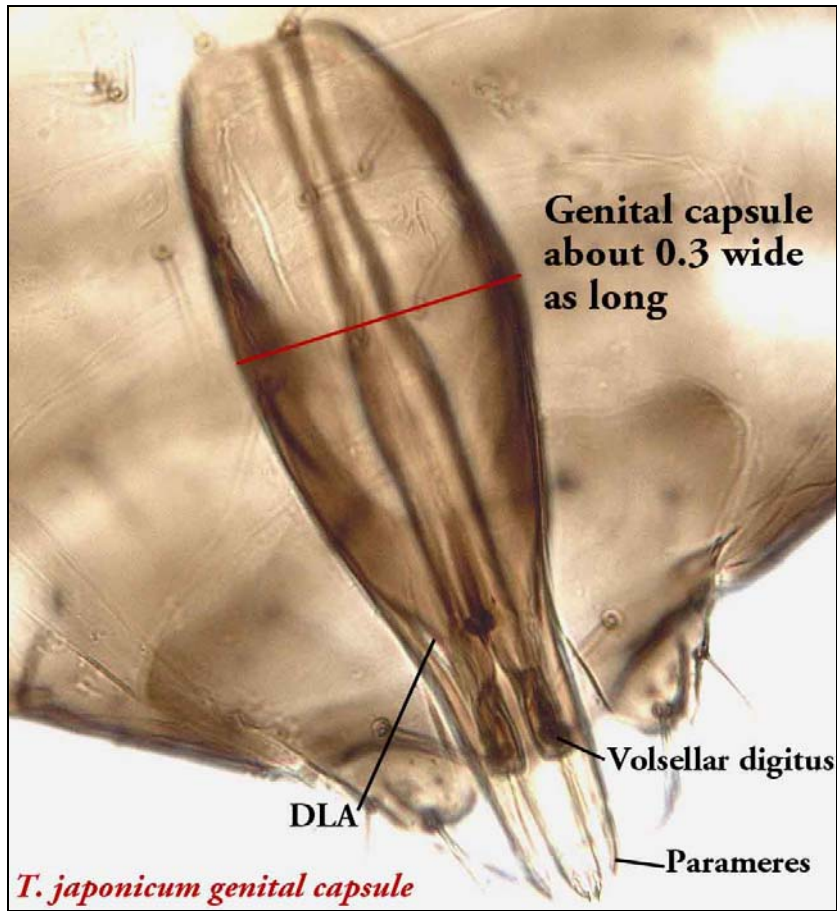
7. Posterior extension of dorsal lamina (DLA) elongate, spatulate, extending beyond apex of parameres (PM).....*flandersi*



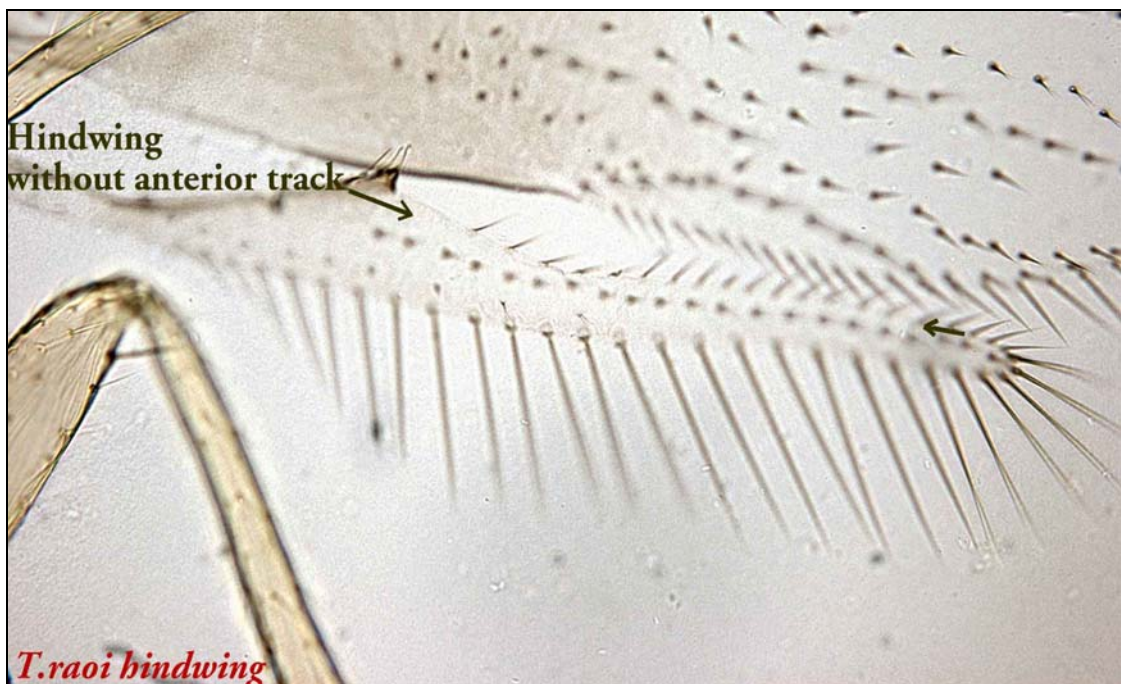
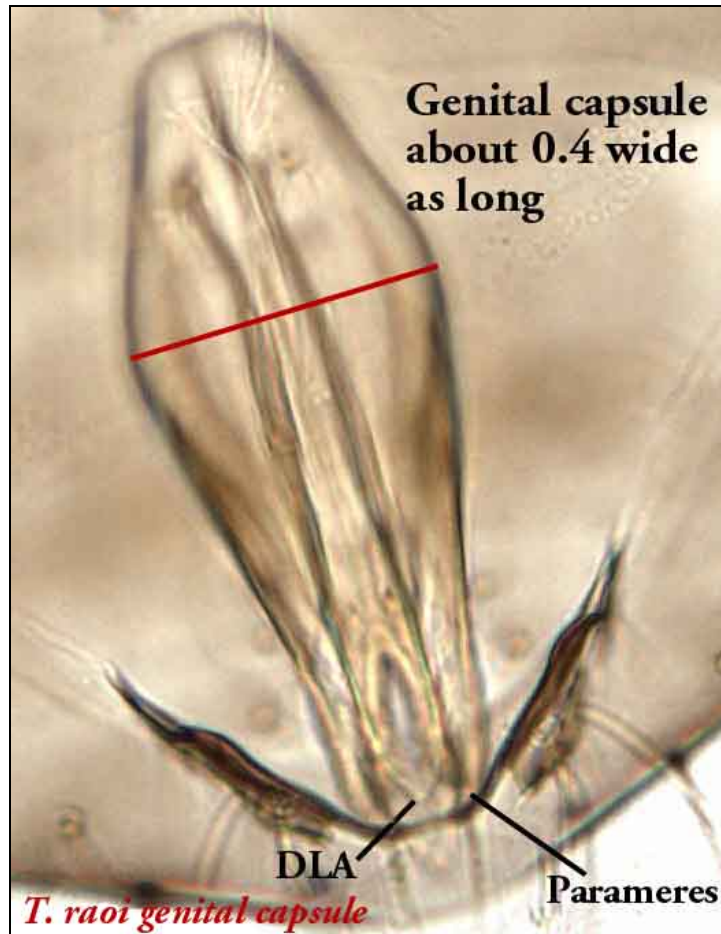
- Posterior extension of dorsal lamina not spatulate and shorter, not extending beyond apex of parameres.....8



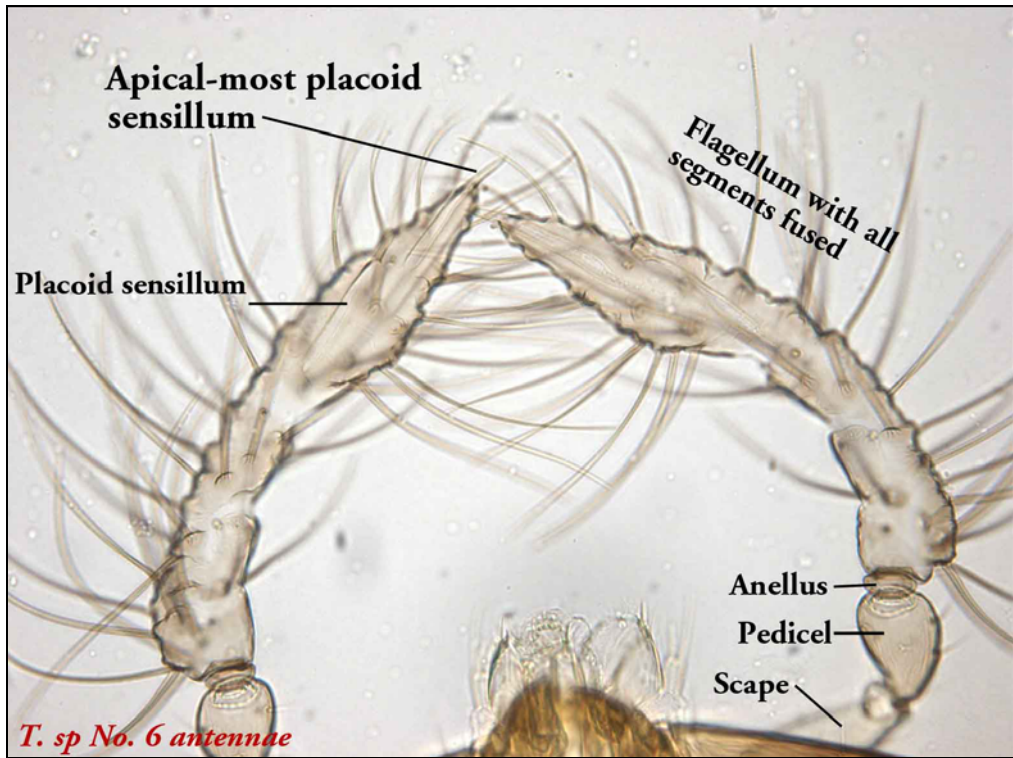
8. Genital capsule elongate, narrow, about 0.3 as wide as long; volsellae (VS) not approaching apex of parameres, occupying only about 0.6 apical distance (AD); hindwing with anterior track composed of several setae.....*japonicum*



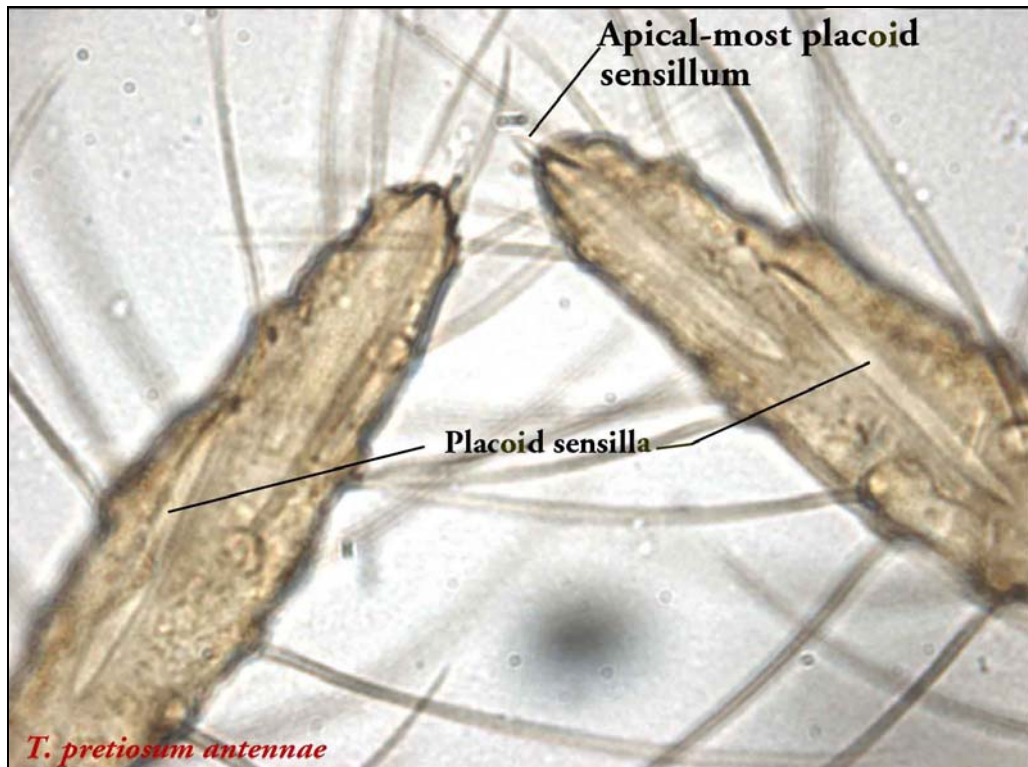
Genital capsule broader, about 0.4 as wide as long; volsellae approaching apex of parameres, occupying about 0.8 apical distance; hindwing without an anterior track .....*raoi*



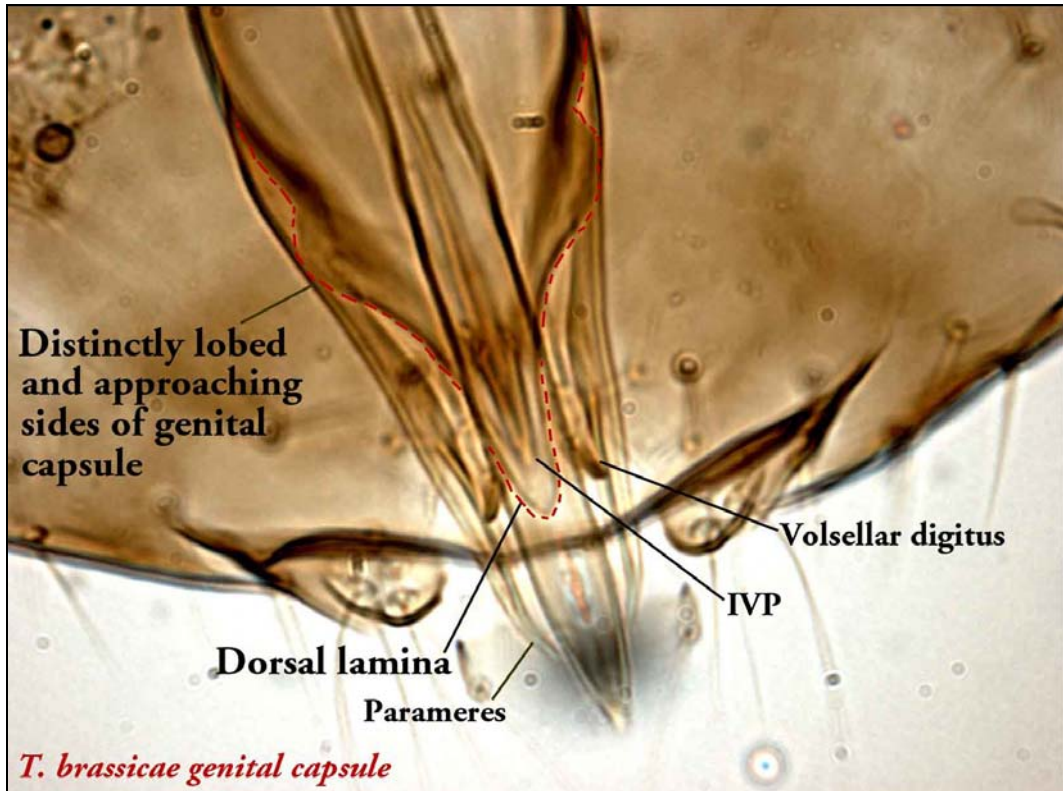
9. Ventral process (VP) protuberant and placed distinctly laterad of ventral ridge (VR); apical-most placoid sensillum of antenna extending well beyond apex of flagellum (about 0.3 of its length extending beyond apex).....sp. #6



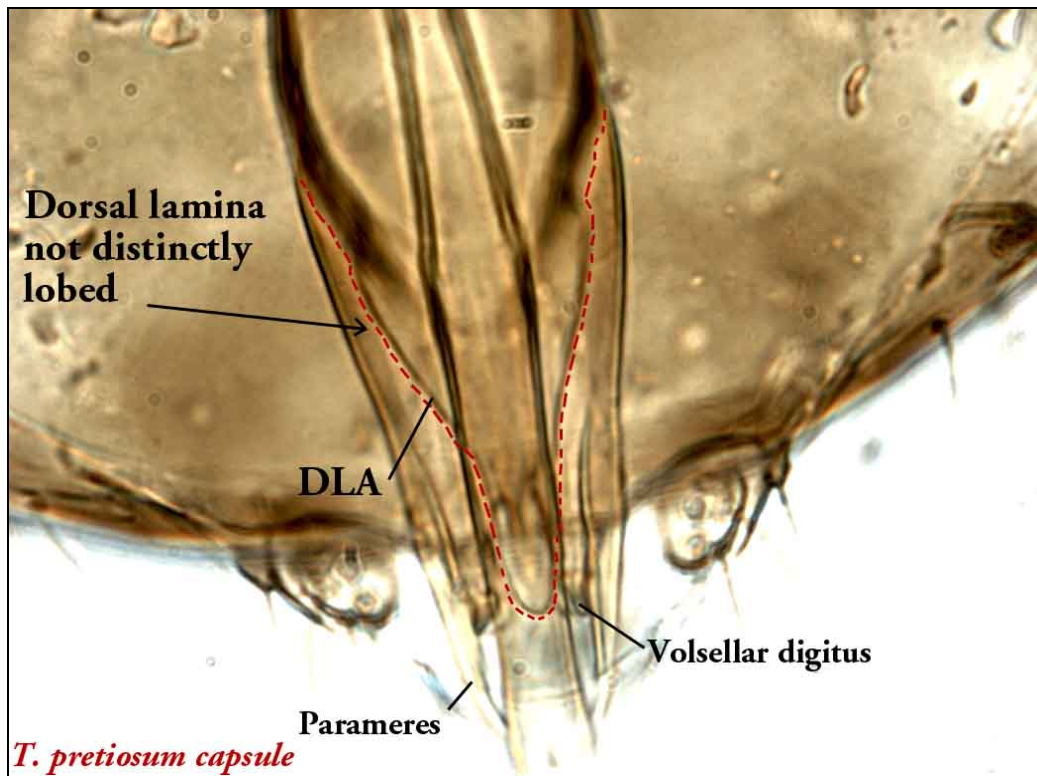
- Ventral process not as protuberant, placed adjacent to ventral ridge; apical-most placoid sensillum extending only slightly beyond apex of flagellum.....10



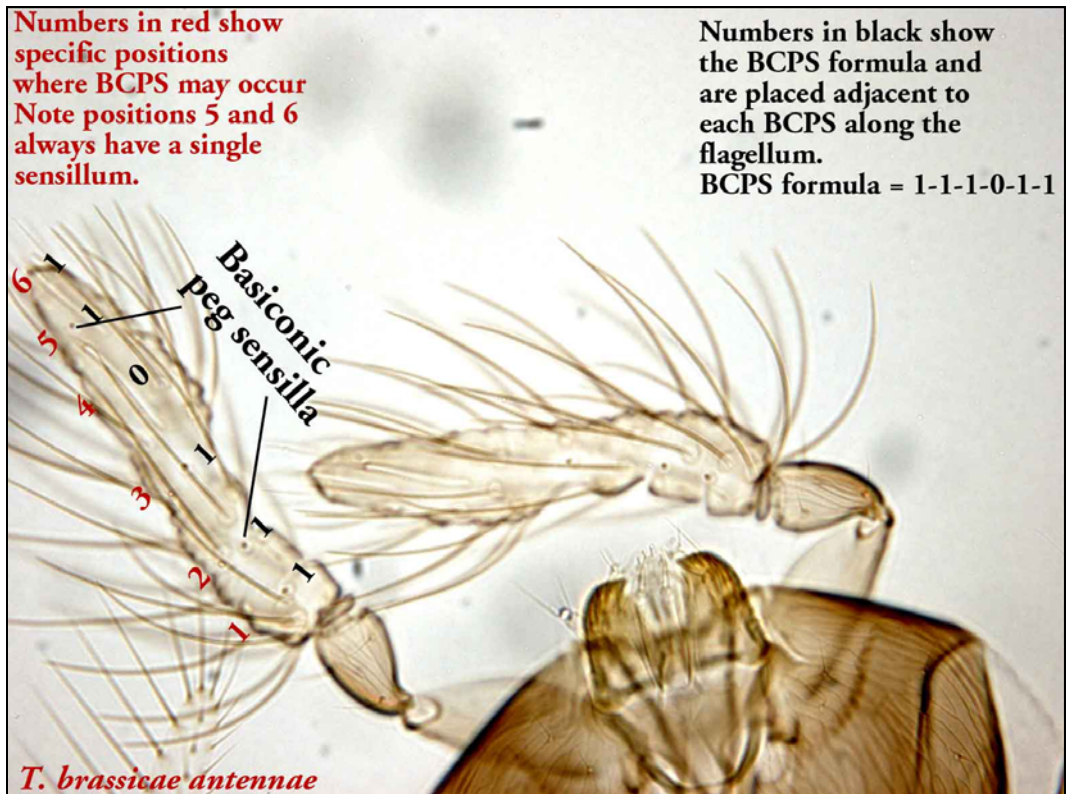
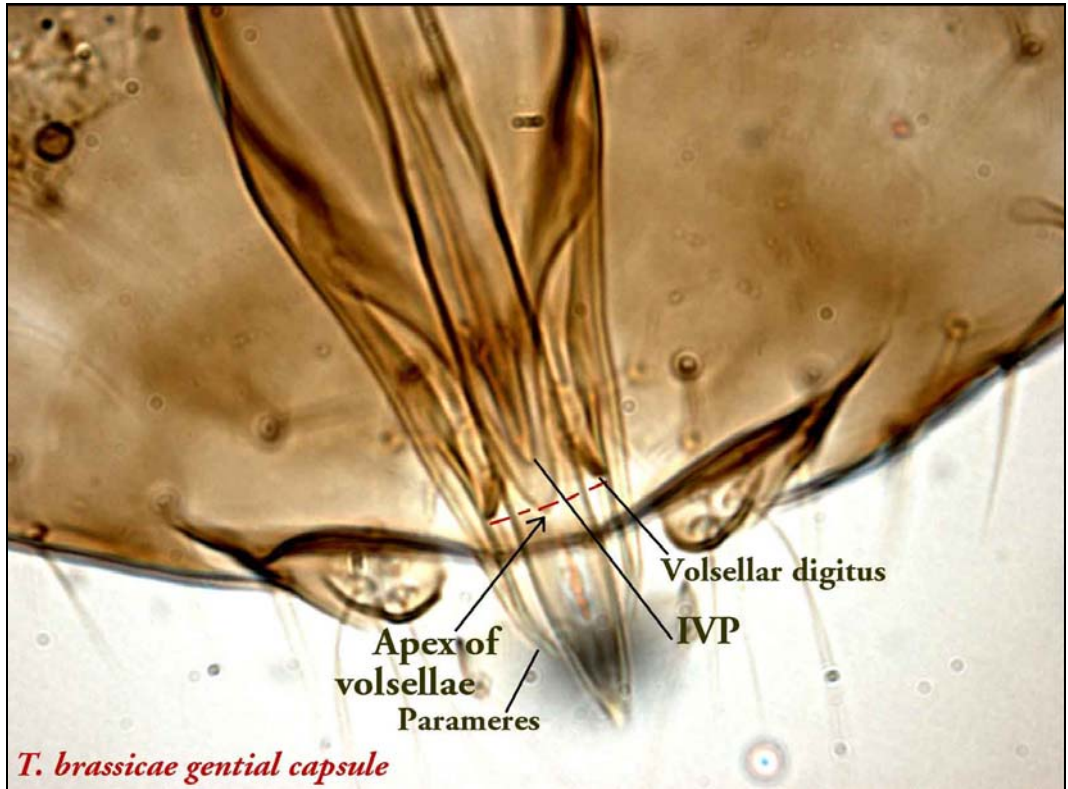
10. Dorsal lamina (DLA) distinctly lobed, with lobes approaching sides of genital capsule ..... 11



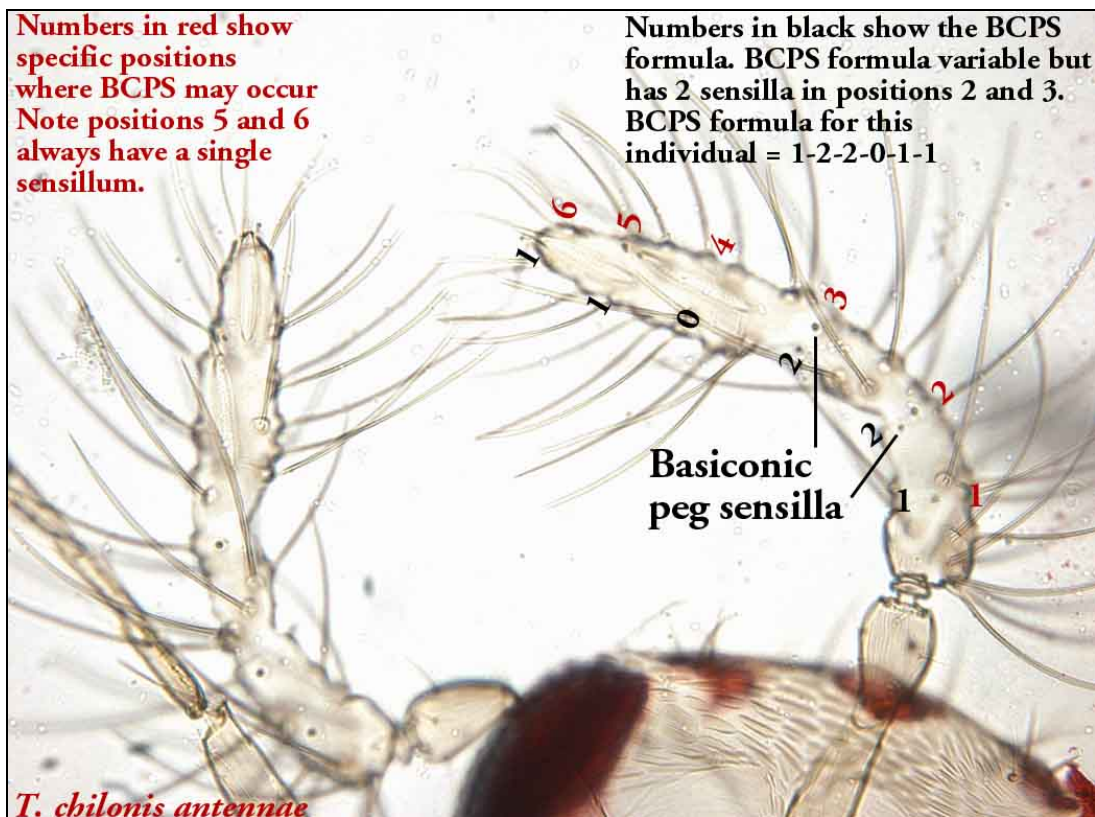
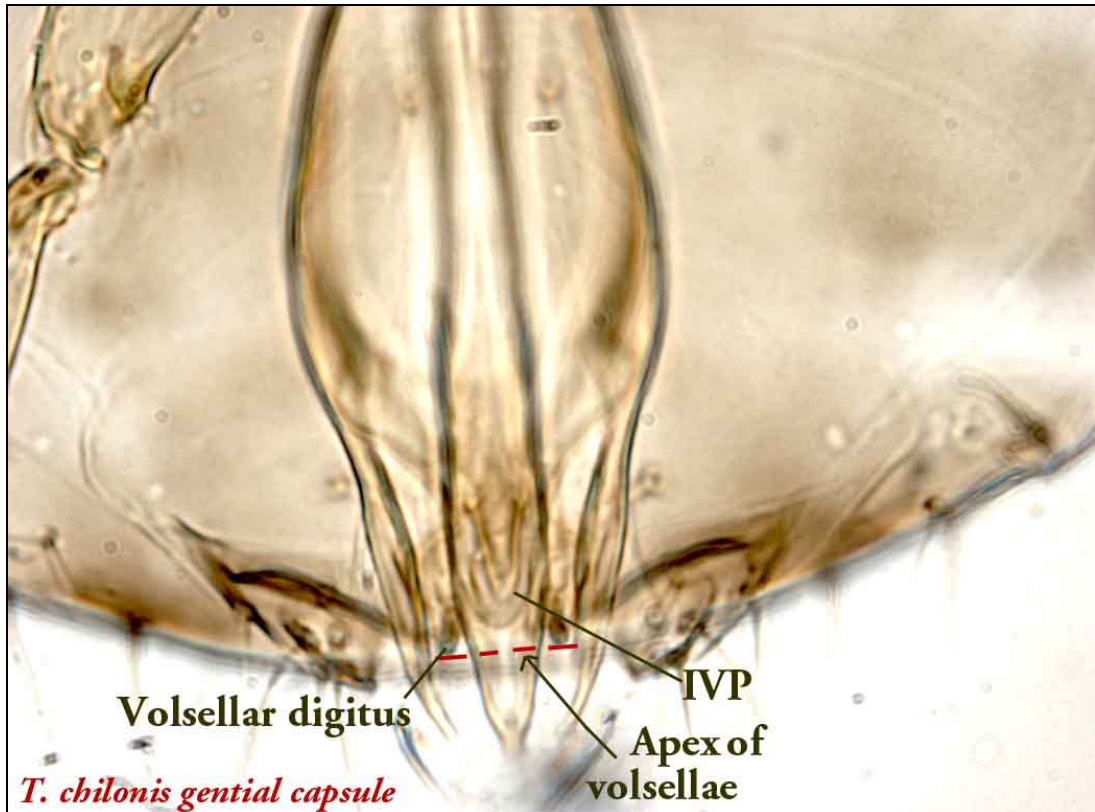
- Dorsal lamina not distinctly lobed, lobes (if present) not approaching sides of genital capsule ..... 13



11. Intervolsellar process (IVP) elongate, 2/3 in length to that of volsellae (VS);  
 BCPS formula of antenna = 1-1-1-0-1-1.....sp. nr. *brassicae*

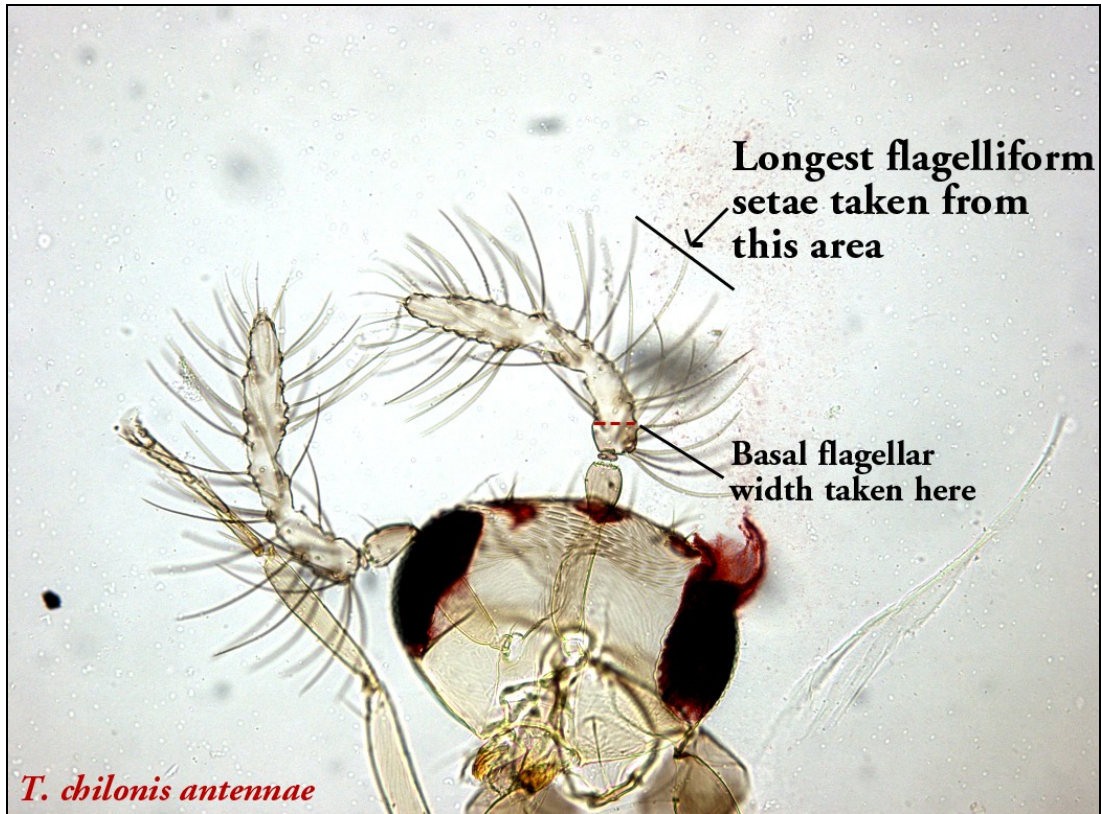


Intervolsellar process half the length of volsellae; BCPS formula of antenna variable but with 2 sensilla at positions 2 & 3.....12

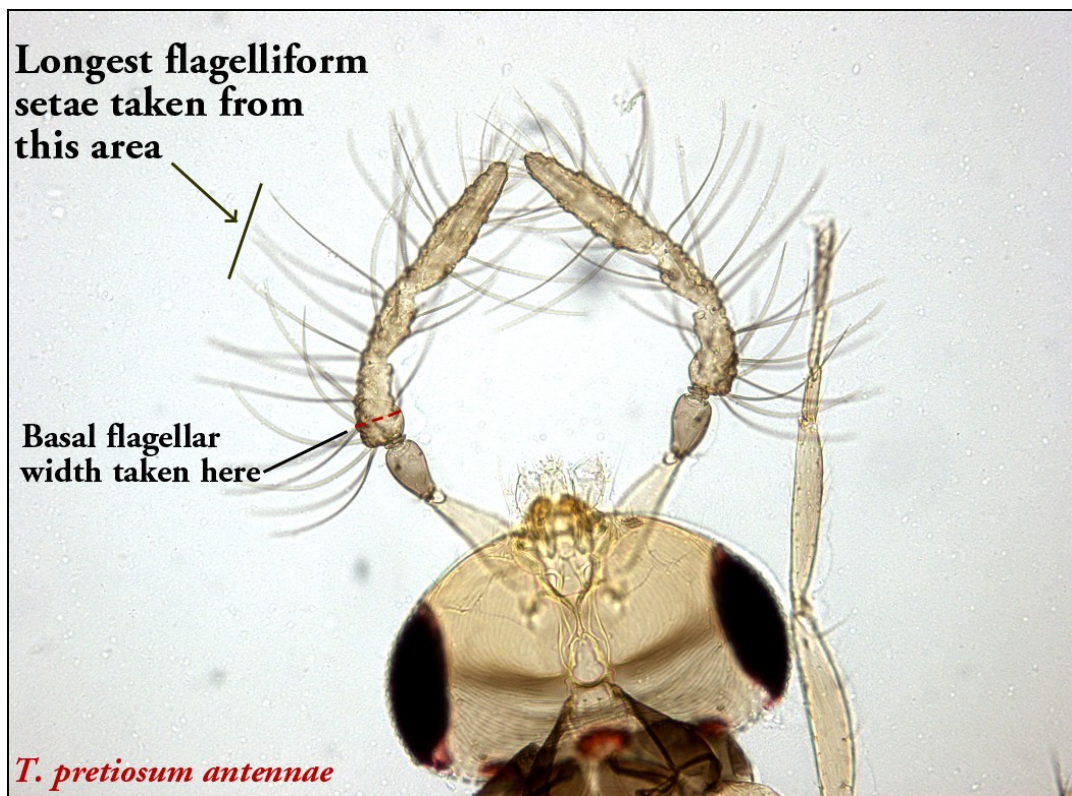


12. Longest setae on flagellum (FSL) less than 2X the basal flagellar width (BFW);  
flagellum with unsocketed setae.....*closterae?*

Longest setae on flagellum 2.5-3.0X the basal flagellar width;  
flagellum without unsocketed setae.....*chilonis*



13. Intervolsellar process (IVP) appearing linear or narrowly triangular; apex of posterior extension of dorsal lamina (DLA) poorly sclerotised (difficult to distinguish under light microscope); ventral ridge (VR) poorly developed, linear from base to apex; flagellar setae elongate, longest setae about 3X basal flagellar width.....*pretiosum*



Intervolsellar process broadly triangular, sides distinctly convergent from base; apex of posterior extension of dorsal lamina well sclerotised (easily distinguished under a light microscope); ventral ridge well developed, distinctly narrowing anteriorly from base of IVP; flagellar setae short, relatively robust, longest setae 2X or less than basal flagellar width.....*australicum*

