1684 The response of cotton to real and simulated mirid damage in Australia

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Green mirids (*Creontiades dilutus* (Stål)) are a problem in Bt-cotton crops in Australia. Management is hampered by poor understanding of the yield response of cotton to damage. This study valuated the effect of real and simulated mirid damage, at a range of intensities and times, on yield in cotton. The effect of simulated damage (injecting bolls with weak pectinase solutions), and real mirid damage (caging mirids on plants) was investigated in the field and glasshouse. Boll injecting allowed more precise levels of damage to be inflicted, but reduced boll development and yield more strongly than mirid feeding. Cotton was able to recover fully from simulated damage inflicted in the early fruiting period (21 d after first flower; DAFF) when 5 or 20 bolls per m were damaged. Damage in the mid (35 DAFF) or late (56 DAFF) fruiting period reduced yield at heavier levels (20 or 50 bolls damaged per m) but had no effect at 5 bolls per m. Yield reductions were due to reduced gin out-turn, resulting from selective removal of damaged lint, reduced boll numbers (in some cases), and reduced boll size of damaged bolls. Damage due to mirids at densities up to 4 per m^2 for 7 d did not affect yield. There was strong evidence of compensation due to retention of extra undamaged bolls from the same cohort as those damaged. These data suggest that cotton can tolerate levels of damage and mirid numbers higher than current thresholds.

Keywords: Secondary pest, boll damage, compensation, yield components

Introduction

In Australia, Bt-cottons containing Cry endotoxins derived from *Bacillus thuringiensis* var *kurstaki* Berliner have dramatically reduced insecticide use against the primary pests *Helicoverpa spp.*. These insecticides controlled coincident green mirid (*Creontiades dilutus* (Stål)) populations. Reduced insecticide use in Bt-cotton allows mirid populations to persist season-long . Related pest species, e.g. *Lygus*, show similar trends in Bt-cotton in the USA and China .

Mirid damage to squares and bolls can reduce yield. found mirid feeding can cause shedding of small and medium squares and of young bolls (< ≈ 10 d old). Damage to older squares causes uneven pollination and boll growth. Developing bolls (10< and < 20 d old) usually do not shed, but may have one or more locules damaged. Bolls older than 20 days are unaffected due to the thick boll walls.

Control of mirid populations is a challenge due to difficulties in sampling, uncertainty of economic thresholds and the broad-spectrum activity of most insecticide control options disrupt beneficials and increase the risk of secondary pest outbreaks . Here we have investigated the response of cotton to real and artificial damage inflicted during boll development.

Materials and Methods

Evaluating the accuracy of mimicking boll damage by mirids using pectinase injection.
This experiment was done in a glasshouse in summer at the Australian Cotton Research Institute, Narrabri, New South Wales, Australia (ACRI; 30°S, 150°E). Cotton plants (Sicot 289BR; B indicates genes for Cry1Ac and Cry2Ab and R or RR indicates genes for glyphosate tolerance) were grown individually in 20 cm diam. pots in a replicated design, with four replications and 13 pots per replicate. When the plants had bolls 10 d old (5-7 nodes) damage treatments were implemented (Table 1). These included caging laboratory reared adult mirids onto bolls of known age or simulating mirid damage by injecting bolls (2 locules per boll) with a pectinase (Sigma-Aldrich Pty Ltd, Sydney Australia)/water solution (2 concentrations) at a known boll age. We diluted the pectinase (in 40% glycerol) with water at 1:10 and 1:20. A 1ml syringe was used to inject 1 or 2 µl of the pectinase solution in each locule to be damaged. This was done for three bolls per plant to increase replication, so the fruiting branches on which damaged the bolls were located at between the 5^{th} and 11^{th} node.

When mature bolls were scored for damage using a 0 to 4 scale with 0 being no damage and 4 being damage causing staining, and stunting of development in at least 3 locules of the boll. For each boll, seed cotton and lint weights per boll were recorded. The weights and locations (node and position e.g. P1, first position on a fruiting branch, P2 second position and so on) of all other bolls on the plant were also recorded to evaluate if reduced weight of damaged bolls was compensated for by increased boll weight elsewhere. Data were analyzed in Genstat 8 (VSN International, Oxford, UK) using ANOVA to compare boll weights between treatments for both damaged and undamaged bolls.

Response of field-grown cotton to simulated mirid damage

The effect simulated mirid damage on yield was investigated in field experiments across several cotton regions. Damage was inflicted at three stages: Early (21 days after first flower (DAFF), 20 nodes), Mid (35 DAFF, 23 nodes), Late (56 DAFF, post-cutout). At the first date, two levels of damage (low, 5 bolls per m^2 and medium, 20 bolls per m^2) and at the later dates three levels of damage (low, medium and high 50 bolls per m^2) were inflicted. In each case two locules per boll were injected for bolls aged 8-12 d old i.e. 20 to 40mm diameter). A randomized block design was used with four replications e.g 36 plots; 9 treatments by 4 replications. Plots were three m long and three rows (3 m) wide.

Eight experiments conducted across four regions; three experiments in 2003-04 , one in the lower Namoi Valley (ACRI – Sicala 189RR and Sicala 289BR), one in the Upper Namoi Valley, 100 km SE of Narrabri (Sicala V3BR) and one in the MacIntyre Valley, 200 km N of Narrabri (Sicot 14B). In 2004-05, there were 5 experiments, one at ACRI (Sicala 289BR), and one in the Upper Namoi Valley, McIntyre Valley, Emerald, 1200 km N of Narrabri and Hillston, 800 km SW of Narrabri, all Sicot 71BR. In all cases cotton was irrigated and managed according to commercial practice.

Yield was measured by hand harvesting a 2 m section from the central row of each plot. The number and weight of damaged and undamaged bolls was recorded separately. Total gin out-turn was measured for the damaged and undamaged bolls combined. Fiber quality was tested using a HVI. Data for all sites were combined and analyzed in Genstat 8 (VSN International, Oxford, UK) using ANOVA.

Comparing the results of experiments with simulated damage to real damage in the field
There were 4 experiments; one at ACRI in 2004-05 and one at ACRI, Hillston, and Emerald in 2005-06. A randomized block design was used, with plots 3m by 3 rows. There were 17 treatments and 4 replications, totaling 68 plots. In addition to boll injection plots, plots were allocated to mirid treatments. Mesh cages were placed over a portion of the plot (2m x 2 rows) and known numbers of adult mirids introduced. The mirids were allowed to feed for a week after which the plants were sprayed with a pyrethrum spray to kill mirids and the cages removed. The adult mirids were captured from nearby alfalfa (*Medicago sativa* L) crops and were of mixed age and sex.

The treatments were mirid densities of 1.5, 3.0, 6.0 and 12.0/m for 1 week at 35 and 56 DAFF, compared with boll injecting (5, 20 and 50 bolls/m) done at the same times. At Hillston only the Mid timing of damage was done. Each time, cages were used to confine mirids, an extra control treatment were also caged to allow for any effect of caging on growth. Yield was assessed by hand harvesting a 2 m section from the central row of each control and boll injection each week, or one m from each of the two caged rows from the start of boll opening. Undamaged and damaged bolls were harvested separately. Gin out-turn was measured for the damaged and undamaged bolls combined.

Results

Evaluating the accuracy of mimicking boll damage by mirids using pectinase injection. Mirids caged on bolls, in the combination of numbers and timing tested here, did not affect boll weight (Fig. 1A). In contrast, injecting bolls either with water or pectinase when bolls were 15 days old reduced boll weight. Injecting bolls at 30 days old had no effect on boll weight. The size of P1 bolls in the same node range as damaged bolls was no different between controls or damaged plants (Fig. 1B), but the size of P2 bolls was often larger (Fig. 1C). There was no difference in size to the controls of P1 bolls from upper nodes of damaged plants (Fig. 1D). Bolls injected with water or pectinase at 15 days old had higher damage scores (Fig. 1E). The number of locules damaged was higher for injected bolls and some mirid damaged bolls than the control (Fig. 1F).

Response of field-grown cotton to simulated mirid damage

There were differences between sites and treatments for each parameter measured but the treatment by site interactions were not significant ($P < 0.05$), so results are presented for the 8 sites combined. Injecting pectinase into small cotton bolls in the Mid and Late periods reduced lint yield (Fig. 2A). Injecting higher numbers of bolls tended to decrease yield more than injecting fewer bolls. Gin out-turn was significantly reduced when 20 or 50 bolls were injected in the Mid and Late periods (Fig. 2B). The number of damaged bolls harvested per m increased with the number of bolls injected (Fig. 2C), though was less than the number of bolls damaged. The number of undamaged bolls per m was less than the control in all except the Early low treatment (Fig. 2C), and the magnitude of difference essentially followed the pattern of the number of bolls injected. The weight of damaged bolls that were retained on the plants was reduced to a mean of 1.1 g per boll compared with about 2 g per boll for undamaged bolls (Fig. 2D). Crop maturity was slightly but significantly delayed in the Early medium, Mid medium and Mid high treatments, though only by a maximum of 2.75 days (Fig. 2E).

Damaged bolls had fiber with lower uniformity, shorter length, slightly reduced strength, higher short fiber content and reduced elongation (Fig. 3). However, when the small number of lighter damaged bolls was included with the larger number of heavier undamaged bolls and the weighted average values obtained, the damage treatments had no effect on any fiber quality parameters ($P > 0.05$ in all cases)..

Comparing the results of experiments with simulated damage to real damage in the field

At Hillston none of the treatments affected yield ($P > 0.05$), and no further data are presented for this site. The data for the other three of the sites, where all treatments were imposed, showed not site by treatment interactions so pooled results are presented. Boll injecting of 20 or 50 bolls per m^2 in the mid season or 50 bolls per m^2 in the late season significantly reduced yield (Fig. 4A). The appropriate caged control was used for comparison as caging tended to increase yield, possibly due to protection from weather. None of the mirid damage treatments reduced yield.

Gin out-turn was reduced by the Mid boll injections and heavier Late boll injections (medium and high) (Fig. 4B). There were more damaged bolls in many damage treatments than in the controls, especially the heavier injection treatments. However, numbers of undamaged bolls and total boll numbers did not differ significantly between treatments (Fig. 4C). Damaged bolls weighed less than undamaged bolls (Fig. 4D).

Discussion

Evaluating the accuracy of mimicking boll damage by mirids using pectinase injection.

Mirid feeding, at the levels evaluated, did not significantly affect boll weight, and bolls showed only minor levels of damage. Simulated damage, using injection of water or pectinase was more severe than damage caused by mirid feeding, for damage of up to 2 mirids per boll for one day or 1 mirid per boll for 2 days. estimated that the volume of a mirids salivary gland (*Lygus hesperus* (Knight)) was about 300 nl and in experiments where they injected this volume of *Lygus* app. salivary gland proteins into cotton flowers they observed damage symptoms similar to that from *Lygus* bugs. Our experiments used three times this volume (1 µl is 1000 nl) hence there may be greater physical as well as biochemical damage.

We found no compensation in boll size in P1 bolls in the same node region as the damaged bolls (nodes 5 – 11) or P1 bolls further up the plant. P2 bolls in this range were, however, often larger in the damage treatments than the control (Fig.. 3D), though not always and in some cases were larger even when boll weights of target bolls were not affected. It is possible that a moderate level of damage to a P1 boll, that doesn't affect its yield, triggers a plant response resulting in increase supply of assimilate to the P2 bolls. There is some evidence for this as P2 bolls on plants where bolls were fed on by mirids tended to be bigger than expected, even though the size of the target bolls was not affected. This would need further experimentation to confirm.

Response of field-grown cotton to simulated mirid damage

The reduced yield of Mid and Late season boll injection treatments was possibly due to several factors. Firstly, the gin out-turn of the more heavily damaged treatments was reduced. found that damaged portions of bolls are often hard and compacted, and was removed in the ginning process, hence reducing the yield. The second factor was the reduction in the total number bolls in some of the damage treatments and the third factor was the reduced weight of damaged bolls that were retained.

The weight of undamaged bolls did not differ between the control and damage treatments (see Fig. 2D); hence there is no evidence for this compensation mechanism. There is evidence of compensation through retention of undamaged bolls that would have been shed. For instance, for the mid high treatment we damaged 50 bolls per m and would expect to have harvested 73.2 undamaged bolls (i.e. the control (123.2) minus 50 damaged). Yet we harvested 95.4 undamaged bolls, so we have retained an extra 22 undamaged bolls. Further, we damaged 50 bolls yet harvested only 21.3 damaged bolls, so 28.7 of the bolls injected must have shed. The difference between the number of undamaged bolls in the treatments and control was always less than the number of bolls injected, indicating a degree of compensation via retention of undamaged bolls, that would have been shed, or alternatively the production of extra fruit in damage treatments. However, crop maturity was delayed by a maximum of 2.8 days. This indicates that suggested compensation occurred through retention of other fruit from the same 'cohort' as that injected, rather than through production of new fruit, as the latter would have resulted in greater delays in maturity as the new fruit would be at least 8 -12 days younger than the fruit injected. The retention of bolls from the same cohort, that would otherwise have been shed is often called 'substitution' and has been widely reported for damage from Lepidopteran pests. It occurs because cotton plants produce two to three times as many fruit as they can mature .

Physiological shedding of excess fruit occurs due to an imbalance between source and sink within the plant. Weak sinks, such as small squares or bolls, are prone to being shed if the plant is unable to meet its needs for growth and for maturation of bigger sinks . The number of damaged bolls harvested per m was always less than the number injected, indicating that some injected bolls were shed. This may have contributed to the plant's capacity to compensate; by shedding some damaged bolls, resources are available retain other undamaged bolls.

Medium damage in the Mid and Late periods caused yield loss, while in the Early period it did not. It is possible that in the Early period the plant's size was large relative to the number of fruit, hence it has more capacity to substitute damaged fruit, whereas in the Mid and Late periods the competition between sources and sinks may be more intense, resulting in reduced capacity to compensate.

Damage bolls had poorer quality than undamaged bolls, but when pooled the overall effect on fiber quality was minimal. This is partially because hard, compacted damaged lint is removed during ginning, thereby reducing the contribution of damaged lint to overall fiber quality and because the proportion of damaged bolls is small, so effects are diluted . Damaged lint will have poorer quality due to the damage caused by pectinase (e.g. , as well as to the reduction in fiber development (eg cell lengthening in the first 20 d after flowering) and thickening (reducing micronaire, strength and elongation) because the seed from which it was growing was damaged or dead. Commercially, the effect of mirids on fiber quality would be influenced by the proportion of total bolls damaged.

Comparing the results of experiments with simulated damage to real damage in the field

Yield loss in the injection treatments was due to reduced gin out-turn and higher numbers of lighter damaged bolls. Again there is evidence of compensation in boll numbers by retention of more undamaged bolls. None of the mirid damage treatments reduced yield despite these treatments having significantly higher numbers of damaged bolls than the controls. This supports research by indicating an economic threshold of 3 mirids per m^2 in the mid season and 4-5 mirids m^2 in the late season.

Conclusions

Cotton can tolerate a degree of mirid damage in the boll filling without resulting in yield loss. This is in contrast to the beliefs of many pest management practitioners in cotton who often treat mirid populations at levels well below threshold . The outcomes of this study support recent thresholds suggested by which are higher than current thresholds and considerably higher than those used by many pest management practitioners .

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Table 1. Treatments imposed to evaluate the accuracy of injecting bolls with pectinase solution to simulate damage from green mirids.

Treatment	Boll age when damaged	Duration of exposure / number of locks injected
Control	-	-
1 caged mirid/boll	15 days	1 day
2 caged mirids/boll	15 days	1 day
2 caged mirids/boll	15 days	1 day
2 caged mirids/boll	15 days	1 day
1 caged mirid/boll	15 days	2 days
1 caged mirid/boll	30 days	1 day
2 caged mirids/boll	30 days	1 day
1 caged mirid/boll	30 days	2 days
1μl of water	15 days	2 locks
1μl of 1:10 pectinase	15 days	2 locks
1μl of 1:20 pectinase	15 days	2 locks
1μl of water	30 days	2 locks
1μl of 1:10 pectinase	30 days	2 locks
1μl of 1:20 pectinase	30 days	2 locks

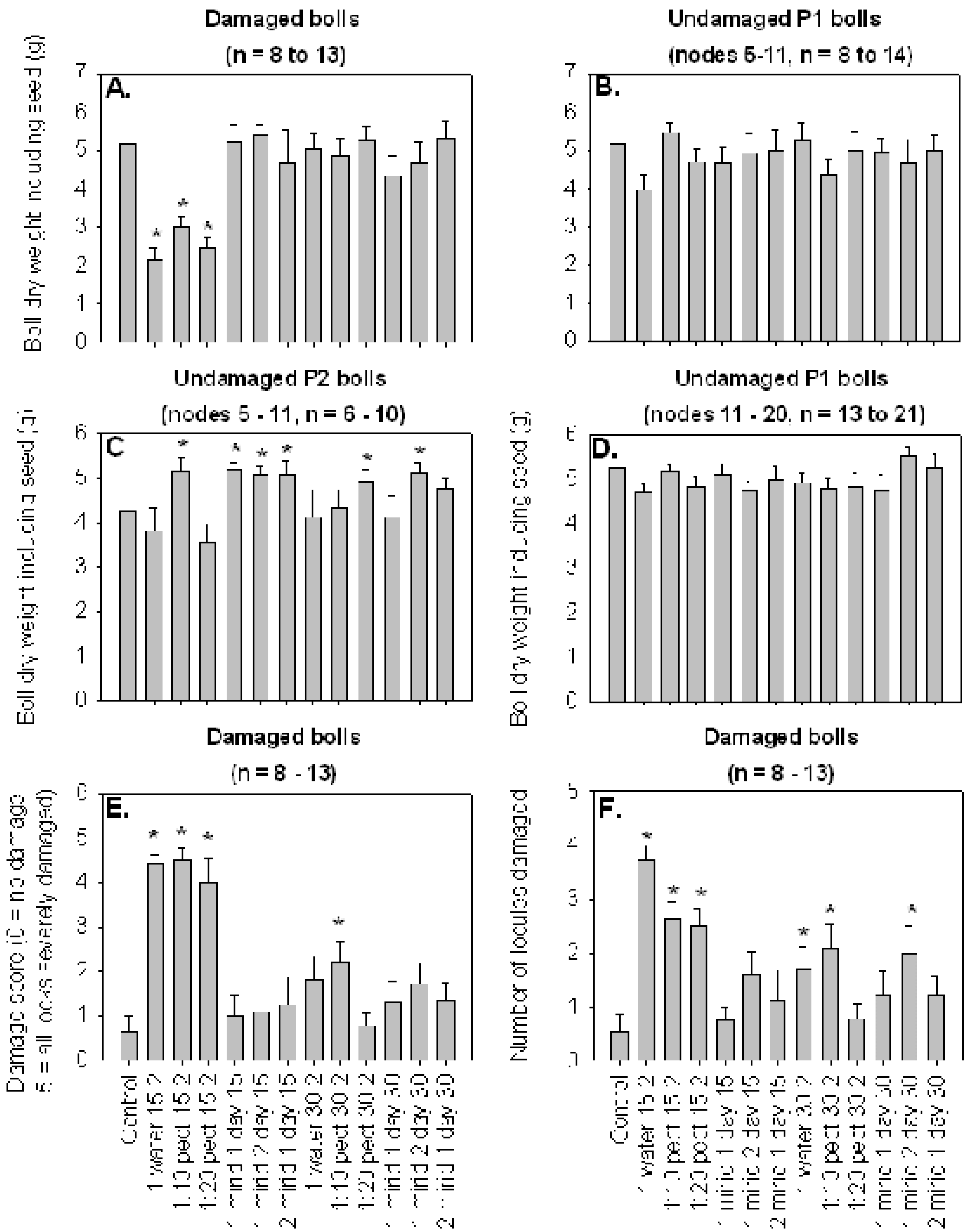
Figure captions

Figure 1. Effect of damage treatments on weights of (A) target bolls (B) undamaged P1 bolls from nodes 5 -11, (C) undamaged P2 bolls from nodes 5 -11 and (D) undamaged P1 bolls from nodes 13-21 (E) damage scores of target bolls and (F) number of locules damaged per boll. The number of bolls contributing to each bar is indicated. Asterisks indicate treatments significantly different from the control using ANOVA at $p < 0.05$.

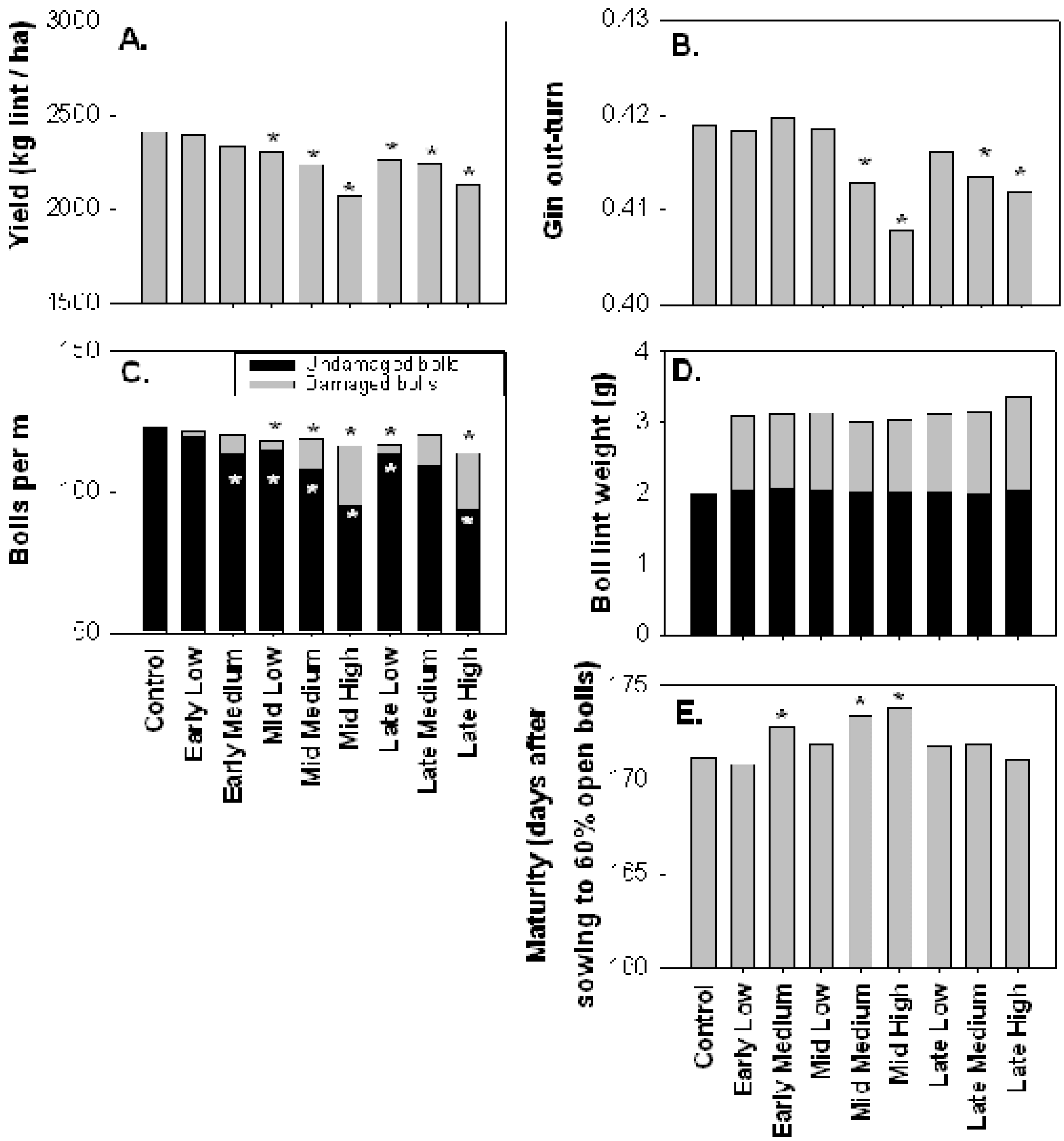
Figure 2. Effect of damage treatments on (A) lint yield, (B) gin out-turn (C) number of bolls per m^2 (E) boll weights and (D) crop maturity date. Trials were conducted at 8 site/years. Asterisks indicate treatments significantly different from the control using ANOVA at $p < 0.05$.

Figure 3. Fiber properties of damaged and undamaged bolls. Asterisks indicate significant difference between damaged and undamaged bolls at that site using ANOVA at $p < 0.05$

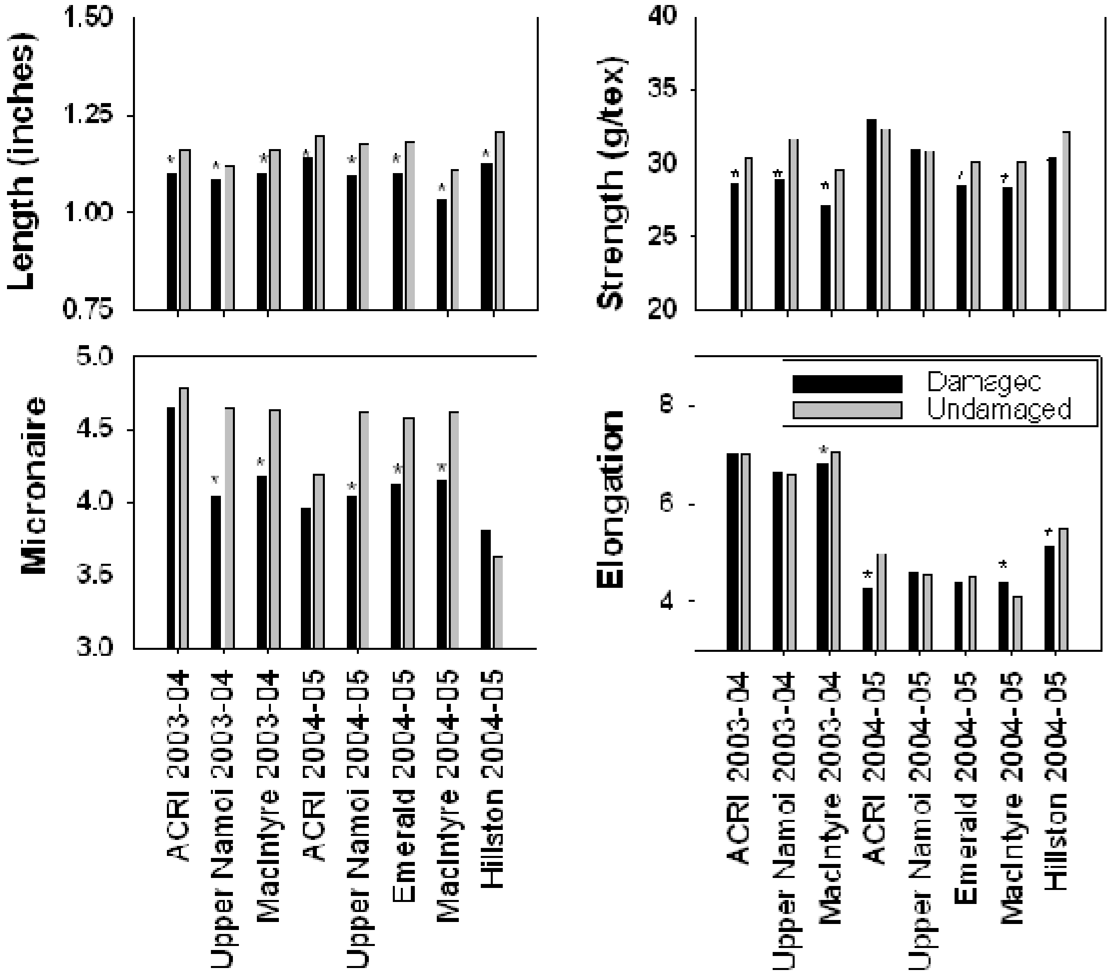
Figure 4. Effect of mirid feeding or boll injecting on (A) yield (B) gin out-turn (C) number of bolls per m^2 and (D) boll weight of cotton. Asterisk indicates significantly different to the control at $P < 0.05$ using ANOVA.



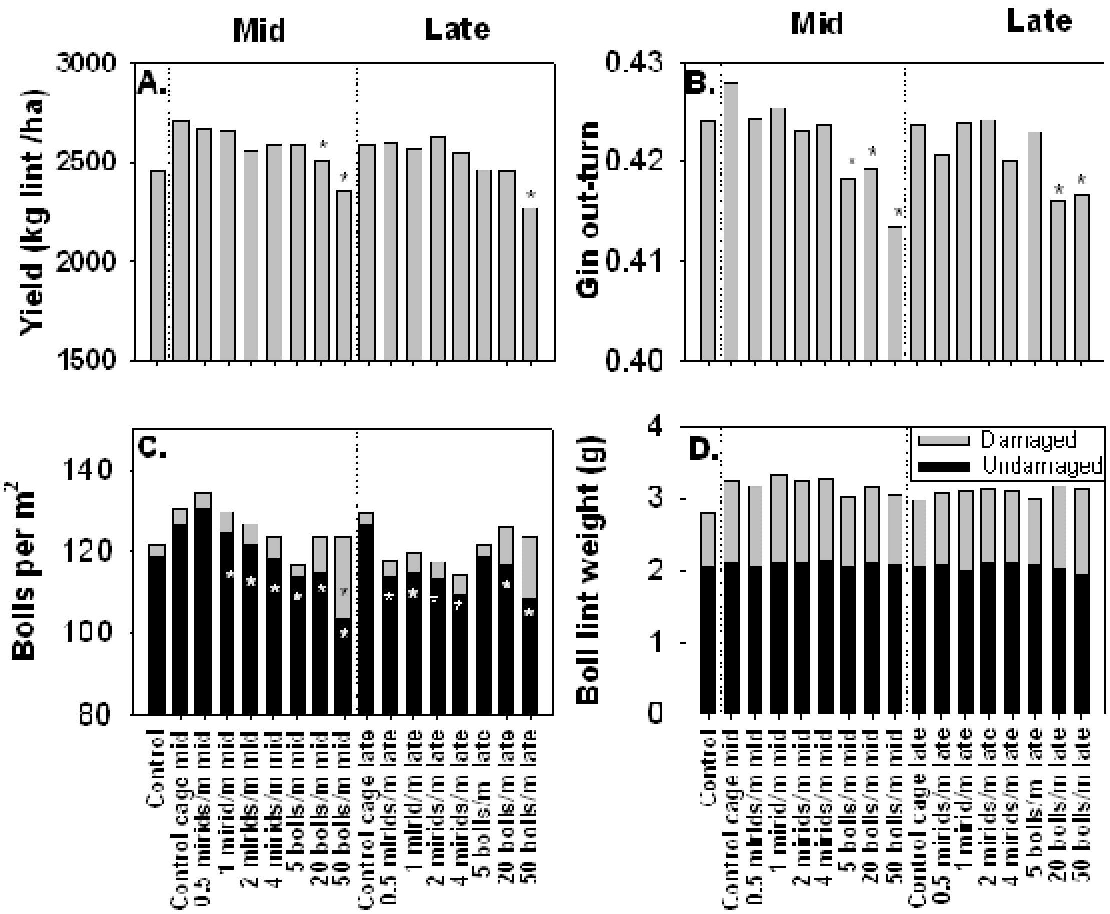
Damaged bolls
(n = 8 to 13)
A.
Boll dry weight including seed (g)
Undamaged P1 bolls
(nodes 5-11, n = 8 to 14)
B.
Undamaged P2 bolls
(nodes 5 - 11, n = 6 - 10)
C.
Boll dry weight including seed (g)
Undamaged P1 bolls
(nodes 11 - 20, n = 13 to 21)
D.
Damaged bolls
(n = 8 - 13)
E.
Damage score (0 = no damage
5 = all locks severely damaged)
Damaged bolls
(n = 8 - 13)
F.
Number of locules damaged
Control
1 water 15 2
1:10 pect 15 2
1:20 pect 15 2
1 water 30 2
1:10 pect 30 2
1:20 pect 30 2



A.
Yield (kg lint / ha)
3000
2500
2000
1500
B.
Gin out-turn
0.43
0.42
0.41
0.40
C.
Bolls per m
150
100
50
Undamaged bolls
Damaged bolls
D.
Boll lint weight (g)
4
3
2
1
0
E.
Maturity (days after sowing to 60% open bolls)
175
170
165
160
Control
Early Low
Early Medium
Mid Low
Mid Medium
Mid High
Late Low
Late Medium
Late High



Length (inches)
1.50
1.25
1.00
0.75
Strength (g/tex)
40
35
30
25
20
Micronaire
5.0
4.5
4.0
3.5
3.0
Elongation
8
6
4
Damegec
Undamaged
ACRI 2003-04
Upper Namoi 2003-04
MacIntyre 2003-04
ACRI 2004-05
Upper Namoi 2004-05
Emerald 2004-05
MacIntyre 2004-05
Hillston 2004-05



Mid
Late
A.
B.
C.
D.
Yield (kg lint /ha)
3000
2500
2000
1500
Gin out-turn
0.43
0.42
0.41
0.40
Bolls per m2
140
120
100
80
Boll lint weight (g)
4
3
2
1
0
Damaged
Undamaged
Control
Control cage mid
0.5 mirids/m mid
1 mirid/m mid
2 mirids/m mid
4 mirids/m mid
5 bolls/m mid
20 bolls/m mid
50 bolls/m mid
Control cage late
0.5 mirids/m late
1 mirid/m late
2 mirids/m late
4 mirids/m late
5 bolls/m late
20 bolls/m late
50 bolls/m late