

Final Report

On Farm Series | Cotton Research & Development Corporation

Part 1 - Summary Details

CRDC Project Number: CSE 110

Project Title: The relationship between local weather, aphid and whitefly numbers and cotton quality

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Part 3 – Final Report Guide (due 31 October 2008)

Background

Internationally, sticky cotton is a major concern for the textile industry (Hector & Hodgkinson 1989). Physiological plant sugars in immature fibres, contaminants from crushed seed and seed coat fragments, grease, oil and pesticide residues are all potential sources. However, all are insignificant compared with honeydew contamination from *Bemisia tabaci* and *Aphis gossypii* (Hector & Hodgkinson 1989; Ellsworth *et al.* 1999a; Hequet & Abidi 2002). The underlying reason is the distribution of the sugars along the fibre. Physiological sugars, grease and oil are usually distributed evenly over the fibre whereas sugars from honeydew tend to be in scattered concentrations (Bruno 1984). In the latter, these sugars lead to uneven yarn which is prone to breaking during weaving and knitting of fabrics (Hequet & Abidi 2002) as well as impeding fibre handling and causing in severe circumstances mill shutdown to clean equipment (Ellsworth *et al.* 1999a). A reputation for stickiness has a negative impact on sales, exports and price for cotton from regions suspected of having stickiness. Reductions in the market value of lint due to stickiness are applied regionally and indiscriminately. In Arizona, perceptions regarding stickiness lead to a -5.63c/lb discount relative to Californian cotton (Ellsworth *et al.* 1999a).

More than 20 different sugars are excreted in honeydew (Hendrix & Wei 1994) and most are insect rather than plant derived (Tarczynski *et al.* 1992; Salvucci *et al.* 1997). The major sugars excreted by *A. gossypii* are melezitose, sucrose, glucose and fructose while for *B. tabaci*, there is the additional sugar, trehalulose. Analysis by Hendrix *et al.* (1992) of aphid and silverleaf whitefly honeydew from insects feeding on cotton indicated around 40% of total sugars present was melezitose in the aphid honeydew, while silverleaf whitefly honeydew exhibited about 40% trehalulose plus about 17% melezitose.

The two sugars that contribute most to cotton stickiness problems are trehalulose and melezitose (Henneberry *et al.* 1995, 1996, 1998a, 1998b; Gamble 2001) and both are produced as a result of transglycosylation reactions involving dietary sucrose (Wei *et al.* 1997). The lint content of both trehalulose and melezitose were quantitatively linked to insect numbers. The composition of honeydew collected directly from *Bemisia* was found to be virtually identical to that recovered from contaminated lint (Hendrix 1995) and poinsettia (Byrne & Miller 1990). For *B. tabaci* first and second instars produce less trehalulose than third and fourth instars while adults produce more than nymphs. In contrast, more melezitose was produced by nymphs than adults (Henneberry *et al.* 1999). While trehalulose and melezitose are significant contributors to sticky cotton the interaction between these, other sugars and cotton stickiness is poorly understood and complicated by the fact that other sugars such as sucrose glucose and fructose occur in both honeydew and cotton lint (Henneberry *et al.* 1998a).

Using the thermodetector method to measure cotton stickiness (Henneberry *et al.* 2000) increases in thermodetector sticky cotton counts were closely correlated with increasing numbers of whitefly nymphs and adults. The experience from the USA suggests that the threshold of concern for sticky cotton is indicated by thermodetector measurement ≥ 5 (Frydrych 1986; Brushwood & Perkins 1993). To reach this level, whitefly numbers needed to be ≥ 8.9 adults per leaf or ≥ 3.2 nymphs/cm² (Henneberry *et al.* 1998a). This is well below the action thresholds for insect growth regulators (Ellsworth *et al.* 1999b). Further, Yee *et al.* (1997) and Henneberry *et al.* (1998) indicated that insecticide applications at an average

of 10 adults per leaf reduced honeydew production as effectively as applications at 5 adults per leaf. However, standardised sampling protocols, and the relationship between levels of stickiness in the field and problems arising in textile mills are still not well understood (Henneberry *et al.* 1998a). This is apparent if one plots the data from the USA against thermodetector readings (Henneberry *et al.* 1998a, 1998b, 2000) (Fig 1). The considerable scatter associated with the correlation underlines the lack of a clear cut decision point with regards to concentrations of trehalulose and melezitose and stickiness.

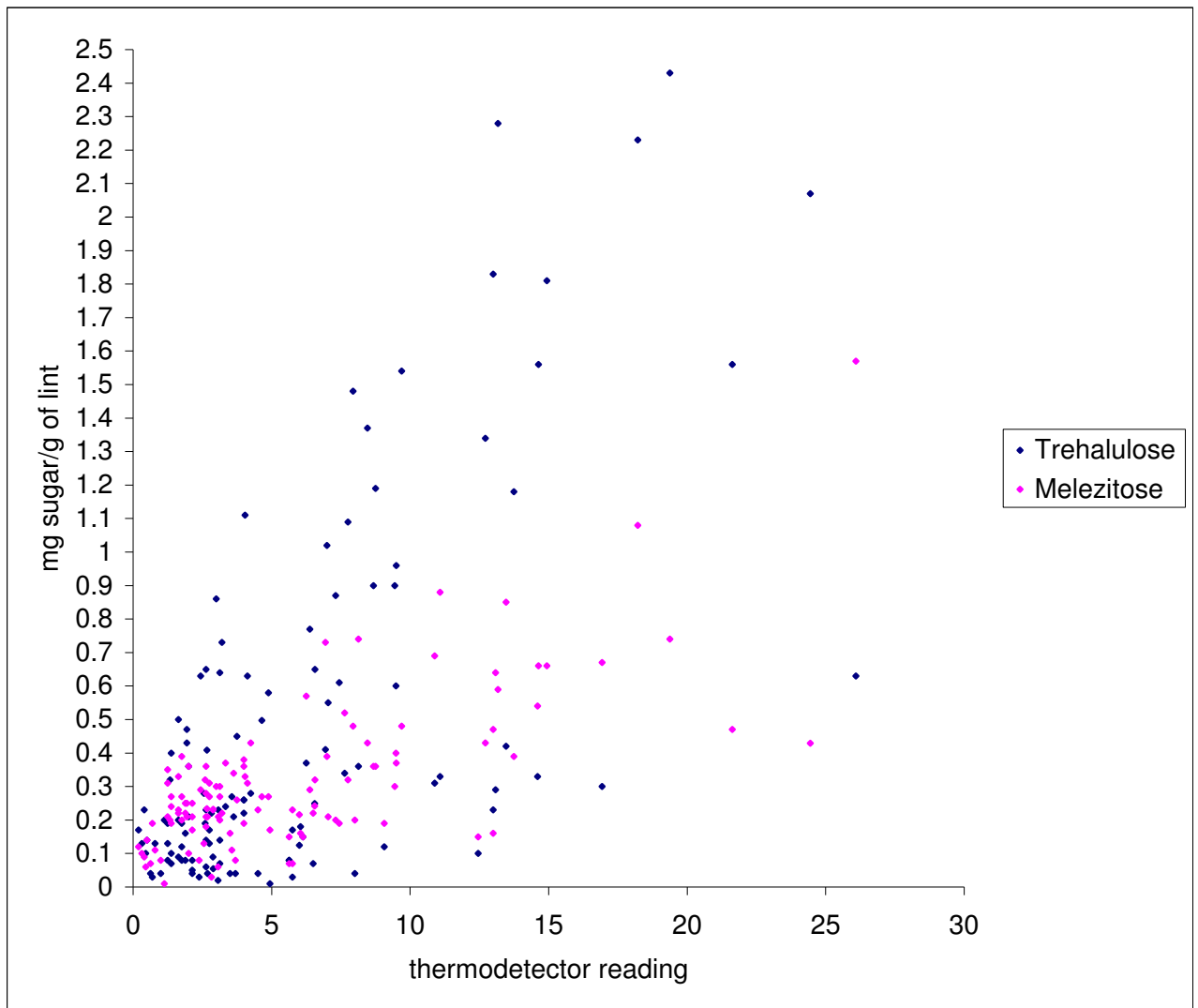


Fig. 1. Correlation between trehalulose (mg of sugar/g of lint), melezitose (mg of sugar/g of lint) and thermodetector readings from a combined dataset from Henneberry *et al.* (1998a), Henneberry *et al.* (1998b) and Henneberry *et al.* (2000).
 Thermodetector reading = 0.212 + 11.918(melezitose) + 3.988(trehalulose), $r^2=0.64$.

Prior to 31 May 2001, SLW was present in Queensland cotton growing regions at very low densities. Since then numbers have steadily increased with the first outbreak occurring in the Emerald Irrigation Area in 2002/3. One of the key threats that this new pest brings is the prospect of sticky cotton which if realised could significantly damage the reputation of Qld and Australian cotton and reduce its marketability. Sticky cotton is caused primarily by the

honeydew of aphids and silverleaf whitefly. A report to CRDC, P. De Barro (2003) Factors leading to stickiness in cotton and the potential for sticky cotton in the Emerald Irrigation Area outlines the problem and the outcomes of a preliminary research on cotton collected in the EIA in early 2003. In summary, the results showed that the makeup of SLW honeydew was very similar to that found in the USA in SLW feeding on cotton being composed of primarily trehalulose, with lesser quantities of sucrose, glucose, fructose and melezitose. Research into sticky cotton indicates that trehalulose (primarily produced by silverleaf whitefly) and melezitose (primarily produced by cotton aphid) are the major contributors to sticky cotton.

However, unlike honeydew contaminated cotton in the USA, the disaccharides sucrose and trehalulose were absent or at very low levels by the time of harvest despite being at higher levels earlier, suggesting that they had degraded. This loss of trehalulose in particular may explain why very few bales of cotton from the EIA were identified as sticky. In contrast to the US results, quantities of melezitose on Emerald cotton were substantially greater than those found in the USA, and at levels which should have led to widespread downgrades yet few occurred.

The lack of agreement between the US and Australian data is a cause for concern. An understanding of the nature of the differences between the US and Australian experiences could be of paramount importance to being able to assess the level of risk posed by SLW to Australian cotton. This is especially the case now that due to sticky cotton, one shipment of cotton has been rejected by an overseas mill. The factors leading to this cotton being sticky are not known.

Objectives

The initial scope was to develop a two year project, however, after discussion with Queensland Cotton, Dunavants, BSES Limited and DPIF there are too many unknowns to allow the development of testable hypotheses. Instead a 6 month period of intensive data collection and analysis is recommended with a view to developing a two-three year program commencing in the 2005/6 season. For the 2004/5 season the following research is proposed,

1. Influence of local weather on sticky cotton.
2. Relationship between whitefly and aphid densities and sticky cotton.
3. Fate of honeydew from cotton crop to classing.
4. Identity of micro-organisms on cotton lint.

The project will focus on a 17 week period from February to the end of May. The current plantings suggest that this will cover the vast majority of area's crop.

Methods

Field collections

A total of 30 fields were selected across the Emerald Irrigation Area. Sites were chosen in consultation with DPIF (Richard Sequeira) and the cotton IDO (Doug Sands) and local cotton consultants. At each site, 2x200g samples of cotton lint for honeydew sugar analysis, 2x200g samples of cotton lint for seed moisture analysis, 1 control sample. Each site was sampled

weekly until harvest. Samples commenced 7 Feb 2005 with the last sample collected 29 July 2005. In total 416 cotton samples and 160 control samples were collected. Whitefly and aphid densities were estimated for the part of the crop from where the cotton samples were collected at the time of lint collection. Estimates of insect numbers were based on the existing estimation methods used to by crop consultants. The control sample were obtained from pieces of plastic that were be placed in the field for 1 week to trap honeydew excreted during this period.

All samples were kept in cold storage prior to being shipped to Brisbane. Each shipment was approximately 30 kg. Immediately prior to harvest, a sample was made to assess stickiness using Dunavaunt's lintronix device. In addition to local weather data based on Bureau of Meteorology data, cotton variety, aphid and whitefly numbers, seed moisture levels and sugar concentrations; date of defoliation and pesticide treatment records were also obtained for each field.

Samples shipped to Brisbane were kept in cold store prior to use. Honeydew samples were extracted using established methodology. After extraction the honeydew extracts were frozen and sent to BSES Limited for sugar analysis. Honeydew extracts were analysed for glucose, sucrose, fructose, trehalulose and melezitose using HPLC. In addition, a smaller sample of 10 will be analysed using GC-MS and HPLC-MS to ensure the identity of each sugar, and to assess the presence of other carbohydrate components which may contribute to stickiness.

Thermodetector estimates

There are several studies related to the measurement of whitefly related in cotton. These studies (Henneberry *et al.* 1998a; Henneberry *et al.* 1998b; Henneberry *et al.* 2000) developed a relationship between trehalulose (mg of sugar/g of lint), melezitose (mg of sugar/g of lint) and thermodetector readings. In Australia we have no thermodetector, however it is possible to obtain an estimate of stickiness by estimating the relationship between stickiness (as measured by the thermodetector) and sugar levels. The model is based on data gathered from a number of sources, namely Henneberry *et al.* (1998a), Henneberry *et al.* (1998b) and Henneberry *et al.* (2000). The data was analysed using a simple linear regression and the resultant equation

Thermodetector reading = $0.212 + 11.918(\text{melezitose}) + 3.988(\text{trehalulose})$, $r^2=0.64$.

was then derived. The resultant formulae enabled us to take the sugar concentration data which we measured and convert it to a measure of stickiness. This enabled us to compare the Australian data to the threshold data from the USA.

Fate of sugars

Synthetic whitefly honeydew based on the formulation of Henneberry *et al.* (2000) was applied to relatively clean cotton lint and pieces of plastic sheeting. Sufficient honeydew was sprayed onto enough cotton bolls to enable samples to be collected at weekly intervals over a three week period from the time of application. In addition, honeydew was applied to pieces of plastic sheeting suspended in the canopy. These acted as the controls. There were four sampling times, each one week apart. Each sample time consisted of 15 x 200 g samples of cotton lint. At the time of application bolls were tagged with 4 different coloured tapes. The initial collection was one hour post-application then 1, 2, 3 weeks post application. After harvest the cotton samples were treated as for the field collected samples.

Data for the following are provided

- 1) Relative humidity data for the period 1 Jan – 31 July.
- 2) Rainfall data for the period 1 Jan – 31 July.
- 3) Cotton seed moisture data for cotton at the point of harvest.
- 4) Concentration of melezitose for each sample collected.
- 5) Concentration of melezitose for each sample collected.
- 6) Thermodetector estimates for pre- and post-defoliation cotton samples.
- 7) Point of harvest estimates for stickiness by Dunavaunt.
- 8) Relationship between aphid and whitefly densities and sugar concentrations.
- 9) Relationship between seed moisture and thermodetector estimates.
- 10) The fate of sugars applied directly to cotton bolls.

Results

Local weather data

Figures 2 & 3 show the relative humidity and rainfall data for the period 1 Jan 2005 to 31 July 2005.

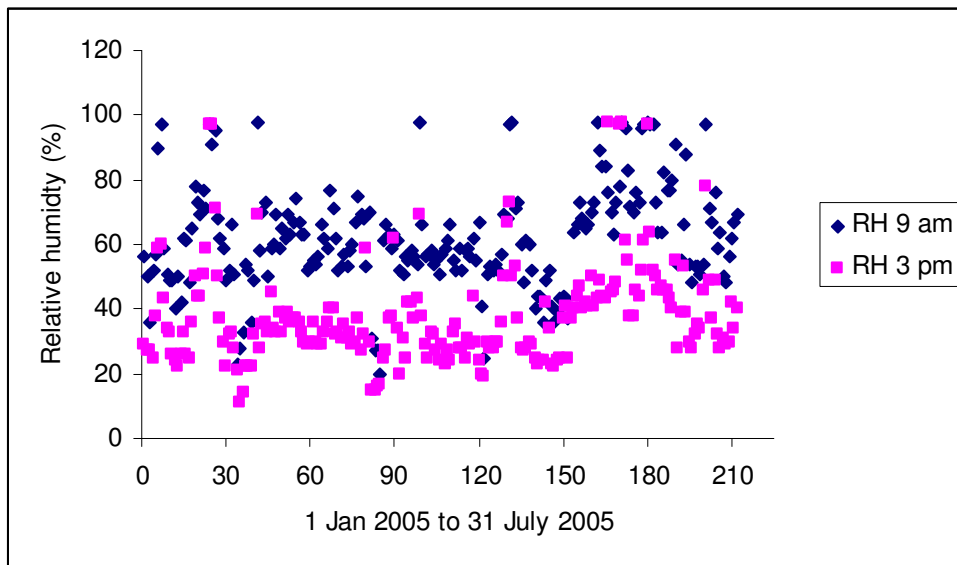


Fig. 2. Relative humidity at 9 am and 3 pm.

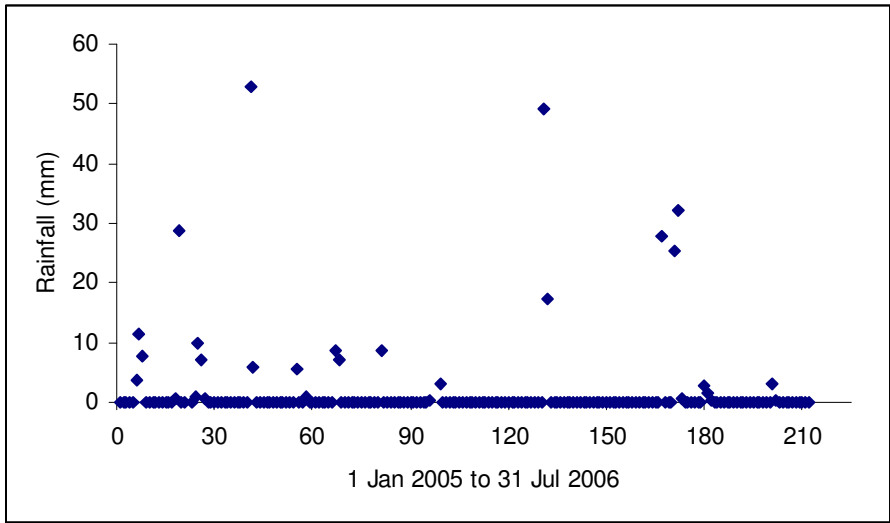


Fig. 3. Daily rainfall (mm).

Cotton seed moisture

Data from the USA indicated that when cotton seed moisture levels increased from an average of between 4% and 5% to between 9% and 10% the incidence of sticky cotton declined markedly. The seed moistures from cotton sampled from each of the 30 sites ranged from 2.9% to 7.5% ($4.9\% \pm 0.2\%$).

Estimates of sticky cotton in the 30 field sites

The estimates of the concentrations of melezitose (Fig. 4) and trehalulose (Fig. 5)

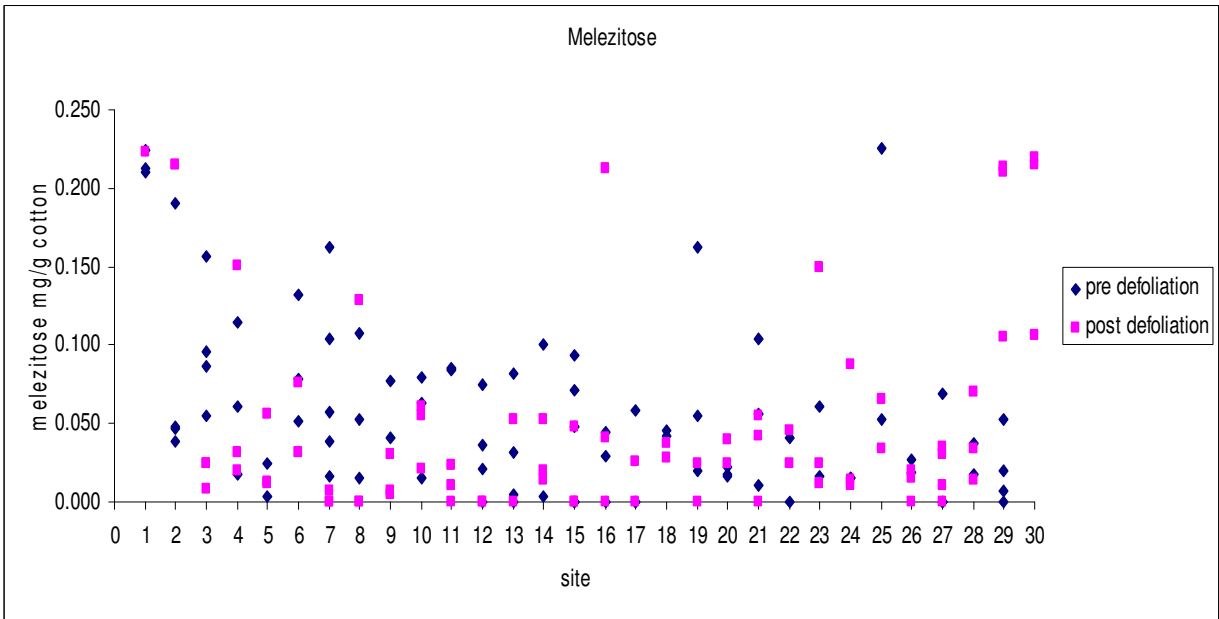


Fig. 4. The concentration of melezitose on pre and post defoliated cotton in each of the 30 cotton crops.

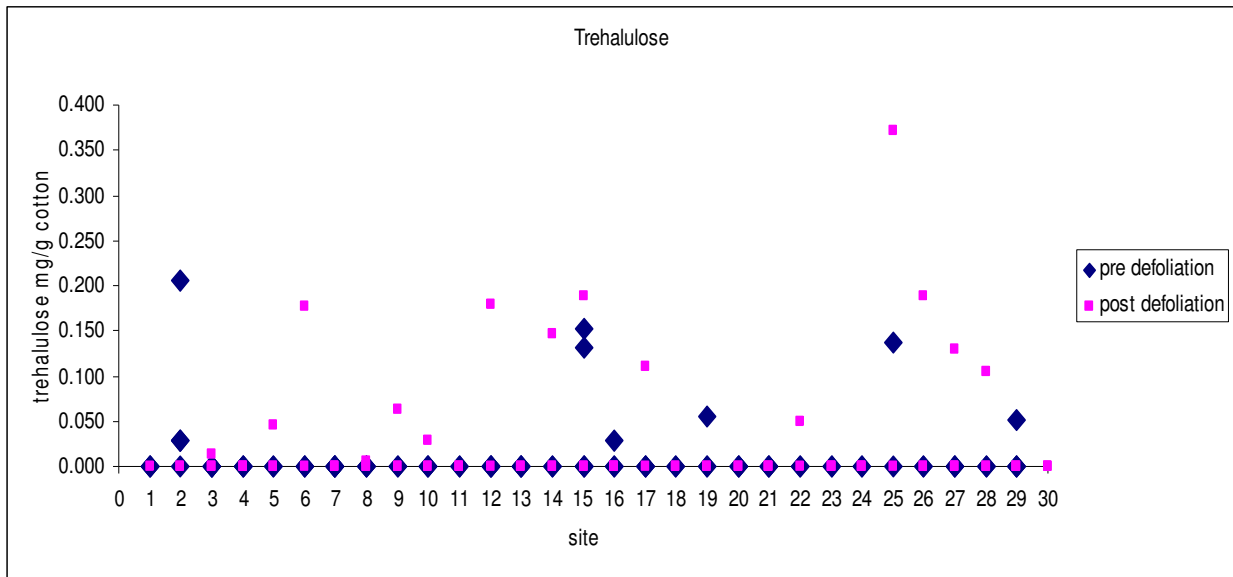


Fig. 5. The concentration of trehalulose on pre and post defoliated cotton in each of the 30 cotton crops.

Across the 30 sites, concentrations of trehalulose were for the most part zero.

Based on the formula,

$$\text{Thermodetector reading} = 0.212 + 11.918(\text{melezitose}) + 3.988(\text{trehalulose})$$

the quantities of melezitose and trehalulose can be used to estimate thermodetector readings and thereby enable us to predict which of the 30 sites faced a threat of sticky cotton. Figure 4 indicates that thermodetector estimates ranged from 0.2 to 3.5 for pre-defoliated cotton and from 0.2 to 2.9 for post-defoliated cotton (Fig. 6). These are all well below the threshold level of 5. This indicates that it was likely that none of the 30 sites faced the risk of sticky cotton.

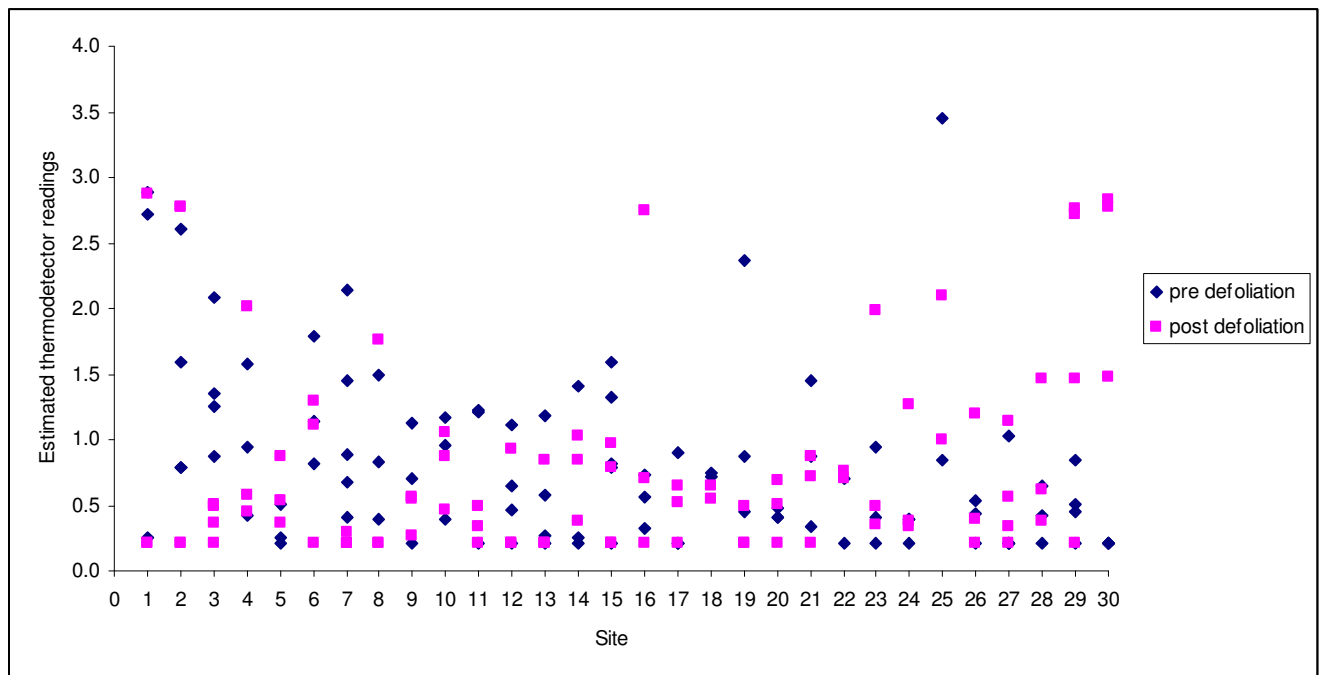


Fig. 6. Estimates of thermodetector readings for pre and post defoliated cotton for each of the 30 sites

Estimates of sticky cotton in the sticky cotton field trial (21 Feb 2005 to 14 Mar 2005)

Figure 7 shows the concentrations of sugar present on cotton bolls. In all cases the application of the synthetic honeydew mix lead to an increase in each of the sugars relative to the background levels. After seven days the quantities of sugars had each declined to less than half that measured at 1 hr post application. There was no further eduction in the sugars. Similarly, the quantities of sugars on the plastic sheets (Fig. 8) declined markedly over the seven days following application and thereafter changed little over the next 14 days. The concentrations of sugars when converted to thermodetector readings (Fig. 9) indicate that readings were maximal, 9.5, soon after application, but then declined to less than half, 3.9, over the following seven days. Over the next 14 days readings remained largely unchanged. The marked decline coincided with 5.6 mm of rainfall which occurred between the application of the synthetic honeydew and the a seven day post application sample. However, 8.6 mm of rain between the 14 day post application sample and the 21 days post application sample saw no similar declines in sugar concentrations.

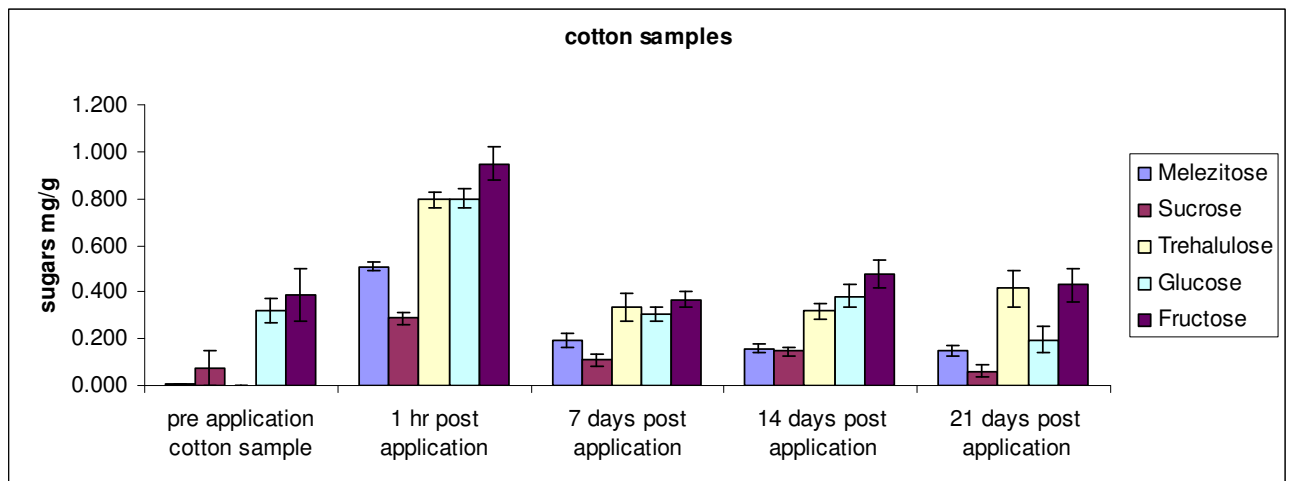


Fig. 7. The mean concentration of sugars present on cotton bolls.

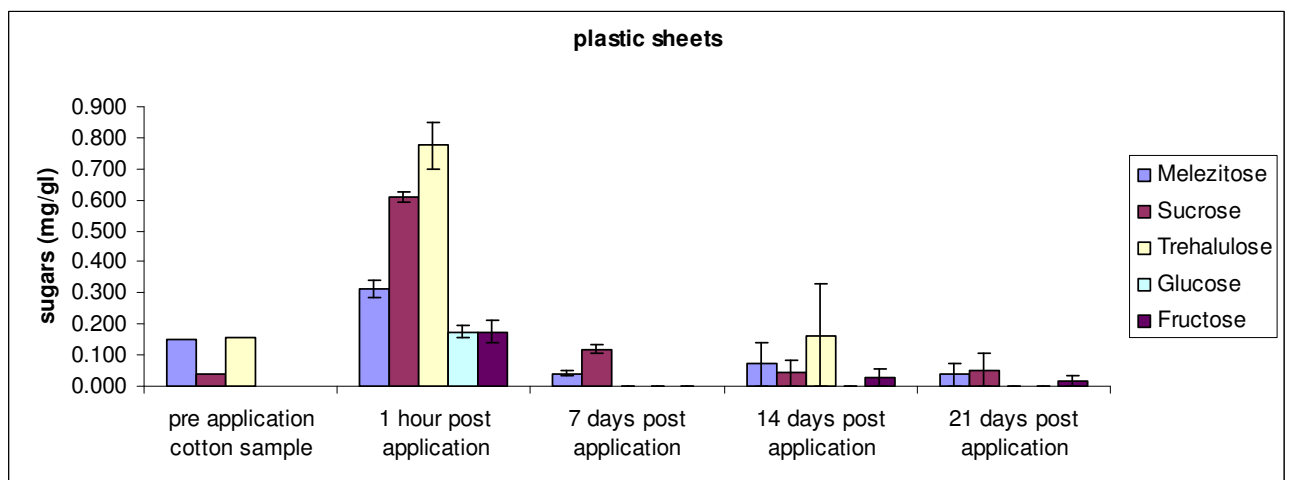


Fig. 8. The mean concentration of sugars present on plastic sheets set out in the trial site.

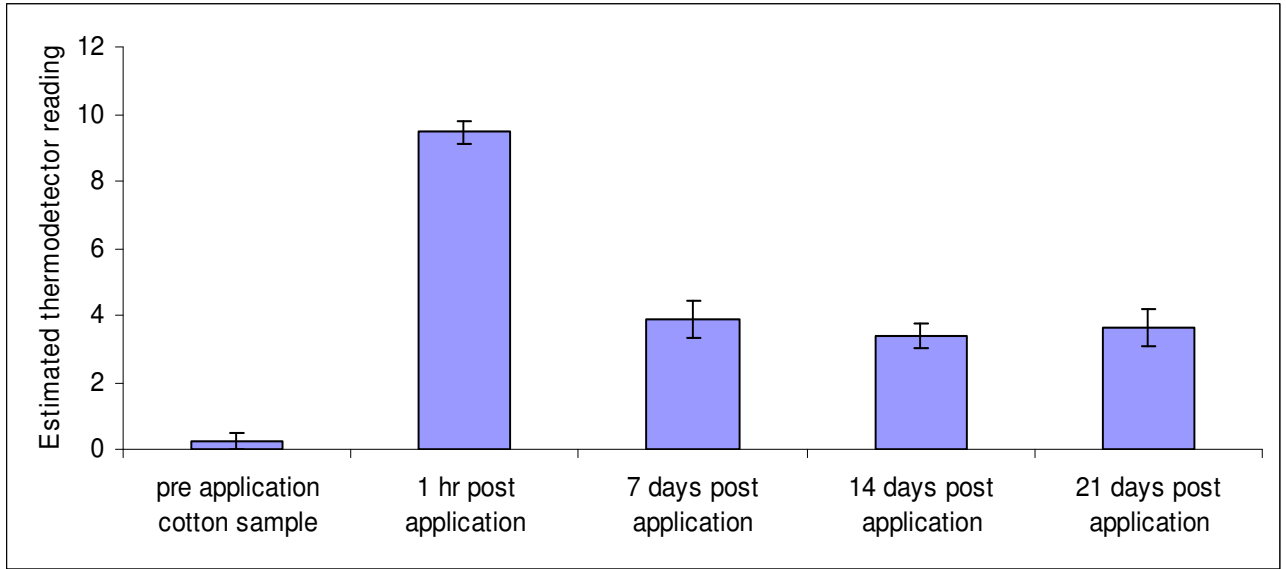


Fig. 9. Estimated thermodetector readings based on the concentrations of melezitose and trehalulose of cotton bolls harvested from the trail.

Stickiness measures by Dunavaunt

Dunavaunt uses a lintronix device to measure stickiness measure. Stickiness is assessed on a scale of 0-200, 0-60 is no or low stickiness, 61-120 is medium stickiness, 120-200 is sticky and 200+ is high stickiness. All sites tested gave measures of between 0 and 11 indicating that there was little or no stickiness detectable in any of the samples.

Relationship between whitefly and aphid numbers and concentrations of sugar

While silverleaf whitefly produces both trehalulose and melezitose, it produces approximately twice as much trehalulose as it does melezitose. Therefore, trehalulose alone provides a good indication of the relationship between insect number and stickiness. Total numbers of whitefly adults across the 30 sites ranged from 0 to 6.1 adults/leaf while nymph densities ranged from 0 to 1.2 nymphs per cm² these are both below the 8.9 adults per leaf or 3.2 nymphs/cm² thresholds for sticky cotton (Fig. 10a,b). The relationship between aphid numbers and sticky cotton has yet to be established, however, as stickiness measures were well below those needed to run the risk of sticky cotton it may be assumed that the numbers of aphids observed were well below that required (Fig. 11). The very low numbers of aphids and whiteflies and the lack of any sticky cotton prevents any further meaningful analysis of the data.

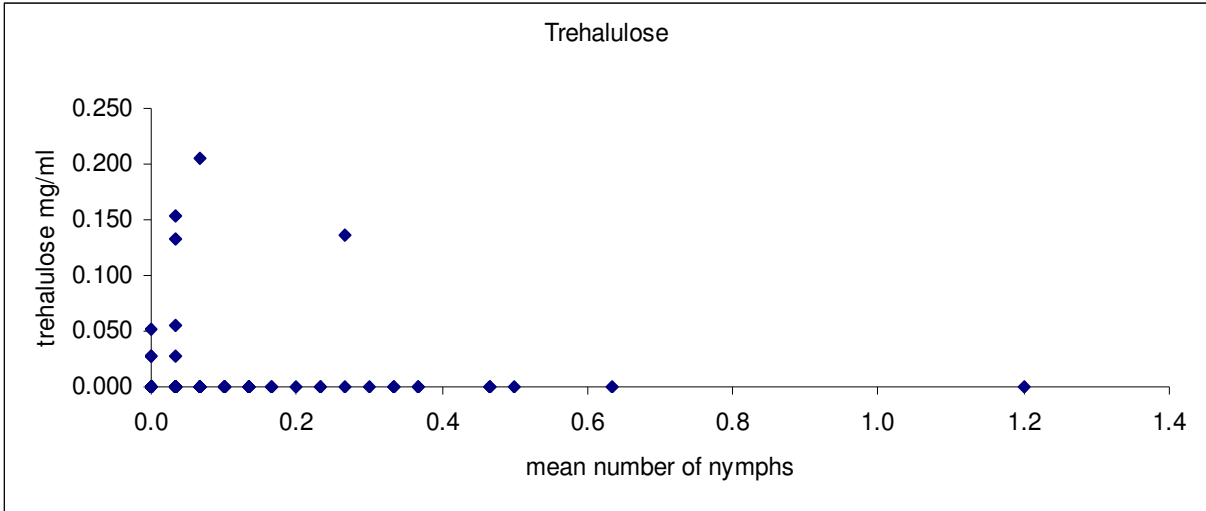


Fig. 10a. The relationship between mean concentration of trehalulose and mean number of SLW nymphs/cm².

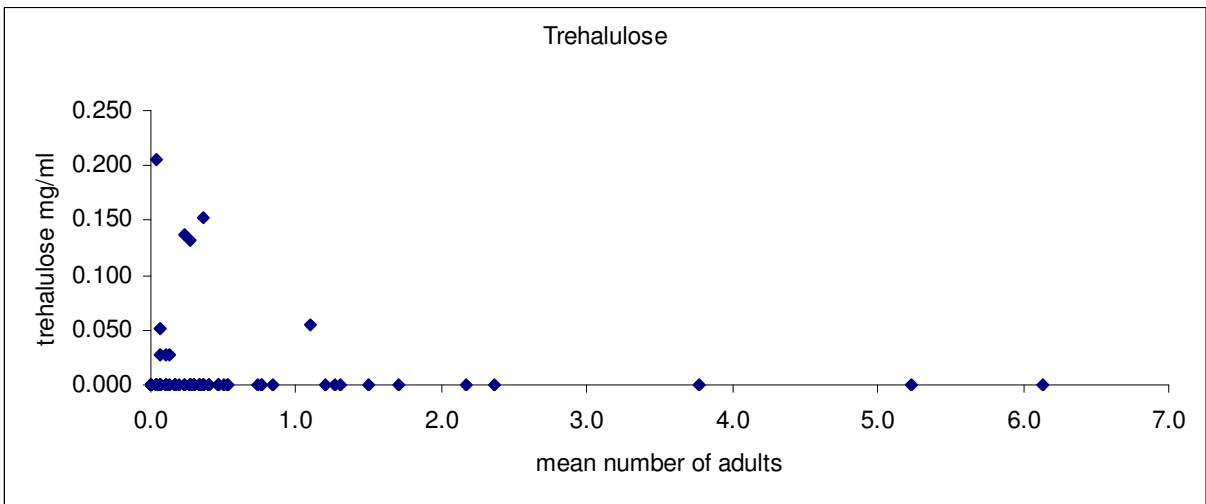


Fig. 10b. The relationship between mean concentration of trehalulose and mean number of SLW adults/leaf.

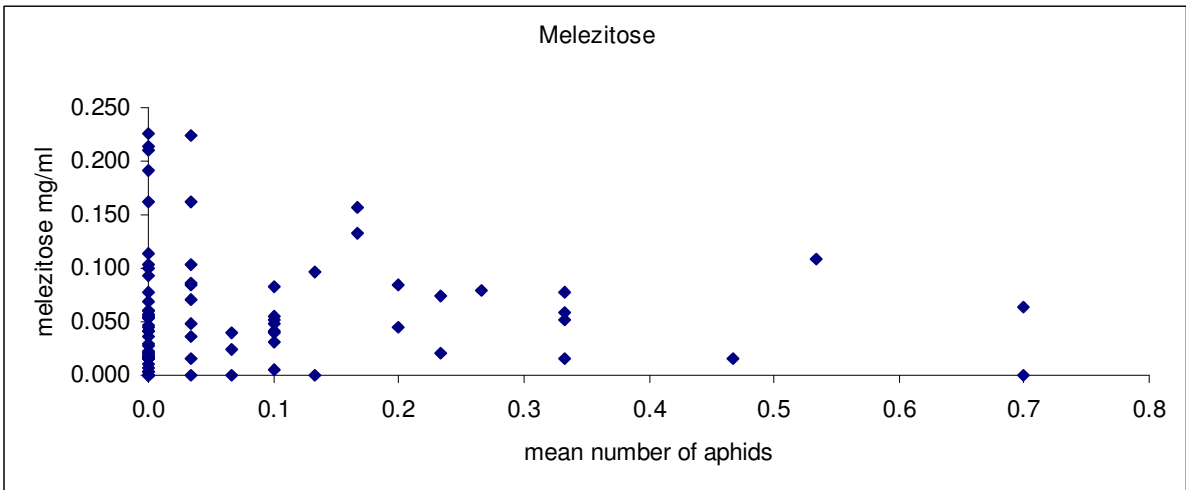


Fig. 11. The relationship between the mean concentration of melezitose and the mean number of aphids.

The relationship between seed moisture and sugar concentrations

Sugar concentrations were converted to thermodetector estimates using the formula; thermodetector reading = 0.212 +11.918(melezitose) + 3.988(trehalucose). The relationship between these estimates and seed moisture can be seen in Fig. 12. The subsequent regression analysis indicated there was no strong relationship between the two.

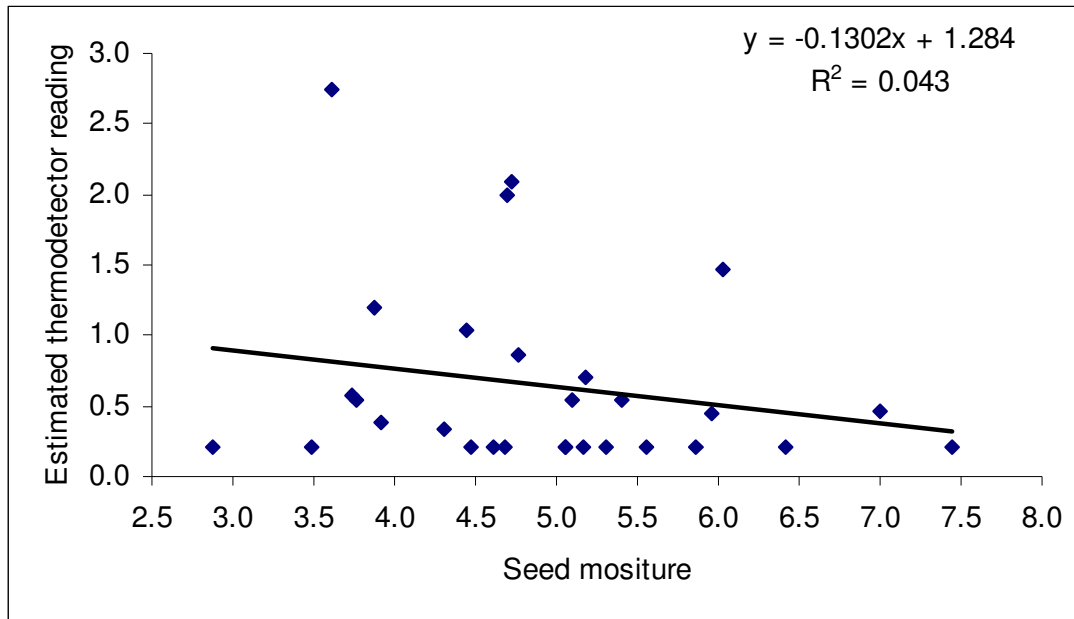


Fig. 12. The relationship between the seed moisture (%) and thermodetector estimates.

Outcomes

Sticky cotton is considered a major threat to the profitability of Central Queensland cotton. This study seeks to understand the level of threat posed by sticky cotton and in doing so provide growers with appropriate steps to minimise the threat.

The low numbers of whiteflies and aphids and the concentrations of sugars resulted in limited outcomes. Few meaningful correlations could be tested. However the synthetic honeydew trial demonstrated that sugar levels did decline and suggests that one or more factors, as yet unknown, are operating that work to rapidly degrade disaccharide sugars.

Conclusion

Influence of local weather on sticky cotton

The very low numbers of aphids and whiteflies has made it difficult to draw too many detailed conclusions. The trial using synthetic honeydew showed an initial rapid decline in the concentrations of all sugars that may have been associated with rainfall. However, subsequent rainfall did not induce further declines. Throughout the study humidity levels were fairly consistent and this combined with the low levels of sugars makes any attempt at developing a correlation meaningless. The cotton seed moisture levels were lower than what is normally expected for Emerald (8%). This may have been due to the generally dry conditions experienced as a consequence of drought. Whether the dry conditions helped to reduce the rate of degradation of disaccharide sugars is not known.

Relationship between whitefly and aphid densities and sticky cotton

The only clear outcome here is that low aphid and whitefly numbers appears to be linked to low concentrations of sugars.

Fate of honeydew from cotton crop to classing

Again the low levels of sugars make it difficult to draw too many conclusions. The best data comes from the field trial and here sugar levels declined markedly over the first seven days post application, but failed to decline further over the remaining 14 days. While rainfall did occur it was relatively light at 5.6 mm. It is doubtful whether this would have been sufficiently heavy to wash the sugars from the lint especially given that 8.6 mm of rainfall 12 days later had no effect of sugar concentrations. The low levels of sugars observed throughout the study may explain the lack of any observable trends in regards to sugar levels time after defoliation. The rapid decline in sugar levels mirrors that observed in previous studies, however the underlying cause is not known.

The most usefull data came from the trial where synthetic honeydew was applied directly to cotton bolls. Here the data showed a considerable decline in disaccharide sugar concentrations. The trail was a very expensive experiment given that we used trehalulose which is hugely expensive. However, as the data showed a decline across the disaccharide sugars it would be possible to get the same outcomes using melezitose and sucrose plus glucose and fructose without trehalulose. Therefore a series of trials could be undertaken throughout the cotton season that focused on measuring the association between local weather and sugars. This could focus more closely on local rainfall and humidity and would avoid the complication of not having suitable insect numbers. The trails could be undertaken at several locations in the EIA.