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

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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.

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 wasps against heliothis.

Here we report on field trials evaluating different release rates of *Trichogramma pretiosum* against *Helicoverpa armigera* in the 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 showed signs of parasitism (turned jet black). 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 planting was in two strips, and 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 only been sprayed once with Costar® (on 30 December 2002) at the time of the trial. The *Trichogramma* were released on 10 February 2003, and *H. armigera* 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 sprayed three times at the time of the trial. The last spray (Affirm®) was applied on the 29th January 2003. The *Trichogramma* were released on 17 February, and *H. armigera* 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* (without application costs) are $25, $50 and $100 per hectare for the low, medium and high rates evaluated in this trial. 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 20th 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 that 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 that 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 this option, 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. .

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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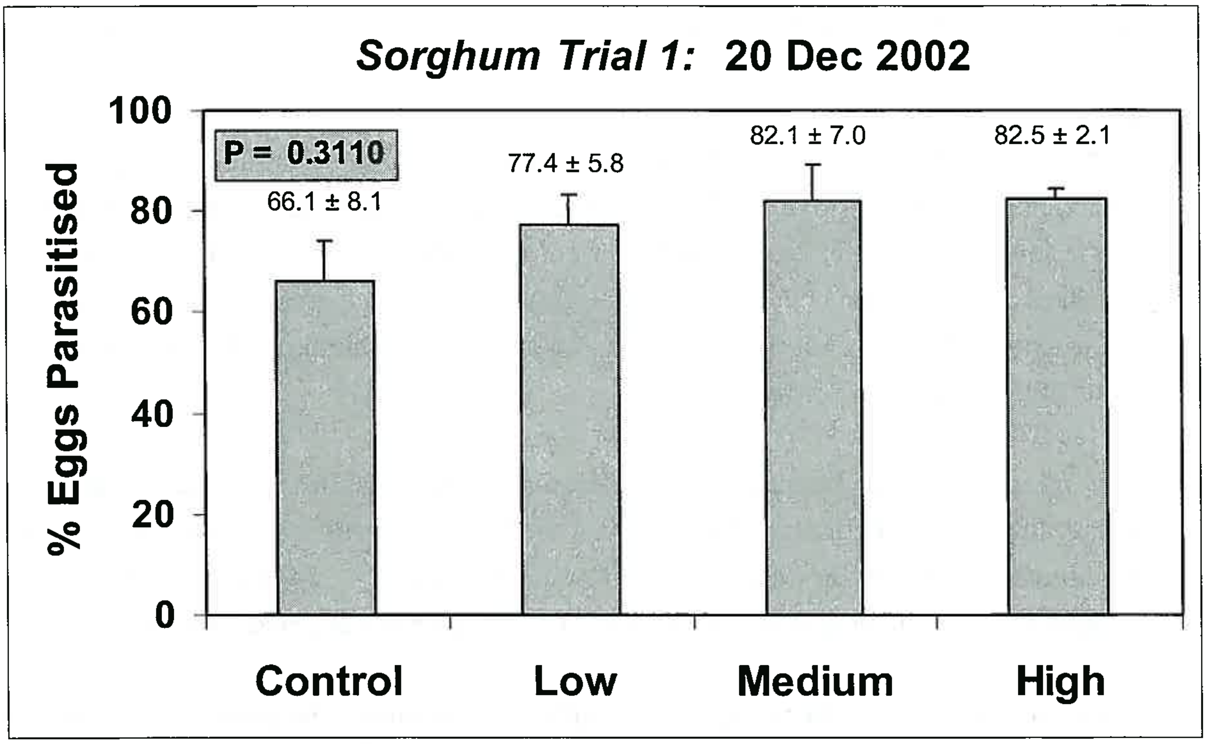

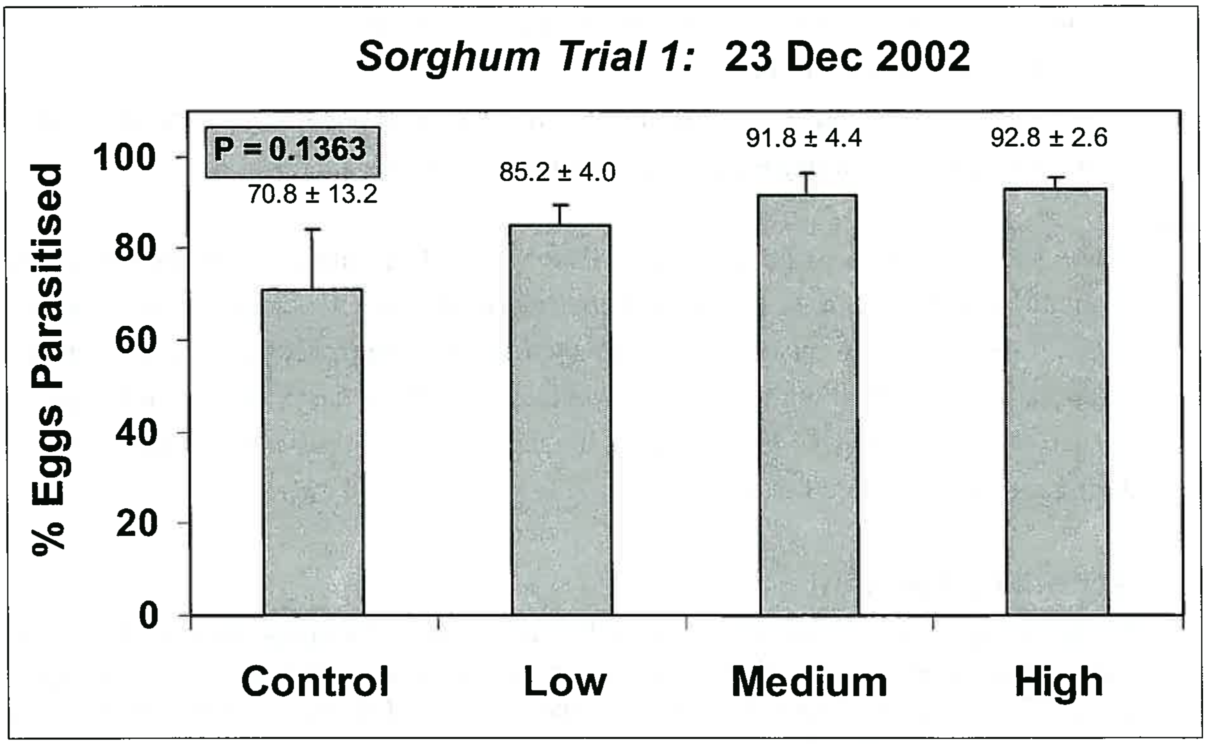

Figure 1: The levels of heliothis (*H. armigera*) egg parasitism following the release of different rates of *Trichogramma pretiosum* into sorghum at Rasmussen's, Evanslea. Values are the mean ± standard error of four replicates, and were arcsine transformed for analyses (ANOVA). No significant differences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

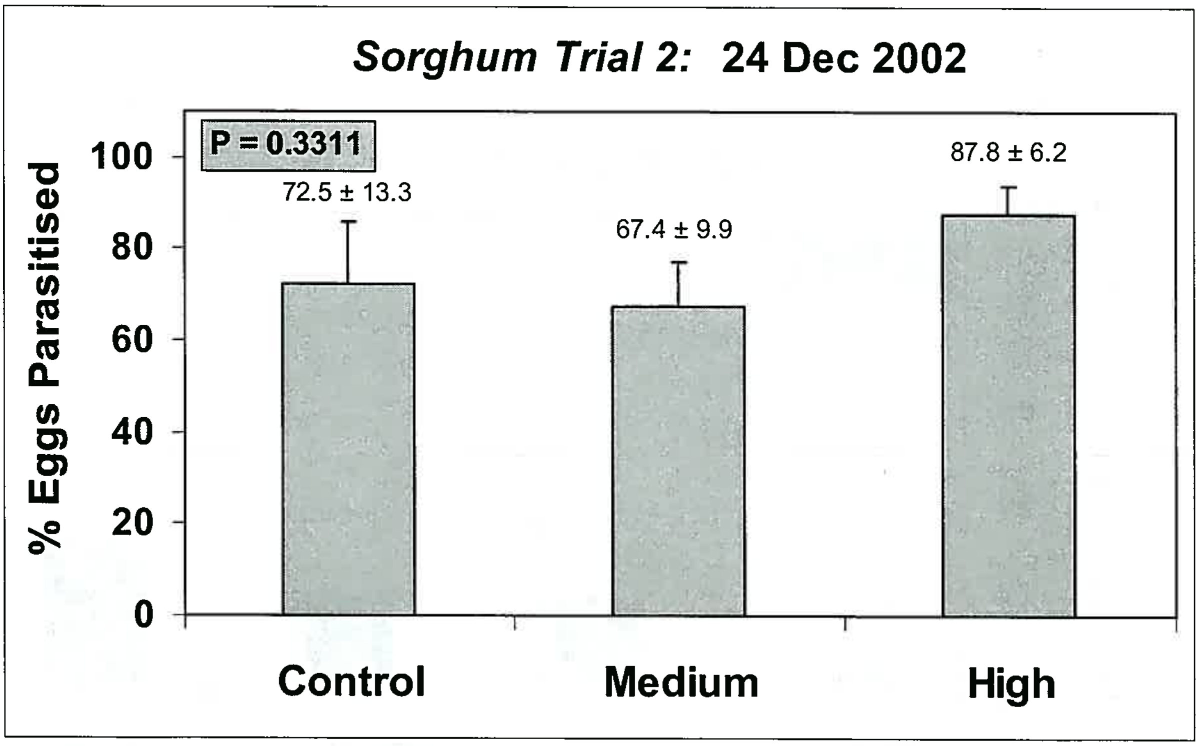

Figure 2: The levels of heliothis (*H. armigera*) egg parasitism following the release of different rates of *Trichogramma pretiosum* into sorghum at Speed's, Evanslea. Values are the mean ± standard error of four replicates, and were arcsine transformed for analyses (ANOVA). No significant differences 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
Sorghum Trial 1	1.42	1.45	1.03
Sorghum Trial 2	1.46	1.53	1.05
Cotton Trial 1	1.08	1.73	1.31
Cotton Trial 2	1.33	2.51	1.88
Mean Difference	**1.32**	**1.81**	**1.32**

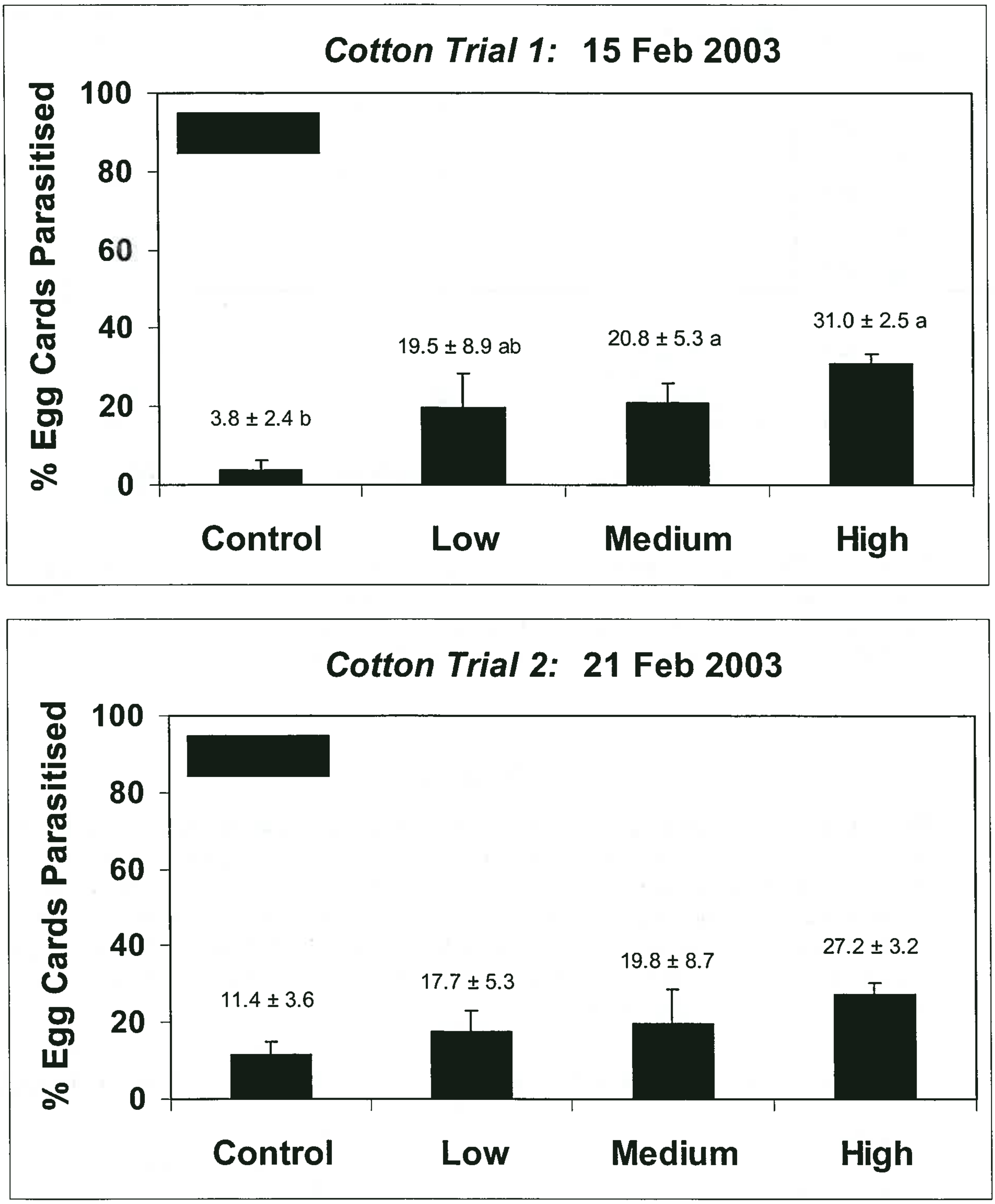

Figure 3: The levels of heliothis (*H. armigera*) egg parasitism on egg cards following the release of different rates of *Trichogramma pretiosum* into conventional cotton. Values are the mean ± standard error of four replicates, and were arcsine transformed for analyses. Means followed by the same letter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

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

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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.

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 30ml 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 25^0C 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, 6th 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 (Trichogramanza) 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, and was also recovered from Goondiwindi. 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.

Acknowledgements

We thank the Cotton Research and Development Corporation for funding the research (project DAQ125C). This assistance is gratefully acknowledged.

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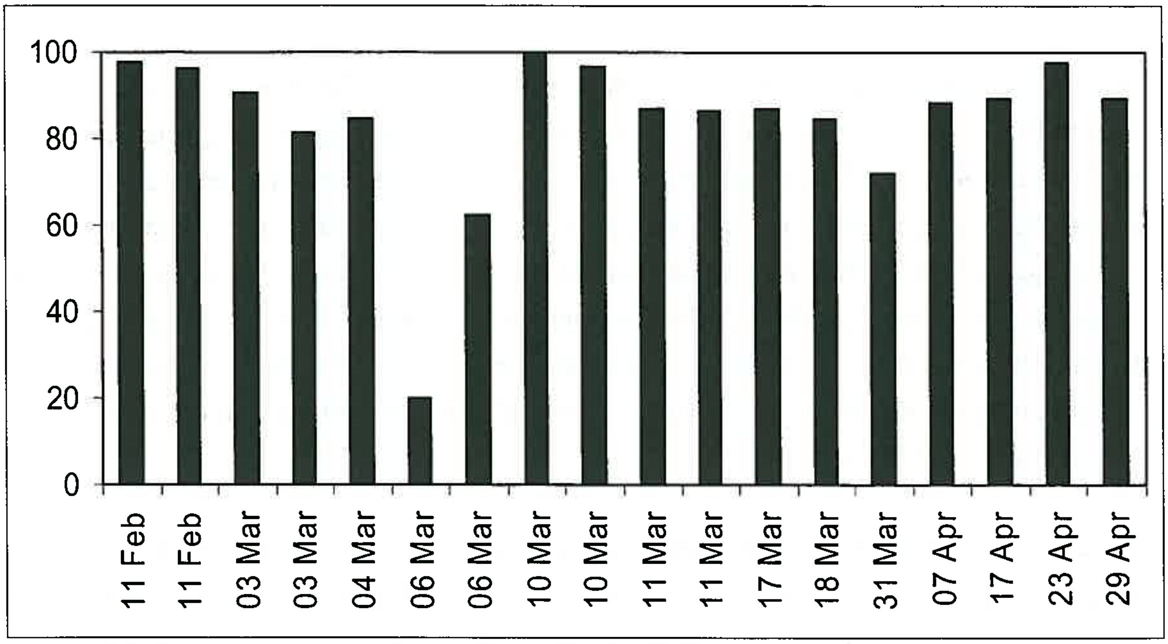

Figure 1. The percentage of heliothis (*Helicoverpa armigera*) eggs parasitised from various sorghum crops on the Darling Downs during 2003. All of the sorghum sampled was on farms were no *Trichogramma* releases had been carried out.

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	26^0 59.31, 151^0 00.52	96	97.6
11 Feb	Dalby	27^0 13.25, 151^0 19.04	96	96.4
03 Mar	Brookstead	27^0 45.38, 151^0 27.60	60	90.9
03 Mar	Millmerran	27^0 51.24, 151^0 19.53	30	81.5
04 Mar	Goondiwindi	28^0 37.25, 150^0 23.06	45	84.6
06 Mar	Warwick	28^0 10.76, 152^0 02.79	29	20.0
06 Mar	Hermitage	28^0 12.20, 152^0 05.99	31	62.5
10 Mar	Kingsthorpe	27^0 23.49, 151^0 44.69	43	100.0
10 Mar	Evanslea	27^0 27.04, 151^0 33.54	41	96.7
11 Mar	Kingsthorpe	27^0 28.79, 151^0 45.68	62	87.0
11 Mar	St Ruth	27^0 23.47, 151^0 19.20	30	86.4
17 Mar	Evanslea	27^0 27.15, 151^0 33.82	43	87.1
18 Mar	St Ruth	27^0 23.68, 151^0 25.42	48	84.8
31 Mar	St Ruth	27^0 23.59, 151^0 21.99	48	72.2
07 Apr	St Ruth	27^0 23.59, 151^0 21.99	60	88.2
17 Apr	Evanslea	27^0 28.38, 151^0 31.91	132	89.1
23 Apr	Evanslea	27^0 28.38, 151^0 31.91	60	97.9
29 Apr	Evanslea	27^0 28.38, 151^0 31.91	60	89.1
	TOTALS		**1014**	**87.1**