Boll damage by sucking pests: An emerging threat, but what do we know about it?

Tom Lei[1], Moazzem Khan[2] and Lewis Wilson[1]
[1] CSIRO Plant Industry, Cotton Research Unit and Australian Cotton CRC, Narrabri NSW AUSTRALIA
[2] QDPI, FSI and Australian Cotton CRC, Kingaroy QLD AUSTRALIA
Correspondence author tom.lei@csiro.au

ABSTRACT

Historically, green mirids are common pests of seedling cotton, but not during fruit set. This is partially because they were controlled by insecticides applied against Helicoverpa *species. With the introduction of the one- and two-gene Bt-cotton, the number of* Helicoverpa *sprays will decline, potentially allowing green mirids to build to levels causing boll damaging later in the season. The effect of damage on yield and fiber quality, and the ability of cotton to compensate from it is poorly understood. Unnecessary control of mirids with insecticides may undermine some of the IPM benefits conferred by transgenic cotton. We addressed these issues by conducting experiments where plants were either artificially damaged or naturally damaged by green mirids. We mimicked boll damage by mirids by injecting pectinase, a component of mirid saliva, into 10-day-old bolls. Piercing the boll wall with a syringe always resulted in a visible external scar and the formation of warts on the inner boll wall. Wart formation, however, was not always correlated with lint damage. We found that termination of fiber development results only when seeds were pierced. The injection of pectinase exacerbated the piercing damage and lint discoloration. Compared to undamaged bolls, the seed cotton mass of individual bolls injected with pectinase was reduced by 38%. Plants responded to such boll damage not by increasing the number of undamaged bolls, but by increasing their size, indicating that allocation of resources from damaged to healthy bolls represents a potential form of compensation. In the field, high mirid numbers (up to 6/m row) over 50 days during the boll filling stage resulted in a yield loss: 6.4 versus 13.4 bales/ha in low-mirid density plots (up to 1.4/m). The reduction in average boll mass caused by mirids (47-54% compared with undamaged bolls) was similar to that caused by injecting pectinase (41-62%), indicating that artificial damage closely mimicked actual damage. Compared to the low mirid treatment, high mirids numbers caused both more damaged locules per boll and more severe locule damage. With high mirid numbers, the majority of damaged bolls had two or more affected locules while at low mirid numbers, only one or two locules were affected. This suggests that individual mirids tended to feed in one region, causing damage to single locules. Bolls with multiple locule damage probably experienced repeated visitation by several mirids. Even when damage was high, 60 to 75% of the damaged bolls had at least one undamaged locule. However, there is no evidence to suggest intra-boll compensation (i.e. undamaged locules attaining greater mass than locules in a healthy boll). The high yield of the low-mirid plots indicates that compensation at the plant level is possible. While increasing mirid damage was associated with a yield decline, we did not find any downgrading of fiber quality. It appears that during the picking and ginning process, damaged locules and lint were selectively removed. This is supported by the lower gin outturn of damaged bolls. Hence, the risk of penalty for low fiber quality may be relatively minor.*

Introduction

Historically, green mirids have been common pests of seedling cotton but not during fruit set, and green vegetable bugs (GVB) are rarely a problem. This is because broad-spectrum pesticides applied against *Helicoverpa* larvae usually control mirids and GVB through the mid- and late season. With the introduction of Bt-cotton however, the number of *Helicoverpa* sprays used has declined allowing green mirids and GVB to build to potentially damaging levels in some instances. Spraying for *Helicoverpa* is expected to drop even further with two-gene cotton (Bollgard II), raising the concern that sucking pests may become a significant problem during the fruiting period. The effect on yield and fiber quality by the increased feeding of mirids and GVB on bolls is not well understood. In one of the few studies to date, Khan and Bauer (2001) found that damage to young bolls (<10 days old) often causes shedding. Damage to older bolls (10-20 days old) usually does not cause shedding but the damaged locules may not develop properly. This has implications for the capacity of the crop to compensate for damage by producing more bolls, making undamaged bolls larger or making undamaged locules in damaged bolls larger.

An understanding of the effect of mirids and GVB on cotton yield and fiber quality is therefore crucial to development of rational thresholds and IPM strategies. To accurately predict the yield consequences of boll damage by sucking pests, we first need to understand the nature of the damage and the response of the cotton plant to the damage. When bolls are fed upon by sucking pests, the causes of lint damage are unclear: was it physical piercing of the immature seed, or the injection of enzymes breaking down the developing fibers or seed, or a combination of these and other factors? We are developing techniques to answer these questions by mimicking mirid damage artificially. This provides a way to precisely quantify the relationship between a given level of damage and its effect on yield parameters. For growers, the impact of sucking pests on yield and fiber quality and whether cotton can compensate from this type of damage is clearly a priority.

Here we report on the first phase of an on-going investigation with the aim of quantifying the ability of cotton to tolerate this type of boll damage, and the mechanism by which this is achieved. In the end, we hope to provide growers and consultants with the necessary knowledge to make better decisions on the management of sucking pests in the age of the two-gene cotton.

Experimental procedure

Mimicking mirid boll damage using injection of the pectinase enzyme

Experiment 1. Sicala V-3i was grown in 50 liter bins that were watered regularly. Bolls were artificially damaged when they reach 25 cm in diameter, which corresponds to 10 days after white flower, were damaged. Damage was imposed by injecting two locules in each boll with pectinase (an enzyme in the saliva of mirids that breaks down complex carbohydrates including cellulose). The pectinase was a commercial extract from *Aspergillus niger* (Sigma-Aldrich Pty Ltd, Sydney Australia). We diluted the pectinase (in 40% glycerol) with water at 1:4. A 1ml syringe was used to inject 20 μl of the pectinase solution into each locule to be damaged. Out of the 30 plants were used for this experiment, 20 received damage and 10 were kept as undamaged control. Plants assigned to receive the damage were treated on two consecutive weeks where all bolls within the target size were injected with pectinase. This method allowed us to produce a range in the number of bolls damaged, i.e. from one to nine damaged bolls per plant. Plants were grown to maturity, when open bolls were harvested. The number and mass of undamaged and damaged bolls were recorded for each plant.

Experiment 2. In this experiment we tested lower volumes and concentrations of pectinase than in Experiment 1. Levels in this experiment may be closer to that injected by mirids. Two cotton varieties (Sicala V-2 and Siokra V-16) were grown in a glasshouse in 42 l pots at two plants per pot. When plants reached a sufficient boll number (about eight per plant), randomly selected bolls about 10 days in age were injected with pectinase at 1 μl or 3 μl of two dilutions (1:4 and 1:10 pectinase to water). Some bolls were also injected with 1 μl or 3 μl of water as controls for the injection process. Two locules were injected in each damaged boll with a Hamilton repeating dispenser and a 50 μl Microlitre syringe (Hamilton Co. Reno, USA). An average of three bolls per plant were injected, with each boll receiving a randomly assigned treatments. There was a total of six bolls per treatment. Plants were grown to maturity and harvested. Damaged bolls were weighed individually. The number of all undamaged bolls per plant was recorded and the bolls were weighed as a pooled sample.

Natural boll damage by mirids

In the 2001-02 cotton season, we undertook experiments at two location in southeast Queensland where mirid numbers were high, to establish the effect of mirids on boll size, damage, yield and fiber quality. The first field was located at Byee in the South Burnett. This field was sown with Siokra V16i at 5-6 plants/m row. Plots were established with low mirid density (where they were controlled with Folimat (100 ml/ha) or Rogor (250 ml/ha)), or with high mirid density where they were left uncontrolled. In the high mirid treatment, mirid numbers increased from 1.5/m row at first square (52 DAS) to a peak of 6.25/m row (105 DAS). In the low mirid treatment, numbers were 1.66/m row at 52 and and 1.16/m row at 105 DAS. GVB was also present at low numbers during late fruiting season, but their effect on boll damage was considered minor. At the end of the season, all open bolls were collected from 20 plants (i.e. 10 plants from each of two replicate plots) in each treatment. Bolls were individually scored for their degree of damage of individual locules), mapped and harvested.

The second field was located at the QDPI Sir Joh Bjelke-Petersen Research Station (JBP) in Kingaroy. Siokra V16i was established in this field at 10-12 plants/m and was left unsprayed to allow mirid damage to accumulate. Mirid numbers increased from 1.3/m at the seedling stage (36 DAS) to a peak of 1.8/m at boll stage (113 DAS). At JBP, we randomly selected 10 pairs of plants comprising of a high and a low damage plant based on the extent of boll damage determined visually. We assigned a categorical score of 0 to 4 (i.e. 0=no damage to 4=severely damaged) to each locule on each boll on each plant. Figure 1 shows the range of damage. Numbers of undamaged and damaged bolls and their weights were recorded for each plant. Seed cotton of all bolls (damaged and undamaged) was combined for ginning and fiber quality test (HVI) to produce a final yield and quality value for each plant.

Results and Discussion

Artificial damage

In a preliminary study, we found that puncturing the outer boll wall always resulted in a visible scar; whether it is produced by a needle or the stylet of a sucking pest (Figure 2 left). Penetrating the inner boll wall always resulted in the formation of warts (Figure 2 right). This is probably a form of defense to seal off the wound against infection. But the formation of warts does not always mean that there will be lint damage. Our results suggest that if the puncture has pierced a seed, this could cause the seed to die and terminate its fiber development, resulting in lint damage. We found light to moderate locule damage simply from puncturing a seed with a pin. But if the puncture misses a seed and only pierce developing lint, no damage results. Since we have no direct means of determining if a pin had penetrated a seed during its entry, these inferences require further validation.

Experiment 1. The injection of pectinase clearly exacerbated the piercing damage resulting in varying degrees of locule damage and lint discoloration (Figure 3). Compared to undamaged bolls, the seed cotton mass of individual damaged bolls was reduced by 38% (Experiment 1, Table 1). Despite the formation of warts, wounding with pectinase injection led to secondary fungal infection and boll rot in 31% of the bolls damaged. Plants responded to boll damage not by increasing the number of undamaged bolls, but by increasing their size (Table 2). We conclude that cotton plants could partially compensate for boll damage by allocating more resources to healthy bolls.

Experiment 2. Based on the size of mirids, in this experiment we reduced the volume of pectinase from about 20 μl (Experiment 1) to 1 and 3 μl to mimic more closely the amount of saliva mirids might inject. We also tested a lower pectinase concentration of 1: 10 dilution in addition to the 1:4. The results indicate that our lowest dose of 1 μl of pectinase at 1:10 dilution was still sufficient to cause heavy damage to locules. Table 3 shows an average reduction in boll mass from 41% (1 μl and high dose) to 63% (3 μl and high dose) compared with the undamaged control. Injection of water alone produced a significant 25% reduction in seed cotton per boll (Table 3). These results support the earlier tests that piercing the boll without pectinase can also cause locule damage but adding pectinase greatly exacerbates the damage. While we do not know how closely our treatments mimicked the actual volume and enzyme content of mirid saliva, it is apparent that the resultant damage was similar to that caused by actual mirid feeding as described below.

Natural mirid damage

The percentage of bolls damaged by mirids per plant ranged from 10% (Byee, sprayed to reduce mirid numbers) to 54% (Byee, unsprayed); the mean for JBP was 43%. In all cases, damaged bolls were substantially lower in boll mass (Table 4) than undamaged bolls. For Byee, the high mirid plants returned a yield of 6.4 bales/ha compared to the 13.4 bales/ha for the low mirid plants (127 kg seed cotton/bale). It is worth noting that the reduction in average boll mass caused by real mirids (at 47-54% in Byee, Table 4) was similar to that induced by artificial damage (at 41-62% with pectinase, Table 3). Natural mirid damage (based on external scars) could occur anywhere on the boll wall but tended to concentrate along the suture region, which corresponds to the center of a locule (i.e. a cracking boll splits down the middle of locules to maximize lint fluffing and seed dispersal). Figure 4 illustrates the typical result of moderate feeding damage. The internal damage of a particular boll is localized and is probably the result of mirid damage killing a single seed. Note that the seed adjacent to the wounds has disintegrated while other seeds in the locule remained unaffected. There are two major differences in boll damage caused by the chewing action of *Helicoverpa* and by the sucking action of mirids. Firstly, while very young bolls (<7 days old) are easily shed following any damage, older bolls attacked by *Helicoverpa* are more likely to be shed soon after damage while those fed on by mirids are more likely to remain on the plant until maturity. Secondly, when bolls are fed upon by mirids, not all locules are damaged to the same degree, while *Helicoverpa* larvae tend to cause significant damage to all locules. Mirid damaged bolls may therefore have some locules that still produce harvestable lint.

We examined the extent of locule damage in the two locations. A range of locule damage existed (Figure 5). Where mirid damage was high (i.e. JBP and Byee high mirids), more bolls showed ¾ or all locules damaged than when mirid numbers were low. But even under high damage, between 60 and 75% of the bolls had at least one undamaged locule. When mirid numbers were low (Byee low mirids), damage to bolls was limited most to one or two locules. This suggests that individual mirids tended to feed in one region, causing damage to single locules. Bolls with multiple locule damage probably experienced repeated visitation by several mirids. Field confirmation of these inferences is required.

In addition to the distribution of undamaged and damaged locules in damaged bolls, we were also interested in the severity of damage between locules, i.e. were they all damaged to the same extent? Using the scoring system shown in Figure 1, we obtained the results shown in Table 4. When damage by mirids affected more than one locule in a boll, in 70% of the cases the level of damage among locules was different. And there is remarkable consistency in this observation regardless of location or mirid numbers. While the cause of this finding is not clear at this point, it will be useful in future prediction of boll damage by sucking pests.

The relationship between seed cotton mass per plant and total boll number in Byee (Figure 6) showed a reduced seed cotton yield per boll in damaged plants (slopes are significant different at $P<0.05$). Figure 6 also showed an overall reduction in boll number per plant for the high mirid damage treatment, which suggests that there was no evidence of compensation for boll damage by increasing boll production. There was, however, a weak but significant positive relationship between the mean seed cotton mass of undamaged bolls and % damaged bolls ($R^2=0.12$, $P=0.015$). This indicates that the reduced resource demand of damaged bolls enabled more resources to be distributed to the development of larger, undamaged, bolls. These results are consistent with those of the artificial damage of Experiment 1. We conclude that there is some potential for compensation in increasing the size of undamaged bolls but not the number of bolls. Whether the inability to increase boll production is related to the fact that damage occurred too late in the season, remains to be investigated.

Gin turnout and fiber quality

Another issue of concern with the retention of damaged bolls is the contribution of damaged lint to the final yield. Discolored and immature lint remaining on damaged bolls could be picked and contaminate clean fiber. Figure 7 shows a clear trend of lower gin turnout with higher damage, which indicates that damaged locules have been largely excluded in the ginning process, i.e. they did not gin properly and end up in trash. This interpretation is supported by fiber quality measurements (Table 5). All measures of fiber quality were similar between plants with high and low mirids, except for a modest decline in short fiber index in the high mirid treatment. These findings indicate that mirid damaged locks which often form a "tight lock" where lint does not expand (Figure 1) has several implications. One, damaged locules may not be picked at all which equates with a yield loss; and one or two, if they are picked, "tight locks" do not go through the gin and do not contaminate lint.

Conclusions and Future Directions

This study shows that sucking pests have the potential to affect yield dramatically. For instance, in our experiment, a substantial mirid pressure of 1.5 to 6.25 mirids/m over a 50-day period resulted in a yield loss of 52%. Because mirid damage usually does not affect whole bolls, plants tended to retain partially damaged bolls without responding by increased fruit production indicating limited compensation due to this mechanism. There is, however, some evidence that damaged plants are able to direct some resources from damaged to undamaged bolls, resulting in increased size of the undamaged bolls as a form of compensation. Our results show that although yield may decline with increasing mirid damage, there may not be an associated degrading of fiber quality since most of the damaged locules are excluded from the final lint. One encouraging result from Byee shows that high yields are still possible at 10% boll damage (or an average of 1.4 mirids/m for 50 days at 55-105 days after sowing). Our next task is to explore the tolerance limits of mirid damage on bolls. This can now be achieved using artificial damage, which closely mimics the effect of sucking pests and by further experiments with real mirid damage.

Since there is evidence indicating that external scars do not strongly associate with locule damage, another challenge ahead is to develop techniques where internal damage can be quantified quickly and accurately, preferably through non-destructive means. In this study we have used mirids as a model species to represent a range of sucking pests including green vegetable bug, red-banded shield bug and harlequin bugs. There is a need to define the feeding behavior of different sucking pests on cotton bolls and some work is underway (see paper by Khan and Bauer in this proceedings).

If we find that cotton has only a limited ability to recover from boll damage by sucking pests, alternative IPM strategies will become more critical.

Acknowledgements

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Reference

- Khan, M. and Bauer, R. (2001). Comparing damage from green mirid and green vegetable bug. *The Australian Cottongrower*, **22**: 16–18.

Table 1. *The effect of simulated and natural mirid damage on seed cotton mass. For Byee means of undamaged bolls with different letters are different at p=0.05.*

	Average mass (g) of seed cotton per boll		% Reduction in boll mass
	Undamaged bolls	Damaged bolls	
Experiment 1	4.2	2.6	38
JBP	5.6	4.3	23
Byee (low mirids)	6.1a	3.3	47
Byee (high mirids)	6.6b	3.5	46

Table 2. *Differences in boll number and seed cotton weight between control plants and those with bolls damaged by pectinase injection. Values are mean ± standard deviation.*

	Control plants	Damaged plants
Number of undamaged bolls / plant	21.7±5.5	15.6±4.1
Mass of undamaged bolls (seed cotton g / boll)	4.2±0.9	4.8±0.8

Table 3. *The effect of varying the dosage of pectinase per locule on seed cotton mass. The values in brackets indicate the % reduction in mass of treatment bolls compared to undamaged bolls.*

Treatment	Average mass (g) of seed cotton per boll	
	1 μ l of treatment / lock	3 μ l of treatment / lock
High pectinase dose (1:4)	2.69 (41%)	1.74 (62%)
Low pectinase dose (1:10)	2.18 (52%)	1.85 (59%)
Water (Control)	3.42 (25%)	3.30 (27%)
Undamaged bolls	4.53	

Table 4. *The proportion of differential damage among locules in a boll from the two Queensland locations with natural mirid infestations.*

	All locules similarly damaged (%)	Locules differentially damaged (%)
JBP	27	73
Byee (high mirids)	28	72
Byee (low mirids)	30	70

Table 5. *Fiber quality parameters for plants with heavy and light boll damage from mirids.*

	Low mirids	High mirids
Staple length (inches)	1.17	1.17
Uniformity index	85.1	85.1
Short fiber index	3.3*	3.9
Fiber strength	31.7	30.4
Elongation	4.0	4.1
Micronaire	4.3	4.7

* Values significant (P<0.05) different between treatments.

Figure 1. *The five classes of locule damage (the bottom locule of each boll) by mirids. The severity of damage increases from right (no damage, damage score=0) to left (damage score=1, 2, 3 and 4, respectively). This picture also illustrates how common it is to have locules of a single boll having different levels of damage.*

Figure 2.
Piercing of the boll wall with a pin led to the development of internal warts.

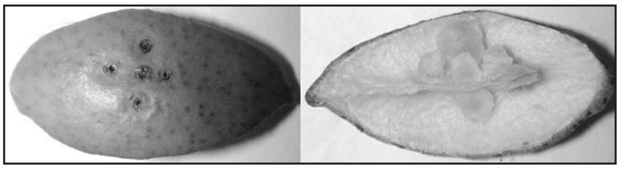


Figure 3.
Boll damage caused by injecting a locule with 20 µl of pectinase (1:3 dilution).

Figure 4.
Natural damage caused by mirids showing for the same boll the external scars and the localised internal damage.

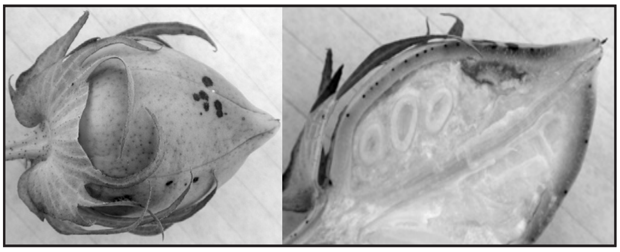
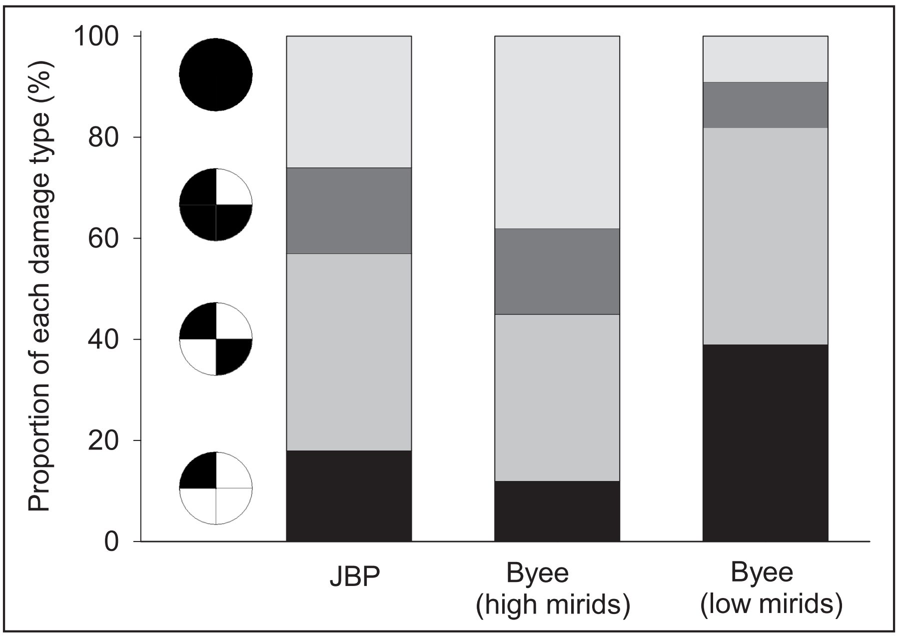


Figure 5.
Proportion of locule damage types in damaged bolls at the two Queensland sites that experienced natural mirid infestations. The solid wedges of the pie charts represent the number of damaged locules in a boll, the four corresponding bar segments are their proportions in all damaged bolls.



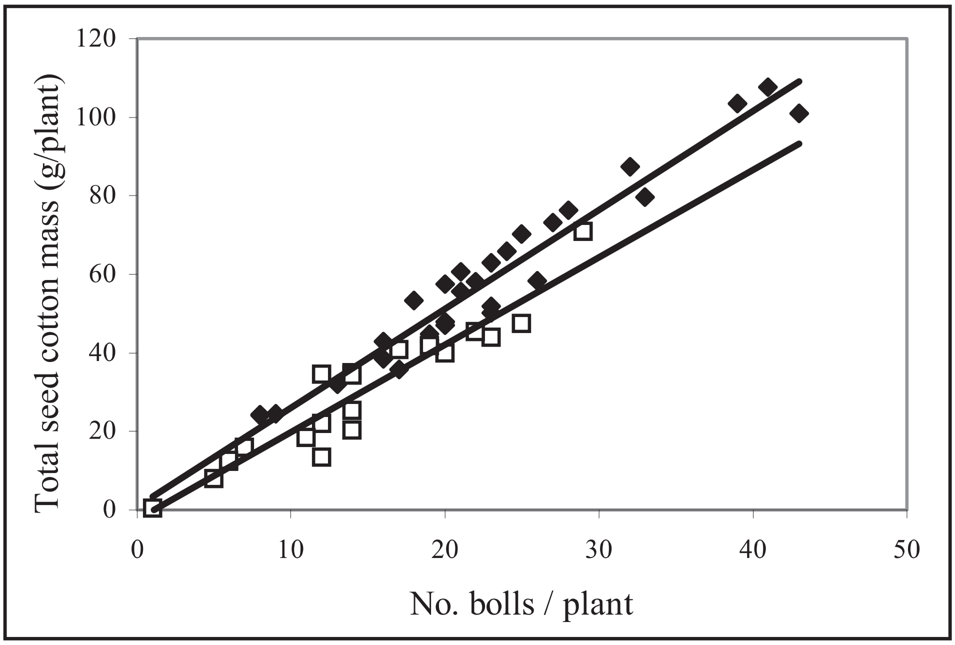


Figure 6. The seed cotton mass to boll number relationship in cotton grown in Byee after low (diamonds) and high (squares) levels of natural boll damage by mirids. The regression lines are for low (upper) and high (lower) levels of damage.

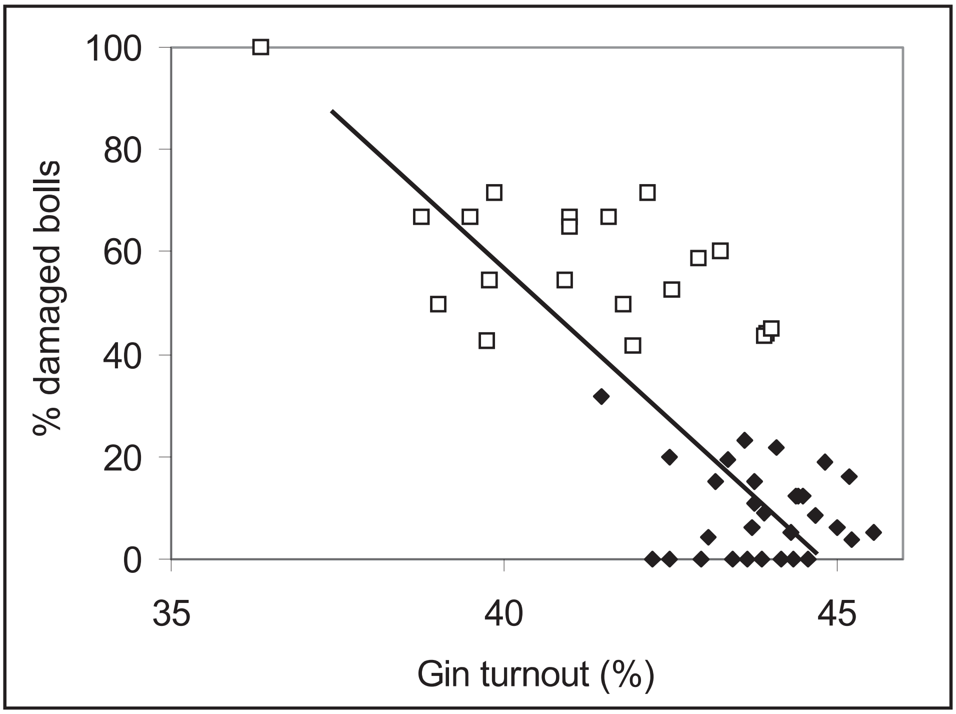


Figure 7. Using data collected from Byee, we show that the higher the damage sustained by a plant, the lower the gin turnout. Symbols: diamonds are low mirids and squares are high mirids.