Mirid damage assessment in Bollgard II - critical damage stage and action thresholds at different stages in irrigated and raingrown cotton

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Two species of mirids - green mirid (GM), *Creontiades dilutus* (Stål) and brown mirid (BM), *Creontiades pacificus* (Stål) - are found in Australian cotton. They are important sucking pests in Bollgard II causing damage from the seedling stage through to the late boll formation stage. During the 2005/06 season, two to four insecticide sprays were required to manage mirids. Preliminary studies showed that the nature of damage to cotton for both mirid species is similar. While GM are abundant throughout the season, BM are usually found in the later part, from mid January onwards. At seedling stage their feeding causes damage to tips, branch primordia and young leaves and at squaring stage, feeding on squares causes abscission. Khan (1999) found that small squares up to 3 cm are the most vulnerable to abscission, but it depends on the feeding site. Squares are shed if mirids feed on the ovule and reduce auxin production, the chemical responsible for retention, or if mirids feed on the soft stalk of the square, where the chemical pectinase, released by the mirids during feeding, causes breakdown of cells surrounding the feeding site. At boll stage, mirid feeding is characterised by black spots on the bolls, warty growth inside the boll wall and brown coloured lint. Small bolls, less than 7 days old, could drop off and bolls with severe lint damage end up with characteristic tight lock which is unharvestable (Khan and Bauer 2001; Lei *et al*. 2002). In the pre-transgenic cotton era, mirids were considered early season pests and broad-spectrum chemicals applied to control *Helicoverpa* usually kept mirids under control, particularly in the later part of the cotton season. While mirids have been studied in detail in conventional cotton by Miles (1995) and Khan (1999), the information on mirids in Bollgard II is limited.

In Australia, current management decisions made by growers or consultants for mirids in Bollgard II are based on treatment threshold levels of 1.5 to 3/m (Farrell and Johnson 2005). These are based on beat sheet sampling and are as determined for conventional cotton. Since physiological and agronomic aspects of conventional cotton are different to Bollgard II, the existing thresholds need to be revised. In conventional cotton *Helicoverpa* is the major pest that causes early fruit loss. Additional fruit loss from mirids is very critical to cotton yield and therefore the threshold level was somewhat conservative. In Bollgard II in the absence of *Helicoverpa* damage, plants retain more early fruit which may lead to early cut out. It is thought in the Australian cotton industry that early cut out cotton yields less with lower fibre quality. This may be more pronounced in raingrown cotton under limited water conditions. There is also some sentiment in the Australian cotton industry that Bollgard II could tolerate more mirids in the early stage, when some fruit loss can be tolerated and which may avoid early cut out. Early mirid management intervention sometimes disrupts natural enemies and results in outbreaks of whitefly, mites or aphids later in the season. It is therefore necessary to clarify these concerns and optimise the mirid thresholds at different stages of cotton growth to achieve better management decisions.

Here we report on the on-going investigation into mirid damage assessment in Bollgard II with the objective to determine action thresholds for mirids at different stages of Bollgard II growth in irrigated and raingrown systems.

Methods

Field trials

Field trials were conducted in three irrigated and raingrown cotton fields. Details of these six sites are given in Table 1. Damage was assessed in three different stages (treatments). There were two more treatments - unsprayed and sprayed throughout the season. Plots were sprayed if mirid numbers reached >1/m in accordance with an IPM strategy. Treatments were replicated three times. Plot sizes for each trial site are given in Table 1. Sizes of the plots were determined according to the grower's spray boom length for ease of management operations. The stages were squaring (from seedling to 60% plants reached 1st flower), early boll (from first flower to 60% bolls reached 20 days old) and late boll (from 20 days old boll to cut out). Each stage was left unsprayed during the defined time period, but before and after that time treatment plots were sprayed to keep mirids and other insects under control.

Table 1. Description of trial sites

Growing System	Trial site	Variety	Plot size (per replication)	Date of planting
Irrigated	1. Glen Fresser (Mayfield), Dalby S 27°14.816; E 151°07.996	Sicot 289BR	50 m X 24 rows	3 Nov. 2005
	2. Peter Bailey, Macalister S 27°03.270; E 151°01.008	Sicot 71BR	50 m X 24 rows	2 Nov. 2005
	3. Neval Walton, Macalister S 27°04.114; E 150°59.382	Sicot 71BR	50 m X 24 rows	30 Oct. 2005
Raingrown	4. Richard Dowsett (Wyobie), Jimbour S 26°55.273; E 151°07.740	Sicot 289BR	50 m X 24 rows Single skip row	31 Oct. 2005
	5. St. John Kent (Coondarra), Jimbour S 26°56.713; E 151°08.175	Sicot 289BR	20 m X 18 rows Single skip row	1 Nov. 2005
	6. Neil Wegener, Macalister S 27°02.157; E 151°04.977	Sicot 71BR	50 m X 20 rows Double skip row	2 Nov. 2005

Fields were sampled once a week. Up to the 8/9 node plant stage, one 20 m row section/replication was sampled with a suction machine. Collected insects were returned to the laboratory for further processing and for recording data. After the 9 node plant stage, sampling was done on 3 x 1 m row sections/replication with a beat sheet. The beat sheet method was used at this stage since this method was more efficient than any other method for sampling mirids and other predatory insects. For each stage, estimated fruit loss for all stages and boll damage for early and late boll stage, were assessed twice (3 x 1 m row section/replication); one at the last sampling date of the stage and the other at cut out stage. In unsprayed and fully sprayed treatments, damage was assessed every time as with other stages. Yield was assessed by hand picking, three randomly selected 1 m row sections in each plot. Tight lock and brown lint portions were discarded from the hand harvest since mechanical pickers can not pick tight locks and brown lint has quality implications (Lei *et al*. 2002).

Cage Trial

A replicated cage trial was conducted on Bollgard II at Kingaroy Research Station (KRS) with different densities of GM on 1 m row cotton using 1 m x 0.8 m x 1.2 m field cages at different stages of crop growth. The stages were same as in the field trial and densities of GM were 0, 1, 2, 3 and 4/cage with 5 replications. Caged plots were marked just after emergence for each stage and sprayed in accordance with an IPM strategy (such as reduced rate of fipronil plus salt) to keep plots insect free until caging. Insects were allowed to feed throughout the stage. Thereafter cotton was sprayed until maturity to avoid further damage. Male GM were used to avoid further build up of the population and dead individuals were replaced. Fruit loss and boll damage were assessed twice for each stage as described for the field trial. Plots were hand-harvested and cotton weight was recorded as in the field trial.

Analysis

Data were analysed by ANOVA in MINITAB and means were compared using LSD at 5%. Regression analysis was performed for mirid density and damage relationships for cage trials and threshold levels were determined from the equations.

Results

Field Trials

Mirid numbers on different sampling dates for each stage in different sites are given in Figures 1 and 2. At all sites mirid numbers in the unsprayed control and at each stage were higher than the respective sprayed control and were significantly different ($P<0.05$) at squaring stage at sites 1, 2 and 5 and at early boll stage at sites 2, 4, 5 and 6. The average mirid number/m with range at squaring and early boll stage for all sites is presented in Table 2. At late boll stage at all sites, mirid numbers were very low, less than 1/m, and differences between treatments were not significant ($P>0.05$).

The percentage fruit loss at each stage (Obs. 1) and at cut out (Obs. 2) and number of damaged bolls for irrigated and raingrown cotton are given in Tables 3 and 4 respectively. The results showed that for observation 1 fruit loss at squaring stage and at early boll stage was significantly higher ($P<0.05$) than sprayed control at sites 2, 3, 5 and 6 and at sites 2 and 4 respectively. At early boll stage at sites 3 and 6, fruit loss in observation 1 was significantly lower ($P<0.05$) than unsprayed control. At late boll stage, fruit loss in observation 1 was lower than the unsprayed control at almost all sites and the difference was significant ($P<0.05$) at sites 3 and 5. The results also showed that irrespective of percentage of fruit loss in observation 1, percentage loss at each stage in observation 2 was not significantly different ($P>0.05$) from unsprayed or sprayed control except at site 3. Number of bolls damaged at early boll stage in both observations was significantly higher ($P<0.05$) than sprayed control and was significantly lower ($P<0.05$) than unsprayed control at late boll stage.

Figure 3 shows yield (bales/ha) at different stages for each site. The results showed that yields at squaring stage were higher than unsprayed control and almost similar to sprayed control except at site 3 and the difference was significant ($P<0.05$) at sites 1, 4 and 5. At site 3, however, yield was significantly lower ($P<0.05$) than sprayed control. At early boll stage, yields were significantly lower ($P<0.05$) than sprayed control except at site 5 where yields were lower but the difference was not significant ($P>0.05$). At late boll stage at all sites yields were higher than unsprayed control with significant differences ($P<0.05$) at sites 1 and 3.

Table 2. Average mirid number/m along with range at squaring and early boll stage at all sites

Site	Crop growth stage	Mirid/m (range)
1	Squaring stage	2.3 (0 – 7)
	Early Boll stage	1.3 (0 – 3)
2	Squaring stage	1.6 (0 – 7)
	Early Boll stage	7.6 (0.33 – 16)
3	Squaring stage	2.2 (0 – 6)
	Early Boll stage	1.7 (0 – 4)
4	Squaring stage	1.4 (0 – 6)
	Early Boll stage	0.6 (0.33 – 1)
5	Squaring stage	3.3 (0 – 7)
	Early Boll stage	1.8 (0.67 – 3)
6	Squaring stage	1.5 (0 – 4)
	Early Boll stage	1.5 (0 – 5)

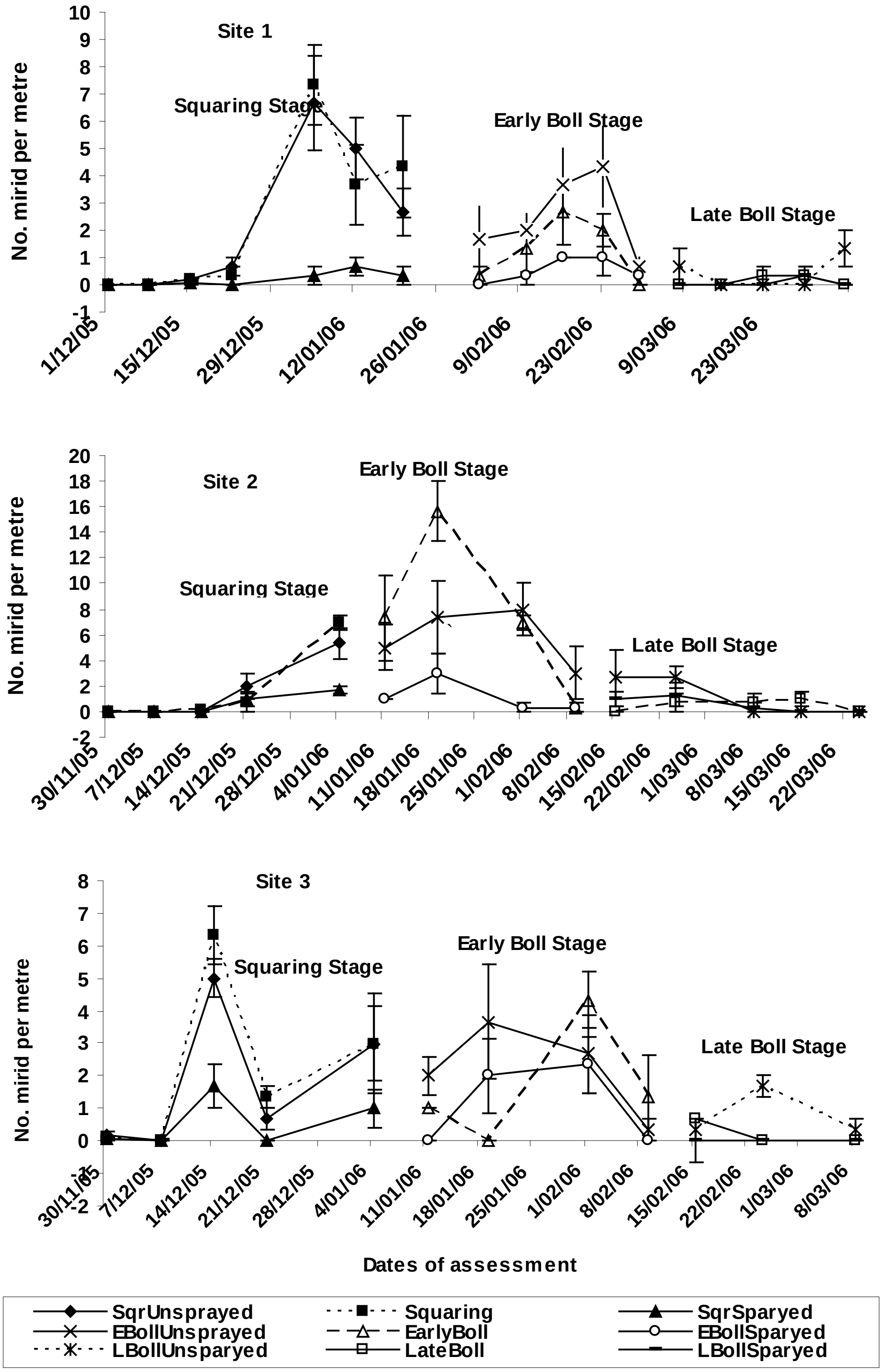

Figure 1. Mirid numbers at different crop growth stages in irrigated cotton. Error bars indicate standard error of mean.

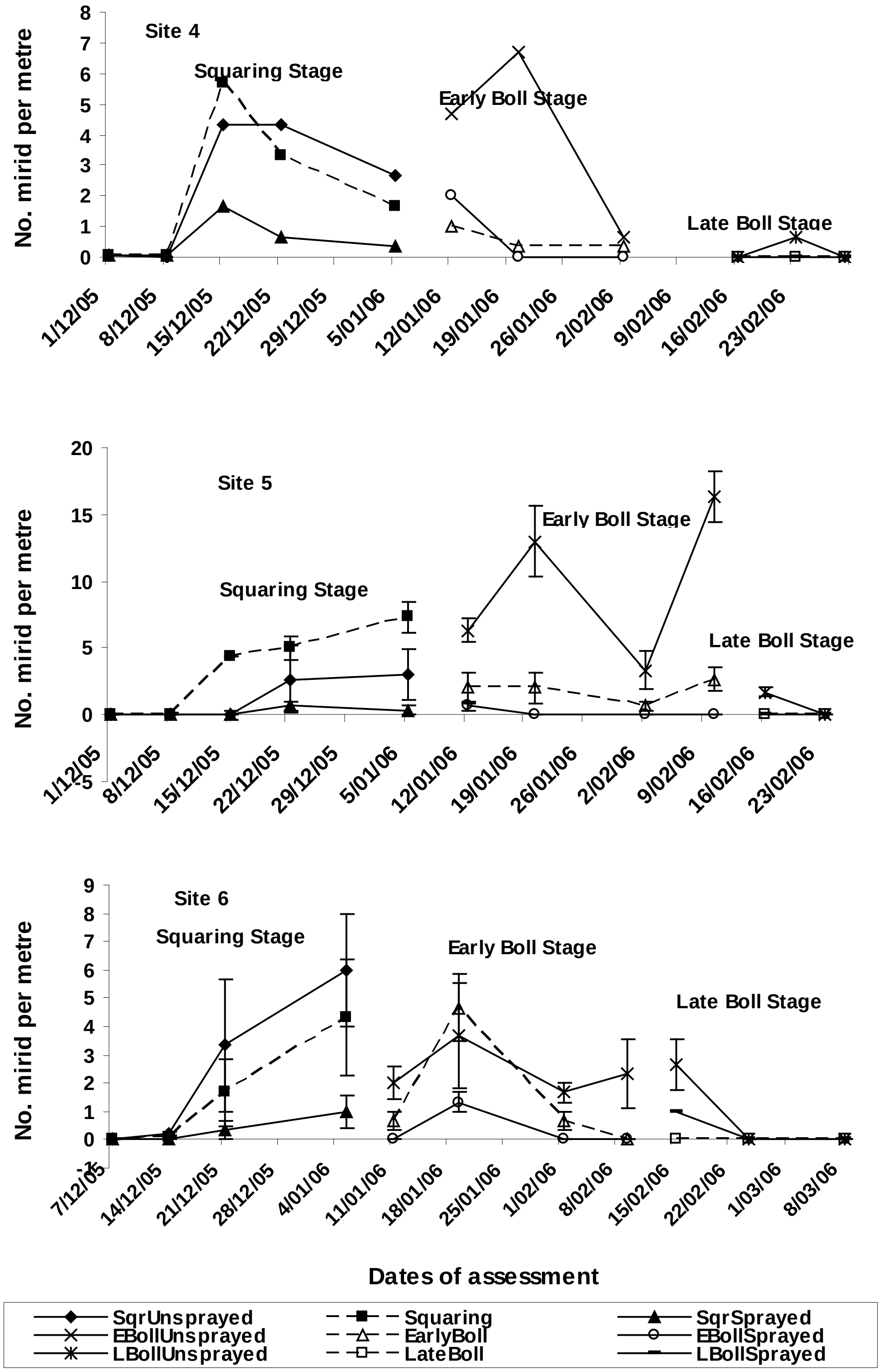

Figure 2. Mirid numbers at different crop growth stages in raingrown cotton. Error bars indicate standard error of mean.

Table 3. Percentage fruit loss and boll damage in irrigated cotton. * indicate value significantly different (P<0.05) from sprayed control and + indicate value significantly different (P<0.05) from unsprayed control.

Trial Site	Treatments	% fruit loss		No. bolls damaged/metre	
		Obs. 1	Obs. 2	Obs. 1	Obs. 2
1	Squaring stage	08.25	43.44	-	-
	Early Boll stage	22.44	41.52	20.00 *	12.67 *
	Late Boll stage	47.98	38.94	06.00 +	05.00 +
	Unsprayed Squaring	09.71		-	-
	Unsprayed E. Boll	24.13	46.54	31.00	11.33
	Unsprayed L. Boll	55.11		16.33	
	Sprayed Squaring	07.45		-	-
	Sprayed E. Boll	20.62	35.28	08.33	05.00
	Sprayed L. Boll	49.07		09.00	
2	Squaring stage	16.71 *	43.36	-	-
	Early Boll stage	35.58 *	50.56	25.67 *	17.00 *
	Late Boll stage	57.20 *	41.93	20.33 +	06.00 +
	Unsprayed Squaring	21.19		-	-
	Unsprayed E. Boll	32.00	43.24	22.33	10.67
	Unsprayed L. Boll	49.08		40.33	
	Sprayed Squaring	06.29		-	-
	Sprayed E. Boll	18.76	44.35	02.33	05.00
	Sprayed L. Boll	25.35		13.67	
3	Squaring stage	61.75 *	59.09 *+	-	-
	Early Boll stage	25.68 +	55.99 *+	04.00	11.67 *
	Late Boll stage	48.65 +	50.65 *+	06.33 +	04.00 +
	Unsprayed Squaring	69.64		-	-
	Unsprayed E. Boll	54.10	70.33	03.67	14.00
	Unsprayed L. Boll	63.25		14.00	
	Sprayed Squaring	11.05		-	-
	Sprayed E. Boll	22.07	47.57	04.67	03.67
	Sprayed L. Boll	48.21		07.33	

Cage Trial

The results are summarised in Table 5 and Figure 4.

Table 5 shows that the fruit loss for observation 1 at squaring and early boll stage was significantly higher (P<0.05) than control (0 mirid). At squaring stage, 3 mirids/m caused significant fruit loss while at early boll stage 1 mirid/m caused significant loss. Losses at squaring stage recovered by cut out and differences were not significant (P>0.05) between treatments (Table 5). For damage at early boll stage, there was some recovery by cut out but 3 mirids/m still caused significant loss ($P<0.05$) compared to control. At late boll stage for both observations differences between treatments were not significant ($P>0.05$) (Table 5).

Table 4. Percentage fruit loss and boll damage in raingrown cotton. * indicate value significantly different ($P<0.05$) from sprayed control and + indicate value significantly different ($P<0.05$) from unsprayed control.

Trial Site	Treatments	% fruit loss		No. boll damage/ metre	
		Obs. 1	Obs. 2	Obs. 1	Obs. 2
4	Squaring stage	29.92	53.33	-	-
	Early Boll stage	31.06 *	55.99	23.00 *	07.00
	Late Boll stage	50.28	52.57	15.33 +	05.67 *+
	Unsprayed Squaring	15.52		-	
	Unsprayed E. Boll	27.53	51.66	29.67	10.00
	Unsprayed L. Boll	53.31		29.33	
	Sprayed Squaring	11.27		-	
	Sprayed E. Boll	21.19	51.19	04.67	10.00
	Sprayed L. Boll	49.74		10.00	
5	Squaring stage	19.47 *	55.90	-	-
	Early Boll stage	29.40	46.77	29.33 *	10.00 *+
	Late Boll stage	49.17 +	52.70	07.00 +	08.00 +
	Unsprayed Squaring	14.97		-	
	Unsprayed E. Boll	28.63	52.50	35.00	19.33
	Unsprayed L. Boll	60.27		20.67	
	Sprayed Squaring	11.83		-	
	Sprayed E. Boll	26.57	50.33	08.33	03.33
	Sprayed L. Boll	46.97		09.67	
6	Squaring stage	34.53 *	59.29	-	-
	Early Boll stage	31.95 +	56.14	05.67	06.67 +
	Late Boll stage	51.25	53.04 +	08.00 +	02.67 +
	Unsprayed Squaring	40.64		-	
	Unsprayed E. Boll	58.89	61.91	08.67	12.33
	Unsprayed L. Boll	59.69		32.67	
	Sprayed Squaring	13.32		-	
	Sprayed E. Boll	25.27	55.21	08.33	04.00
	Sprayed L. Boll	55.97		10.00	

Figure 4 shows that at early boll stage 3 mirids/m produced significantly less yield ($P<0.05$) than control. The figure also shows that there was no significant yield difference ($P>0.05$) between 3 and 4 mirids/m at early boll stage. At squaring and late boll stage there was no significant yield difference between treatments ($P>0.05$). The regression equations used to predict yield loss/mirid were $Y = 5.54 - 0.166X$ ($R^2 = 0.12$), $Y = 4.28 - 0.236X$ ($R^2 = 0.41$) and $Y = 7.60 - 0.154X$ ($R^2 = 0.06$) for squaring, early boll and late boll stage respectively. Indicative yield loss for 1 mirid/m at squaring, early boll and late boll stage were 0.166, 0.236 and 0.154 bales/ha respectively.

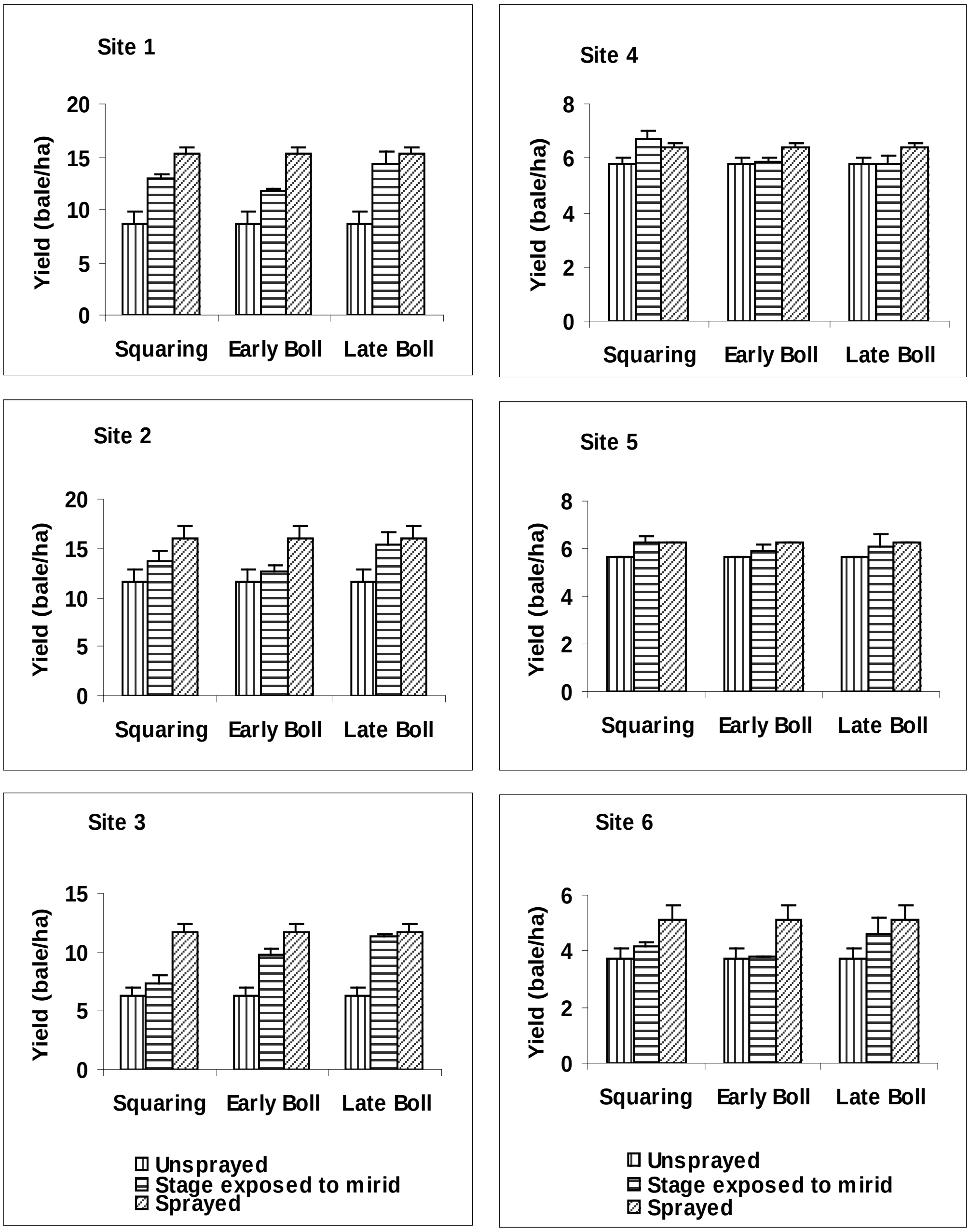

Figure 3. Yield (bale/ha) at each stage at different sites. Error bars indicate standard error of mean.

Discussion and conclusions

The field study showed that irrespective of the growing system, early boll stage (from 1st flower to bolls reaching 20 days old or more (about 5 weeks) is the most critical period for Bollgard II to suffer yield loss from mirid infestation (see Figure 3). At early boll stage, mirid populations in the trial sites reached ≥3/m on at least one sampling date except at raingrown site 4, where the maximum population reached 1/m (Figure 1 and 2). Even with this low number of mirids at site 4, cotton suffered a significant yield loss, indicating raingrown cotton's vulnerability to sustain damage. Mirids, however, were not the only contributing factor. Soil moisture condition in the field perhaps played an important role in this case. The raingrown cotton sites had their last substantial rain (80, 50 and 20 mm at sites 4, 5 and 6 respectively) in 3rd week of January when at sites 4 and 5 50% of bolls reached 20 days old. Lack of subsequent rainfall resulted in a quick crop finish associated with indiscriminate fruit loss.

Table 5. Percentage fruit loss at different stages in Bollgard II in a field cage trial. Means within a column followed by the same letter are not significantly different (*P*>0.05); Fisher's protected least significance difference test. No letters indicate no significant difference (*P*>0.05).

Mirids/m	Squaring Stage		Early Boll Stage		Late Boll Stage	
	Obs. 1	Obs. 2	Obs.1	Obs. 2	Obs. 1	Obs. 2
0	11.50 b	42.59	48.37 c	56.64 c	54.99	48.65
1	13.58 b	42.03	53.74 b	58.45 bc	56.03	45.90
2	11.74 b	41.82	59.11 a	59.68 bc	56.65	49.06
3	19.85 a	46.63	58.23 ab	64.38 ab	57.03	50.71
4	23.69 a	44.37	61.45 a	67.55 a	57.47	49.69

At early boll stage, percentage fruit loss at observation 1 recovered to some extent by cut out, suggesting that the level of fruit loss was not sufficient to result in yield loss in this study (see Tables 3 and 4). The percentage fruit loss was low at observation 1 (≤30%) at all sites. Another factor was that damaged bolls with tight locks also contribute to yield loss. This is in agreement with the findings of Lei *et al.* (2002).

It is well documented that cotton plants usually compensate for fruit losses prior to first flower (Brook *et al.* 1992; Sadras 1995) provided plants do not further suffer from moisture or any other stress. In our study, where percentage fruit loss at squaring stage (60% plants with 1st flower) was 10 to 30%, plants recovered well. At site 3 where fruit loss was 62%, plants failed to fully recover and suffered significant yield loss. These data indicate that yield loss could result from high square loss (>60%) at squaring stage even with ideal growing conditions. At this site, however, conditions were not ideal, with water shortages preventing at least 2 irrigations. The average mirid number was the same as site 1 and maximum mirid number was the same as other sites, but site 4 still suffered more fruit loss than the others. Khan (1999) found that the relationship between mirid

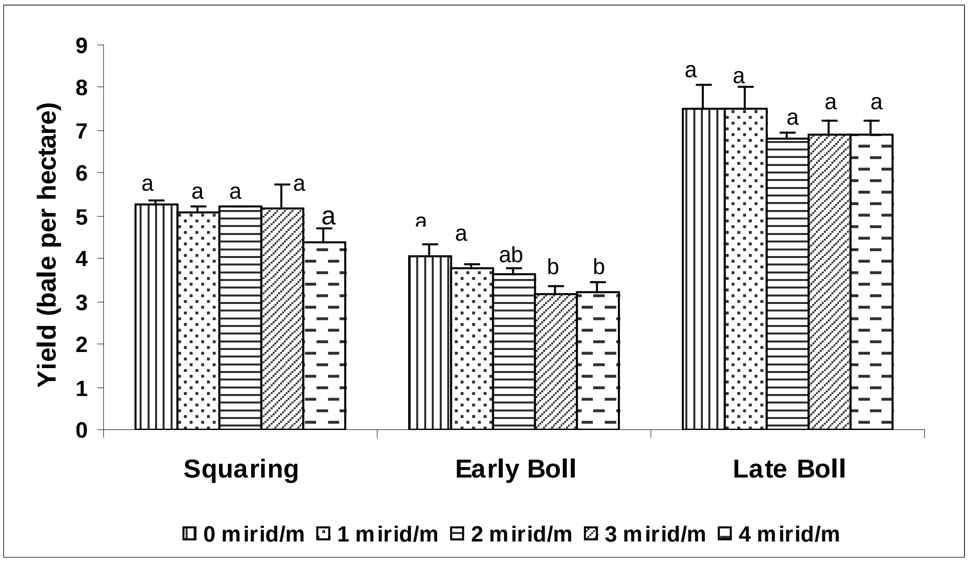

Figure 4. Yield (bales/ha) for each treatment at different crop stages in a field cage trial. Error bars indicate standard error of mean. The same letter above the bar for each crop stage indicates no significant difference (P>0.05); Fisher's protected least significance difference test.

number and square loss was poor and square loss depends on feeding site and square size. Mirid feeding on the stalk of the squares and ovule usually result in square loss. Squares less than 3 cm are usually shed due to mirid feeding. This may be the case in our study at site 3. Consultants have reported this phenomenon with low mirid numbers associated with high fruit loss and vice versa.

As with the field study, the cage study showed a poor relationship between fruit loss at squaring and early boll stage and yield loss (see Table 5 and Figure 4). Plants recovered from fruit loss at squaring stage irrespective of mirid density. Percentage fruit loss, however, was low at squaring stage (24%) even with 4 mirids/m. In comparison, 4 mirids/m at early boll stage caused 61% fruit loss and plants failed to recover. At early boll stage, 3 to 4 mirids/m also significantly reduced yield.

In conclusion our results clearly indicate that in Bollgard II, early boll stage is the most critical period for mirid damage. Three mirids/m or more than 50% fruit loss at early boll stage caused significant yield loss. At squaring stage, plants compensated for damage from 4 mirids/m if fruit loss was less than 30%. If fruit loss was more than 50% other stress factors such as water shortage affected yield loss. At late boll stage, mirids failed to cause any significant yield loss.

Based on this season's results, we propose the following action thresholds for mirids in irrigated Bollgard II:
Squaring stage – 4 to 5 mirids/m or/with 50% fruit loss
Early boll stage – 3 mirids/m or/with 50% fruit loss
Late boll stage - >5 mirids/m or/with 50% fruit loss

Considering the fact that rainfall and moisture availability play an important role in determining yield of raingrown crops, we propose the action threshold for mirids in raingrown cotton as 3/m or/with 50% fruit loss for all stages. The thresholds for mirid numbers are based on the beat sheet sampling method. If other sampling methods are used (visual, sweep net), appropriate conversion factors (Farrell *et al.* 2005) should be applied.

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