



FINAL REPORT 2018

For Public Release

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: CSP1703

Project Title: Investigating the relative contributions of weathering, insect honeydew and fungal agents to cotton colour grade changes and discounts

Project Commencement Date: 01/07/2016 Project Completion Date: 30/06/2018

CRDC Research Program: 1 Farmers

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Date Submitted: _____

Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

Along with length, strength, micronaire and leaf content, the colour grade of cotton is a critical component of the set of characteristics used to assess the overall quality of a sample of cotton, and thereby determine its value. The current ‘base grade’ for colour for Australian cotton is Middling (31). If the colour grade falls to even the next lower grade of Strict Low Middling (41) then a significant discount to the value of the cotton will be applied. Wet, cloudy weather at harvest, a factor beyond a grower’s control, is one of the main causes of deterioration in the colour grade. The extent of the impact of wet weather on colour grade may also be influenced by a range of factors including presence of honey dew, the type / source of the honey dew, crop architecture, crop stage (degree of boll opening), amount of sunshine following the wet and cloudy weather that the crop is exposed to, trash levels in the seed cotton, the moisture of the lint when harvested and the length time between harvest and ginning.

Honeydew studies conducted as part of CSP 1401 “Enhancing IPM in cotton systems” provided information on honeydew characteristics and behaviour under various weather conditions and was followed by a pilot study on the effect of rainfall on cotton colour during 2015/16. This study showed that extended exposure of cotton to rainfall had a marked negative effect on colour. The two-year project herein was developed to continue research into the factors affecting cotton colour in the field and reports on the results of the pilot project and further investigations into the impacts of weather, sooty mould, fungicides and effects on yarn quality.

Objectives

2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.

Achievements are listed against the milestones in Table 1.

Objective 1: To investigate the relative importance and interplay of the factors that contribute to colour changes in cotton

- a) Rainfall – determine the effect of cumulative rainfall and exposure on cotton lint colour
- b) Weathering – investigate the effects of rainfall and exposure on honeydew contaminated cotton lint in the field

Objective 2: To determine the impact of sooty mould on cotton colour

- a) Create sooty mould and develop a system that describes lint contamination
- b) Investigate if sooty mould reduces the sugar content of honeydew contaminated cotton bolls
- c) Test if production line processes contribute to the physical removal of mould spores and improve cotton colour
- d) Assess effect of sooty mould on lint colour and determine the proportion of contaminated lint required in the total pick to incur colour downgrades

Objective 3: To discover novel management options for avoiding and mitigating colour downgrades

- a) Test a range of fungicides to reduce sooty mould in the field

- b) Screen a range of fungicides for their activity against sooty mould fungi in the laboratory
- c) Evaluate the effect of early defoliation on sugar concentration of bolls and sooty mould development
- d) Investigate the role of water repellents
- e) Investigate the role of yeast in sugar degradation

Objective 4: To establish whether a relationship between colour grade and yarn quality exists

- a) Measure the effect of cumulative rainfall on yarn/fabric quality of downgraded lint
- b) Measure the effect of sooty mould affected and downgraded cotton on yarn/fabric quality
- c) Assess the effects of sooty mould on fibre surface properties
- d) Investigate the effect of cellulases on fibre surface properties
- e) Assess the extent to which discoloured, graded cotton can be bleached back to a higher grade

Table 1: Project milestones and achievements

MS No.	Milestone Description (what are the key steps in answering the question)	Performance Indicator (one Output per Milestone)	Expected science/industry outcome (one S/I Outcome per Milestone)	Start Date (dd/mm/yy)	Finish Date (dd/mm/yy)	Comments
1. What is the relative importance and interplay of the factors that contribute to colour changes in cotton?						
1.1	Experiments to investigate the effect of rainfall (total / duration / subsequent conditions) on fungal growth and the lint grade of both clean bolls and bolls contaminated with honeydew.	Experiments established annually at ACRI and in Emerald (contingency for low or no rainfall in Namoi Valley).	Clarification and if possible quantification of the factors that cause or contribute to discolouration of cotton.	01/07/2016	30/06/2018	Accomplished in full
1.2		Experimental results evaluated, and reported to CRDC annually, highlighting the factors that contribute to colour changes, their interaction and relative importance. Presentation of results at Cotton Conference 2018.	Clarification and if possible quantification of the factors that cause or contribute to discolouration of cotton.	01/07/2016	30/06/2018	Accomplished in full
1.3		Extension materials developed based on experimental results and discussions with CRDC.	Extension materials and participation in industry forums such as CCA meetings will help growers understand the factors that can devalue their crop.	01/07/2016	30/06/2018	Accomplished with some additional material that can be published
2. What is the impact of sooty mould?						
2.1	Experiments to determine the relationship between spore load and colour grade	Cotton samples infected with <i>Alternaria</i> collected or created; role of <i>Alternaria</i> reviewed and refined.	Understanding of the role and modus operandi of <i>Alternaria</i> in lint discolouration.	01/07/2016	30/06/2018	Not accomplished, no <i>Alternaria</i> found (dry conditions)
2.2		Experiments undertaken to assess the proportion of sooty – mould covered bolls (pre-ginning) needed to noticeably	Understanding of the tolerance limits of sooty moulds in maturing crops.	01/07/2016	30/06/2018	Accomplished in full

2.3		contaminate clean lint samples, results reported annually to CRDC. Experiments undertaken to determine whether spore load diminishes along the fibre production chain, results reported annually to CRDC.	Understanding how the fibre processing chain may affect downgrading of coloured /contaminated cotton by altering contamination levels through mechanical processes.	01/07/2016	30/06/2018	Accomplished in full
2.4	Investigation of impact of sooty mould on lint.	Report provided to CRDC on the impact of sooty mould on lint, including do sooty moulds break down sugar (and if yes, at what rate); do yeasts, sooty moulds or <i>Alternaria</i> produce cellulases that affect the surface of the fibre?	Determination of the risk that sooty moulds cause for lint discolouration as a trade-off for possible benefits of honeydew degradation. Defining the parameters of sugar degradation by sooty moulds and yeasts.	01/07/2016	30/06/2018	Partially accomplished
3. Are there novel management options for avoiding or mitigating colour downgrades?						
3.1	Identification and preliminary investigation of novel options.	Literature review undertaken that identifies potential novel options for further investigation; review provided to CRDC.	Information about possible methods to mitigate the effects of honeydew related mould contaminations.	01/07/2016	30/06/2018	Accomplished
3.2		Review potential options discussed with CRDC, focus for investigations agreed and appropriate experiment(s) established.	Identification of novel options to mitigate cotton discolouration.	01/10/2016	30/06/2018	Options discussed
3.3						
4. What is the relationship between colour grade and yarn quality?						
4.1	Collection, analysis and spinning of lint samples.	Samples are harvested, collected, ginned and HVI tested annually.	Establishment of database for analysis of treatment effects.	01/07/2016	30/06/2018	Accomplished in full
4.2		Samples are provided to CSIRO Canberra for microscopy and analysed once or twice depending on sample quality available.	Analysis of the effects of weathering or sooty moulds on fibre surface properties.	01/07/2016	30/06/2018	Accomplished for one season

4.3	Samples are provided to CSIRO Geelong and spun into yarn; yarn parameters are assessed when suitable samples are available after harvest each season.	Identification of the possible relationship between colour grade and fibre quality.	01/07/2016	30/06/2018	Accomplished in full
4.4	Annual report provided indicating the relationships between causal agents for colour downgrades, HVI results, yarn surface properties and yarn parameters. Summary report compiled and available to CRDC, information extended to industry via Australian Cottongrower or at Cotton Conference 2018.		01/07/2016	30/06/2018	Accomplished in full

Methods and Results

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

4. Detail and discuss the results for each objective including the statistical analysis of results.

1. To investigate the relative importance and interplay of the factors that contribute to colour changes in cotton

Warm and dry weather conditions during boll opening and harvest ideally preserve the creamy colour, lustre and quality of cotton. Exposure of cotton fibre to weathering by the elements, either alone or in combination with factors such as insect honeydew contamination and fungal activity, can change fibre colour to varying shades of dull grey. In most cases the exact cause of discolouration cannot easily be pinned down since the potential contributing factors are almost always confounded. Industry concerns about the apparent associations between rainfall, UV weathering, insect honeydew, sooty moulds and grade discounts have prompted us to investigate the changes that occur in lint colour due to these factors and to establish quantitative descriptions of the causes and effects.

While it would be convenient to separately investigate each factor, cotton lint colour changes often arise out of a combination of these factors. For example insect honeydew may affect lint directly but it is also a substrate for sooty mould fungi which produce black spores that can give lint a greyish appearance. We have previously demonstrated that rainfall washes off the honeydew but the humidity it generates in the crop may then favour sooty mould growth on residual sugars. High volumes of rainfall or extended wet periods also favour weathering and the growth of other fibre damaging microorganisms. For these reasons we attempted to design experiments that answered several questions at the same time.

a) Rainfall – determine the effect of cumulative rainfall and exposure on cotton lint colour

Colour Post Rain Experiment (CPR) ACRI 2015/16 F1 (Pilot) and 2016/17

Rain falling on open bolls is one of the factors that can cause discolouration of lint. While this is well known in the industry, there has been no record of how cumulative rainfall affects colour grade and how much rainfall is needed to drop lint colour by one grade. In 2015/16 we, experienced a wet finish to the season and took advantage of cumulative rainfall events in a pilot project that assessed the effects of increasing exposure to rainfall on cotton lint colour.

Methods

A field experiment was set up at ACRI in the 2015/16 season prior to first boll opening (24/02/16) to assess + and – Rainfall effects on lint. Two 3 x 6 m plastic greenhouses (Fig. 1) in the field provided 36 m of cotton for the – Rainfall treatment. Sufficient ventilation ensured that the atmosphere in the greenhouse would not lead to excessive humidity and cotton discolouration through moulds. A control for the effect of the tent (no RF, no cover) was not possible as any uncovered cotton in the field would have to be exposed to rainfall. Cotton adjacent to the greenhouses was used for the + Rainfall treatment. During boll opening, between the 24/02/16 and the 10/04/16, the field received 20.4 mm of rainfall in 4 separate events, none of which exceeded 8 mm. On the 12/04/16, after receiving 22.4 mm rainfall on the previous day (a cumulative total of 42.8 mm), 10 x 20 open bolls from the field and 5 x 20 open bolls from each tent were picked. For the following 12 weeks, bolls were sampled from the field after every subsequent rainfall event. An extra sample was collected from the tents on the 06/05/16 to check if colour in the controls was deteriorating.

At every sample date, bolls were collected from strata that had been exposed to rain, about 1/3 of the way up the stem of the plants, to reflect cumulative rainfall and time in the field since opening.

All cotton samples were ginned on a small saw gin and after calculating ginout %, lint for each sampling date was pooled and 3 subsamples of about 150 g were used for HVI testing and manual colour grading at Australian Classing Services (ACS) in Wee Waa. Data was analysed in Genstat13 and graphed. Samples were pooled again for spinning, weaving and testing at the CSIRO Fibre Unit in Geelong (see Section 4a for results).

The experiment was repeated during the 2016/17 season though there was uncertainty about whether there would be sufficient autumn rainfall to replicate the treatments. Sample collection aligned with the same timeframes of exposure as in 2015/16, which was dictated by that season's rainfall pattern, to ensure a basis of comparison (i.e. 7.5, 8.5, 9.5, 11, 15, 16.5, 19.5 and 21.5 weeks after first open boll). Samples were ginned and graded using the HVI at Myall Vale but were not sent for further fibre testing, and no top pick was collected.



Figure 1: Plastic greenhouse in the cotton field to simulate the –Rainfall treatment.

Cotton classing – commercial cotton is classed after ginning to provide information about various fibre characteristics to determine its price to the grower and to enable the merchant to sort bales into even lots. Spinning mills will use the information to blend cotton into yarn of consistent quality. In Australia, cotton is classed by the HVI (High Volume Instrument) which assesses length, strength and micronaire. Colour, leaf content and extraneous matter are manually assessed by a classer. The HVI will also determine colour grade but manual grades are still routinely used in Australia (see Appendix 1). Colour and leaf are visually assessed under specific light conditions and compared to the “Universal Standards for Grades of American Upland Cotton”, sample boxes of which are replaced annually. Fibre length is expressed as staple length in 100^{ths} or 32^{nds} of an inch. Fibre strength is largely determined by variety though environmental factors may have some influence. It is a measure of the strength required to break a bundle of cotton fibres. A third measurement, micronaire, is an approximation of fibre thickness but may also indicate fibre maturity. Australian growers typically produce cotton of the following characteristics:

- Grade: Strict Middling (21) and Middling (31)
- Length: 1-5/32” – 1-1/4” (37-40), 29-32 mm
- Micronaire: G5 (3.5-4.9)
- Strength: 29-34 GPT (g/tex) strong – very strong

- Uniformity: 81-83 (average to high)

Results

Pilot 2015/16: The first bolls opened around the 20/02/16 and all bolls were open by the 29/03/16. Rainfall and colour grades are shown in Figure 2. The initial rain events, totalling 42.8 mm at 7.5 weeks of boll exposure, did not affect the colour of lint which was graded 11, both by the classer and the HVI, when compared to the unexposed controls which were also graded 11 (Fig. 3). An initial 20.4 mm fell in small amounts, followed by a further 22.4 mm in one day. Another 1 mm of rainfall (total 43.8 mm) did not affect colour grade as assessed by the classer but the cotton was downgraded to 21 by the HVI. By week 11 after first open boll (May), a further 27.2 mm of rainfall caused a downgrade to 31 by both the classer and the HVI, and cotton colour remained at that grade during the following 4 weeks in the field and an additional 20 mm of rain (Week 15, 91.0 mm). A further 51 mm by week 16.5 (June) brought the rainfall total to 142.0 mm, and grades worsened further, to 41 by the classer and 51 by the HVI. Cotton colour remained at those grades over the next 3 weeks (to 19.5 weeks, July) even though they received another 44.2 mm of rain, bringing the total rainfall to 186.2 mm. A further 2 weeks in the field with 16.8 mm of rain (Fig. 4) brought cotton colour to a grade of 51 by the classer and 61 by the HVI (Week 21, total rainfall 203.0 mm). In comparison, the cotton colour grade of controls picked at 11 weeks from the tents had not changed during that time and was still 11 (not shown on graph). Tents ripped during the following rain period and were removed from the field so no protected control bolls were sampled after week 11. Cotton that opened late and was picked from the top bolls on the last sampling date (Fig. 5 and right hand columns in Fig. 2) was not downgraded as severely as it received less rainfall (unknown) and field exposure than cotton bolls in the lower crop stratum (Fig. 6).

Fibre Quality

ACS HVI results 2015/16 – Samples for HVI testing were pooled to ensure a large enough sample for processing therefore we did not have true replication. Data were regressed against rainfall and graphed to determine relationships (Appendix 2). A reduction in reflectance (R) was strongly correlated with increasing amounts of rainfall ($R^2 = 0.96$) while the relationship between yellowness (b+) and rainfall was weaker but still statistically significant ($R^2 = 0.71$). Assessment of colour grade was more reliable when performed by HVI ($R^2 = 0.93$) compared to a professional classer ($R^2 = 0.88$). Micronaire was negatively correlated with rainfall ($R^2 = 0.74$) and ranged from 4.83 at the beginning of the experiment to 4.45 towards the end (after 21.5 weeks in the field after first open boll). Elongation was also negatively correlated with rainfall ($R^2 = 0.61$) and ranged from 7.8 at the beginning to 5.8 at the end of the experiment. Staple, fibre length, uniformity and short fibre index (SFI) were not correlated with rainfall and fell into the ranges below:

Staple	37-40
Length	1.14-1.26
Uniformity	82.6-86.1
SFI	6.3-9.5

Post-Gin Cotton Colour Grades - Effect of field exposure and rainfall on lower canopy bolls, ACRI, 2015/16

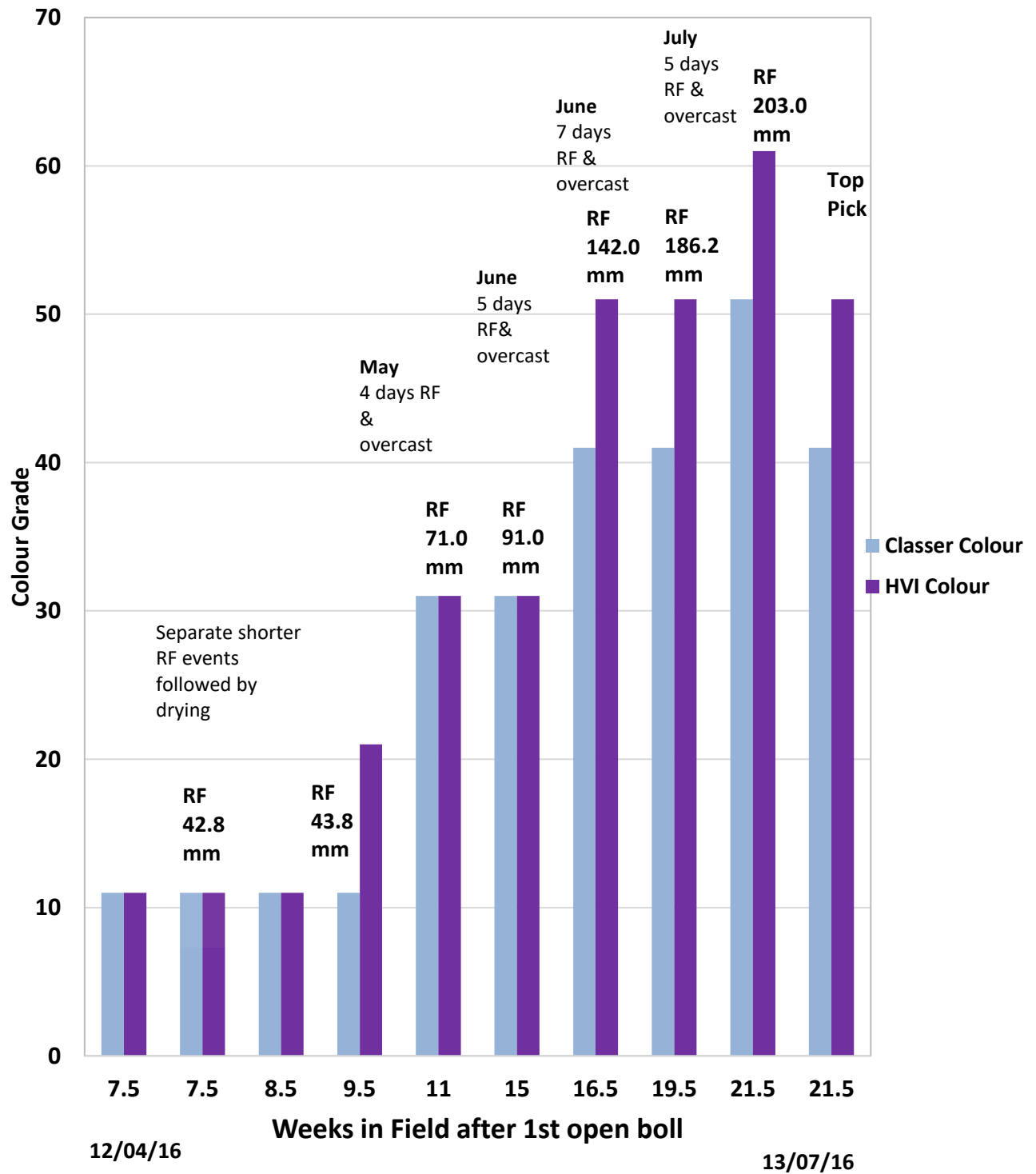


Figure 2: Effect of field exposure and rainfall on cotton colour grades. Controls are represented by the first two columns.



Figure 3: First pick 12/04/16, after 42.8 mm rain



Figure 4: Last pick 13/07/16 after 203.0 mm rain



Figure 5: Top Pick 13/07/16



Figure 6: Low Pick 13/07/16

CPR 2016/17: First bolls opened around the 13/03/17. Rainfall and colour grades are shown in Fig. 7. The first 88 mm of rain fell shortly after first bolls opened, totalling 114.6 mm from very small individual falls by 7.5 weeks after first open boll. Lint grade worsened from 11 to 31 due to that exposure. A further 18.4 mm between weeks 8.5 and 9.5 brought total cumulative rainfall to 141.8 mm and the colour grade to 41. For the subsequent 5 sampling events over the next 12 weeks, cotton grades remained at 41 despite an additional 54 mm of rainfall during that time.

Post-Gin Cotton Colour Grades - Effect of field exposure and rainfall on lower canopy bolls, ACRI, 2016/17

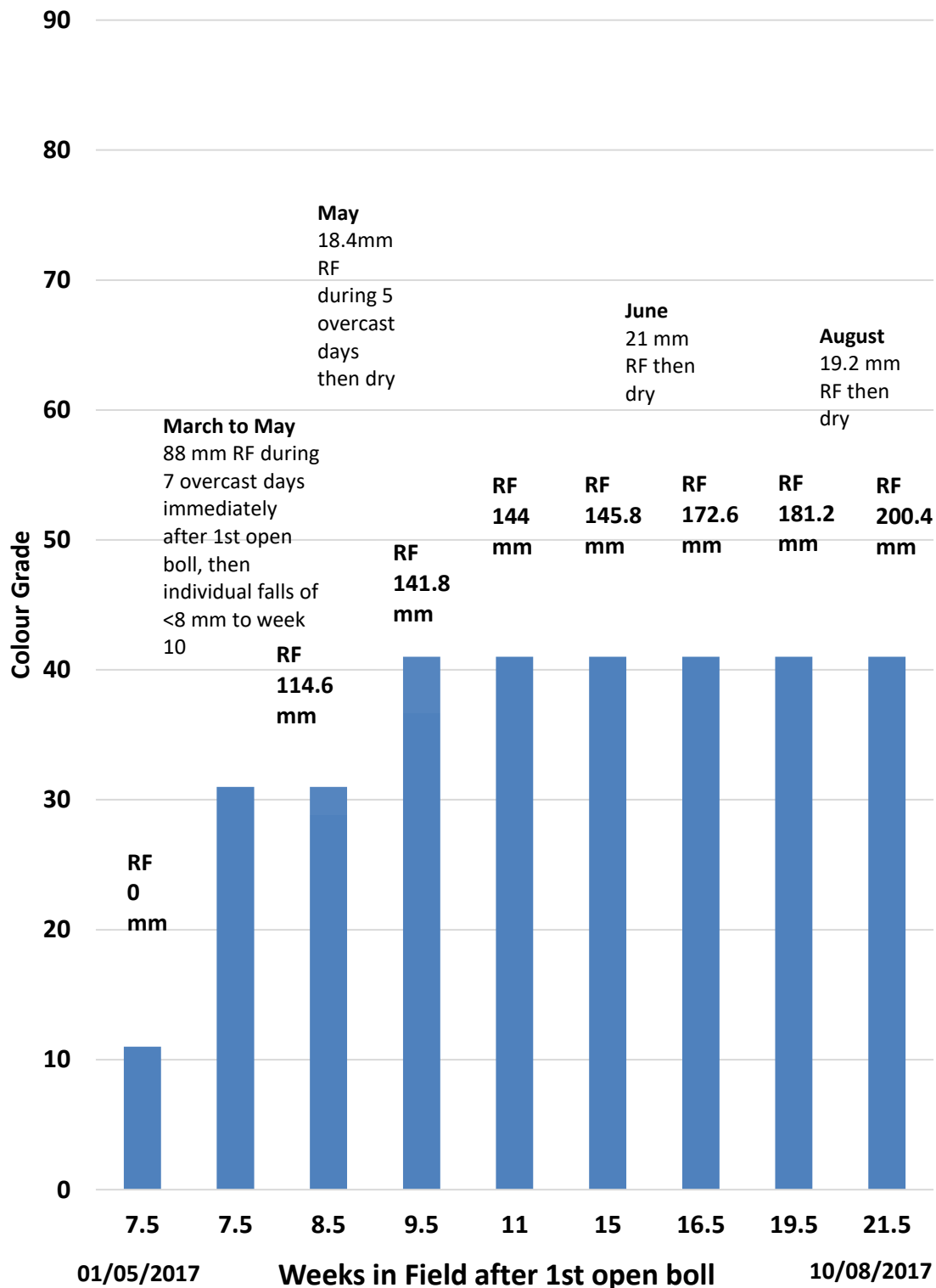


Figure 7: Effect of field exposure and rainfall on cotton colour grades. Controls are represented by the first column.

Discussion

This experiment clearly demonstrated the detrimental effects of cumulative rainfall on exposed cotton lint colour. During the initial rainfalls in 2015/16, there were periods of drying and possibly bleaching between rainfall events which may have reduced effects on colour. From May onwards, the rainfall events that occurred were accompanied by cloud cover and 4-7 wet days, extended periods where drying of the crop was not possible, and after each of those periods, colour grade dropped. Classers graded cotton in favour of the grower, whereas, HVI picked up colour changes earlier and graded worse, especially as lint grade deteriorated.

While the same amount of rain fell in 2016/17, its pattern differed somewhat from that in 2015/16. The first drop in colour grade (11 to 31) occurred after 88 mm of rainfall which fell during 7 overcast days during early boll opening. This corresponds with an equivalent drop in 2015/16 after 71-91 mm of rainfall and similar conditions. The next drop in 2016/17 (31 to 41) occurred after 5 overcast days when 18.4 mm of rainfall brought the cumulative total to 141.8 mm. Again, this corresponds with a drop from 31 to 41 in 2015/16 after 7 overcast days brought the cumulative total to 142 mm. While the total rainfall during the 21.5 weeks after first open boll reached about 200 mm in both seasons, the final colour grades differed. In 2015/16, the final colour grade of cotton exposed to 200 mm of rainfall over 21.5 weeks was 51-61, while in 2016/17 it remained at 41 after 141 mm of rainfall. The difference was in the way the remaining 54-58 mm of rain precipitated. In 2015/16, another 5 days of overcast and rainy weather again worsened the colour grade while the remaining 54 mm in 2016/17 fell in small amounts during individual events, allowing cotton – by that time defoliated – to dry out quickly.

Coarser fibres have a lower surface area and higher micronaire. Conversely, finer fibres have higher surface area and lower micronaire. Towards the end of the experiment, fibres were finer than at the beginning though all values fell within the G5 range (4.3-4.8). Finer fibres tend to be weaker, prone to breakage, increased neps and uneven dyeing. Micronaire is affected by the environment which in turn affects the way the plant manufactures carbohydrates which are the basis of cellulose deposition within the fibre. Any factors that reduce carbohydrate metabolism, such as temperature extremes, impaired light conditions (cloudiness, dense canopies), water stress, high boll load, etc. may negatively affect micronaire. While the crop was subjected to several periods of cloudiness, this mostly occurred after bolls had opened and it is unlikely that low radiation affected sugar metabolism at that stage. Also, care was taken to consistently collect bolls from the lower stratum which had been exposed to cumulative rainfall events so no late bolls were collected. During flowering and boll setting between January and March, the crop experienced several episodes of high temperatures (10 days in the high 30ies in January, 10 days in the high 30ies-low 40ies in February and half of March, and generally high night temperatures). These heat stress periods could have affected micronaire when the lower bolls were formed. Overall, however, the usual micronaire standard for Australian cotton (G5) was not affected and hence the rainfall received in this experiment did not affect quality parameters other than colour.

Conclusion

These experiments established a methodology that compared the effects of various rainfall and exposure scenarios on cotton lint colour. However, the method depends on natural rainfall and is therefore not reliable. It should be noted that these rainfall effects were observed in cotton that was not contaminated with honeydew and hence, sooty mould fungi were not present on the majority of collected sample bolls.

We demonstrated that:

- (i) the extended exposure of open bolls under dry conditions is unlikely to negatively affect cotton lint colour,
- (ii) open bolls exposed to rainfall may experience changes in colour grade,

- (iii) the nature of rainfall events is critical in their effect on lint colour changes, with overcast, rainy periods of more than 4 days and about 20+ mm of rainfall effecting a drop in colour grade,
- (iv) rainfall events of about 20 mm are unlikely to cause colour grade changes if they fall in a short period of time with a subsequent drying period, and
- (v) cotton classed manually by a professional classer was graded more favourably than cotton classed by the HVI. Colour grade classed by the HVI dropped down to the next grade one sampling date prior to the classer dropping it.
- (vi) it is unlikely that the extended exposure and rainfall experienced after boll opening affected micronaire and elongation.

b) Weathering – investigate the effects of rainfall and exposure on cotton lint colour in the presence or absence of honeydew on open bolls in the field.

We have had considerable discussions with consultants and growers about the potential relationship between the presence of honeydew and sooty moulds (SM) on bolls in the field and reductions in grades for that crop when harvested. The belief is that honeydew increases the risk of sooty moulds on open bolls which in turn can affect cotton lint colour. To assess the risks from honeydew and rainfall, we designed an experiment that employed artificial honeydew mixtures (composed of various sugars as described in previous CRDC IPM project reports) and used the tents from the CPR project to keep bolls dry during rainy periods. Again, control for tent effects was not possible (see Section 1a).

Weathering Experiment Pilot 2015/16 – This experiment was set up using bolls collected from the previous season and stored through winter in a cool room. The bolls from this collection were either treated with artificial honeydew (+HD) or left untreated (-HD). We also set up 3 x 6 m plastic greenhouses in the field to provide protection against rainfall. This allowed us to have non-rainfall (-RF) or natural rainfall (+RF) treatments. Honeydew bolls received 5 puffs of honeydew each. The experiment was replicated to allow for sampling after (ideally) two different rainfall events: RF event 1 - a small, sharp rainfall event (about 6mm) and RF event 2 - several events (totalling about 32mm) (Fig. 8). All four combinations with intended treatment effects and expected outcomes are listed in Table 2. For each treatment, 110 individually identified bolls were pinned into the field in mid-canopy (Fig. 9) in 11 replications of 10 bolls each, except for the freezer controls (100 bolls each), totalling 1080 bolls.

Table 2: Summary of intended treatment effects and expected outcomes (red): Weathering Experiment, ACRI, 2015/16 & 2016/17

Treatments	- HD	+HD
Reference in freezer (-Rain)	-HD Control No sugar, no SM	+HD Control Sugar, no SM
-Rain (Controls)	Control for Rain 1 – expect no SM Control for Rain 2 – expect no SM	Dry HD effect for Rain 1 – Some SM development? Dry HD effect for Rain 2 – some SM development?
+Rain 1	Rain without HD – negligible SM effect?	Rain + HD – will sugar be washed off and bolls dry out, therefore no SM?
+Rain 2	More rain without HD – possibly other MO effects - Alternaria?	Rain + HD – will residual sugars and moisture cause SM & possibly other MO effect?
Total Bolls	1080 bolls	

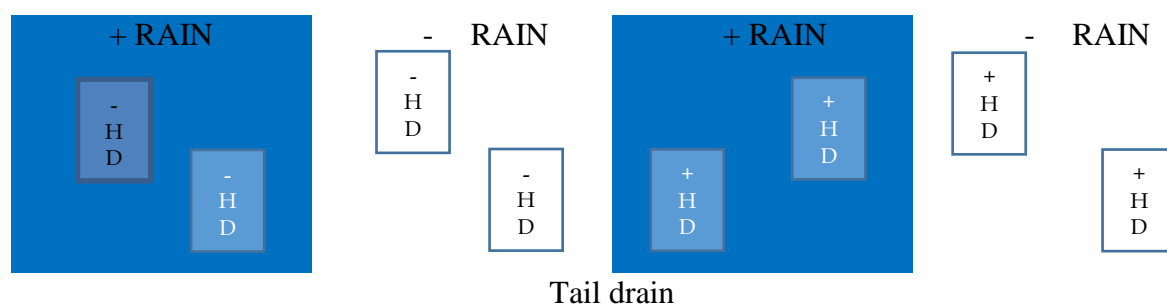


Figure 8: Field plan with greenhouse areas (-Rain) and designated sample areas (+Rain), one for each expected rainfall event.



Figure 9: Example of tagged boll placed in the field or greenhouse (left) and leaves and bolls with developing sooty mould where honeydew was sprayed (right).

The experiment was placed in the field in mid-February as bolls began to open. The first rainfall events occurred mid-March and the first lot of bolls were collected after receiving 10.4 mm of rainfall. By mid-April two further rainfall events (2 x 5 mm and 22.4 mm = 32.4 mm) brought the total rainfall received to 42.8 mm and the second lot of bolls was harvested. After each rain event samples were collected and divided into:

- Reps 1, 5 and 11 – the 10 bolls from each rep were placed into a plastic bag and stored in the freezer to be washed and filtered for sugar analysis by Donna Glassop (CSIRO, QBP, St. Lucia) with a subsample for Linda Smith (QDAF) to help us develop a method to quantify mould spores
- Rep 2 – the 10 bolls from this rep were placed in individual bags (1 boll per bag) and stored in the fridge for sooty mould spore assessment as discussed with Linda Smith (see below).
- Reps 3, 4, 6,7,8,9 and 10 – the 10 bolls from each rep were placed into a plastic bag and kept in a cool dry place for ginning and assessment at Australian Cotton Classing Services, Wee Waa.

Bolls were photographed and each boll in each replication was scored for honeydew presence based on a rating scale developed from variously contaminated bolls (see Section 2a). Mean scores for each treatment were compared to the sugar analyses.

Assessment of sooty mould spore loads was discussed with Linda Smith and we agreed to send her 10 bolls per treatment so she could help us develop a valid technique/method for calculating the spore load of individual bolls, and use the seeds to test for *Alternaria*. *Alternaria* appears to be transmitted at pollination and becomes incorporated into the bolls (Linda concluded this from checking surface sterilised material). Sugar samples from washed bolls in 2015/16 were sent to Donna Glassop for analysis and quantification of sugars. Sugar samples from the 2016/17 experiment were not analysed as the site experienced power failure over a weekend and all samples thawed out and degraded.

Weathering Experiment 2016/17 – This experiment was a repeat of the 2015/16 experiment. Between the 13/03/17 and the 19/03/17 bolls received 106.4 mm of rainfall termed RF1 after which they were collected. During April, short showers (1.6-5.2 mm), which were termed RF2, occurred on 3 days totalling 10.4 mm and the second set of bolls was collected

(cumulative total of 116.8 mm). Bolls were photographed and scored for sooty mould contamination based on a scale developed in 2015/16.

Results

Weathering Experiment Pilot 2015/16 – The +HD bolls were severely contaminated with honeydew sugars (> 90 mg of total sugars/g of seed cotton) after they had been sprayed (Fig. 10, left column, Control +HD treatment). Control bolls of the –HD treatment contained minute amounts of natural sugars which indicated fibre maturity. In the 22 days between Rainfall event 1 and Rainfall event 2 (including treatments protected from rainfall), sugar concentrations on all samples decreased between 40-60 %, except on the +RF – HD samples which remained the same as the -HD controls. At the same time, honeydew scores increased in the range of 185-633 %. It is noteworthy that columns 2 and 3 (No RF, + HD) in Fig. 10 show a nearly 50% reduction in sugars while the honeydew score tripled. This is so far the most direct evidence we have (in the absence of a more controlled experiment) that sooty mould fungi are consuming and reducing sugars on honeydew contaminated bolls. Fig. 10b shows that the increase in sooty mould presence accounted for 0.73% of the change in sugar concentration, irrespective of rainfall or honeydew. Honeydew contaminated bolls and bolls not exposed to rainfall had higher sooty mould scores (Fig. 11), so provided sugar is present, sooty moulds will still grow in the absence of high moisture. Figures 13 and 14 show the range of sooty mould contamination in each treatment with little effect of 10.4 mm rainfall, irrespective of honeydew contamination. By the second rainfall (32.4 mm), honeydew contamination had become more critical with more sooty mould developing on the +HD bolls since sooty moulds can grow on residual sugars. The samples sent to Linda Smith at QDAF have at this stage not been analysed for mould spore identification and quantification due to a staff shortage that made it impossible for her to take on external work.

Weathering Experiment 2016/17 – Sugar concentrations were not determined this season as QBP experienced a power outage over one weekend and our samples thawed out and perished. Honeydew contaminated bolls and bolls not exposed to rainfall had higher sooty mould scores (Fig. 12), a pattern repeated from 2015/16, though scores were higher in magnitude. This is most likely due to higher rainfall during Rainfall Event 1, which saw 10 times the amount of rain for 2015/16. Figures 15 a-d show the development and degree of sooty mould contamination over the two rainfall events.

The observed outcomes were compared to the expected outcomes in Table 3. No sooty mould formed on clean Control bolls in the greenhouses in either experiment. When honeydew contaminated bolls were pinned into the greenhouses, slight to severe sooty mould developed on sticky bolls in the absence of rainfall. This depended on the relative humidity in the greenhouses which was low during RF 1 (10.4 mm) in 2015/16 and somewhat higher during RF 2 (34.4 mm). This was sufficient to increase sooty mould from severe to moderate. In 2016/17 humidity in the greenhouse was initially high during RF 1 (106.4 mm), which caused severe sooty mould on the honeydew contaminated bolls. Since sooty mould does not degrade once formed, it remained on the bolls and gradually intensified under conditions during RF2 (10.4 mm). Clean bolls without honeydew that were exposed to RF 1 had negligible sooty mould the progressed to slight sooty mould during RF 2 in 2015/16, and slight progressing to some sooty mould, respectively, in 2016/17. While none of the samples was tested for *Alternaria*, it may not have been present during those seasons as none of the photographed bolls shows the typical greying of the whole fibre, seen for *Alternaria*. Sooty mould tends to cover the boll surface with lint underneath still white. Honeydew contaminated bolls that were exposed to RF 1 first showed a small amount of sooty mould on some bolls but this had spread to most bolls after RF 2 in 2015/16. The high initial rainfall in 2016/17 ensured that after RF 1, a moderate amount of sooty mould had spread over all honeydew bolls which increased to severe sooty mould on all bolls after RF 2.

Colour Grades – In 2015/16, colour grades of - HD treatments were 11-2 and 21-2 while those of the +HD treatments were 31-2 (Table 4). Colour was downgraded after the second rainfall event. This same pattern was seen in 2016/17 (Table 5).

Table 3: Summary of intended treatment effects and expected outcomes (red): Weathering Experiment, ACRI, 2015/16 & 2016/17 and observed outcomes for 2015/16 (blue) and 2016/17 (green)

Treatments	- HD	+HD
Reference in freezer (-Rain)	-HD Control No sugar, no SM ✓✓	+HD Control Sugar, no SM ✓✓
-Rain (Controls)	Control for Rain 1 – expect no SM ✓✓ Control for Rain 2 – expect no SM ✓✓	Dry HD effect for Rain 1 – Some SM development? ✓slight ✓severe SM on all bolls Dry HD effect for Rain 2 – some SM development? ✓moderate ✓severe SM on all bolls
+Rain 1 (2015/16 – 10.4 mm) (2016/17 – 106.4 mm)	Rain without HD – negligible SM effect? ✓✓very slight SM	Rain + HD – will sugar be washed off and bolls dry out, therefore no SM? Small amount of SM on a few bolls More than moderate amount of SM on all bolls
+Rain 2 (2015/16 – 32.4 mm) (2016/17 – 10.4 mm) Totals 2015/16 = 42.8 mm 2016/17 = 116.8 mm	More rain without HD – possibly other MO effects - Alternaria? Slight SM on some bolls Small amount of SM on some bolls Possibly no Alternaria	Rain + HD – will residual sugars and moisture cause SM & possibly other MO effect? Small amount of SM on most bolls Severe amount of SM on all bolls Possibly no Alternaria

RF	HD	HVI Grade (ACS) RF 1	HVI Grade (ACS) RF 2
-RF	-HD	11-2	31-3
+RF	-HD	21-2	31-2
-RF	+HD	31-2	41-3
+RF	+HD	31-2	41-3

Table 4: HVI grades (ACS, Wee Waa). Weathering Experiment, 2015/16, 8 samples per treatment, Freezer control = 22-3

RF	HD	HVI Grade (MV) Range RF 1	HVI Grade (MV) Range RF 2
-RF	-HD	31-1 to 41-1	21-2 to 41-1
+RF	-HD	31-1 to 41-1	41-1 to 51-1
-RF	+HD	51-2 to 71-1	51-4 to 71-3
+RF	+HD	51-1 to 61-2	51-2 to 61-2

Table 5: HVI grades (Myall Vale). Weathering Experiment, 2016/17, 4 samples per treatment, No freezer control

Conclusion

The Weathering Experiment established the relationships between rainfall, honeydew and sooty mould in cotton fields with open bolls. In the CPR experiment we showed that cumulative rainfall and exposure could negatively affect cotton lint colour in the absence of honeydew through weathering processes. Here we showed that the presence of honeydew supports the growth of sooty mould fungi which cover the surface of cotton bolls giving the crop a greyish, blackish appearance. The spores produced by the moulds, carry through the ginning process and – if in sufficiently high proportions – reduce the colour grade of lint. Rainfall in combination with honeydew promotes this process as higher humidity levels support fungal growth. While rainfall events wash out a portion of sugar, there are sufficient residual sugars left to support sooty mould growth. Sooty moulds growth was also shown to reduce the sugar concentration on contaminated bolls. Price penalties for lint-greying sooty mould are higher than those for stickiness. Hence, if growers find themselves in a situation where honeydew is a problem after boll opening, rain may only conditionally be the solution to cleaning the cotton up. Natural sugar concentrations on mature lint tend to be below 5 mg sugar/g lint (Figure 10a) and such amounts do not cause stickiness problems. Rainfall needs to be sufficient to reduce any honeydew sugars to such low levels. Honeydew is the result of significant Silverleaf whitefly (SLW) or aphid activity, hence the importance of IPM guided insect management cannot be stressed enough.

While most of the problem with discoloured lint is due to sooty mould fungi, there are situations where *Alternaria* plays a role. In a wet season there can be high spore loads in a field from *Alternaria* infected leaves and bolls, and these can infect lint in open bolls to appear grey. The mechanism for this infection is unclear as *Alternaria* spores in lint are usually observed inside the lumen of the fibre but it is possible that the infection progresses through the pedicel, into seeds and then into fibres arising from the seed surface. This type of fungal quality problem was not seen in the Weathering Experiment and will require separate study.

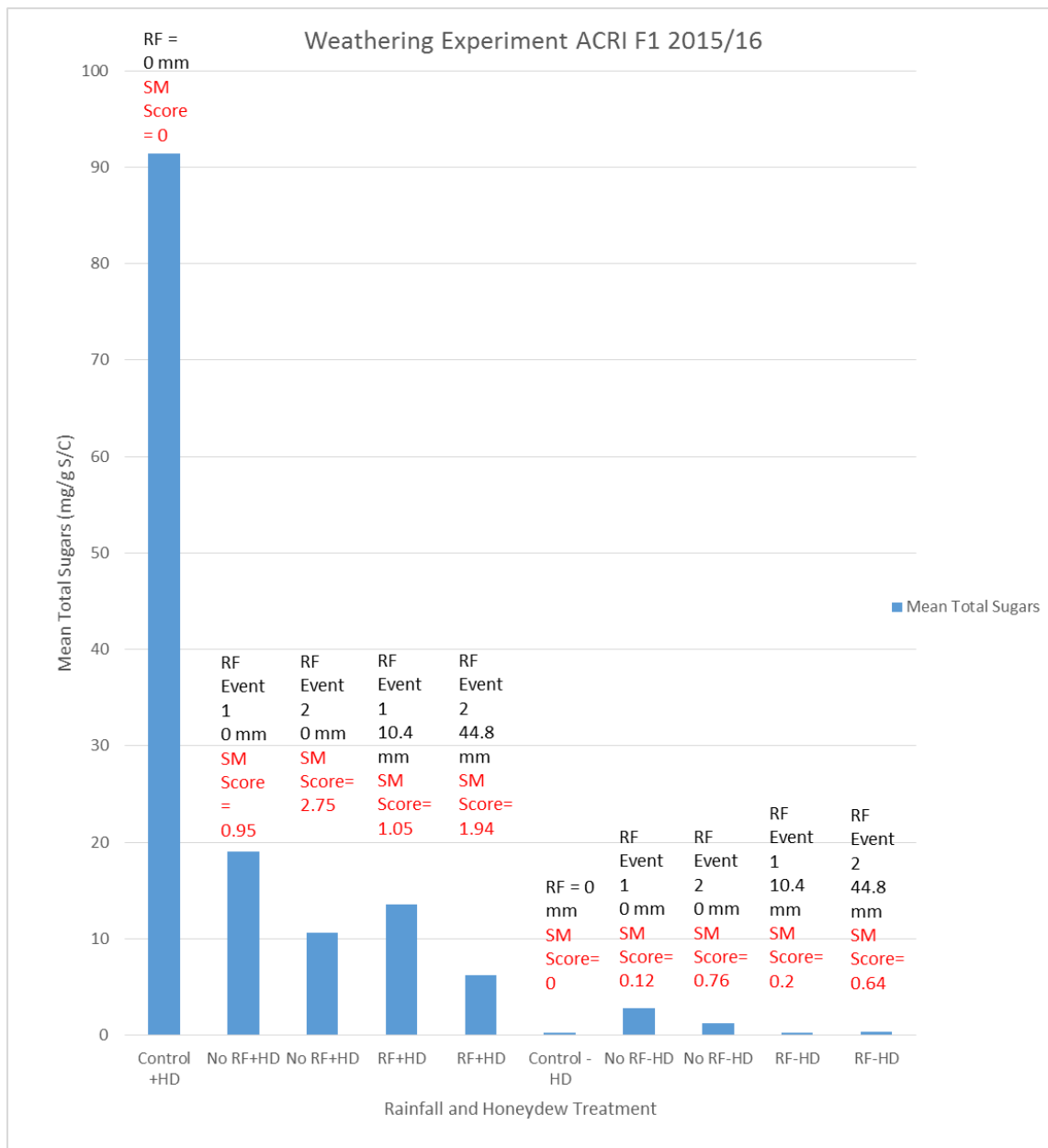


Figure 10: Reduction in honeydew sugars on contaminated lint (left side of graph) and uncontaminated lint (right side of graph), either exposed to rain or protected, and corresponding sooty mould activity. Weathering Experiment, ACRI, 2015/16

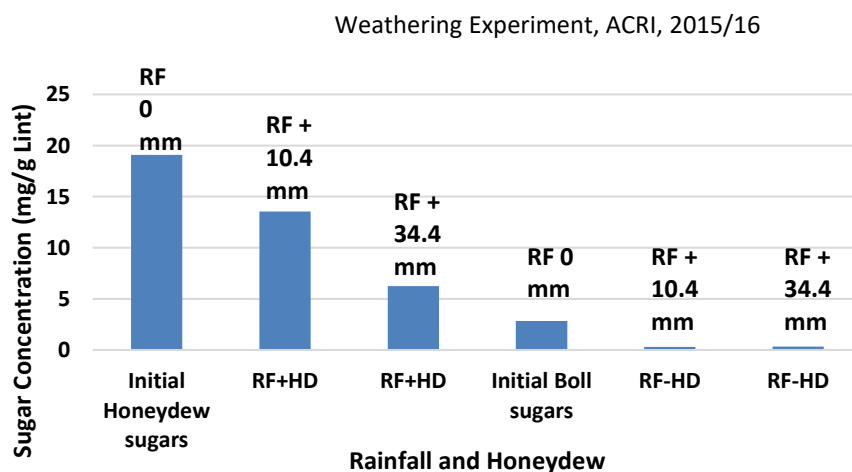


Figure 10a: Extrapolation from Fig.10, reduction in lint sugars (honeydew & natural)

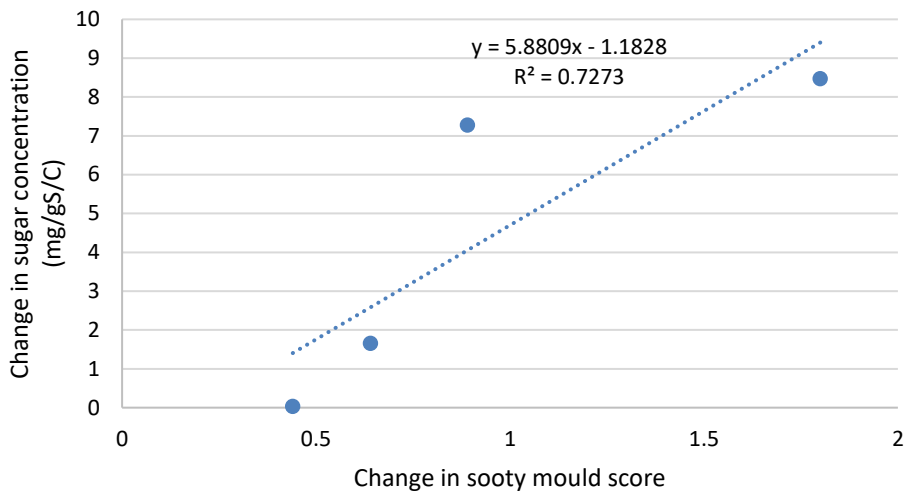


Figure 10b: Extrapolation from Fig. 10, changes in sooty mould score and sugar concentration between Rainfall 1 and Rainfall 2 for each treatment.

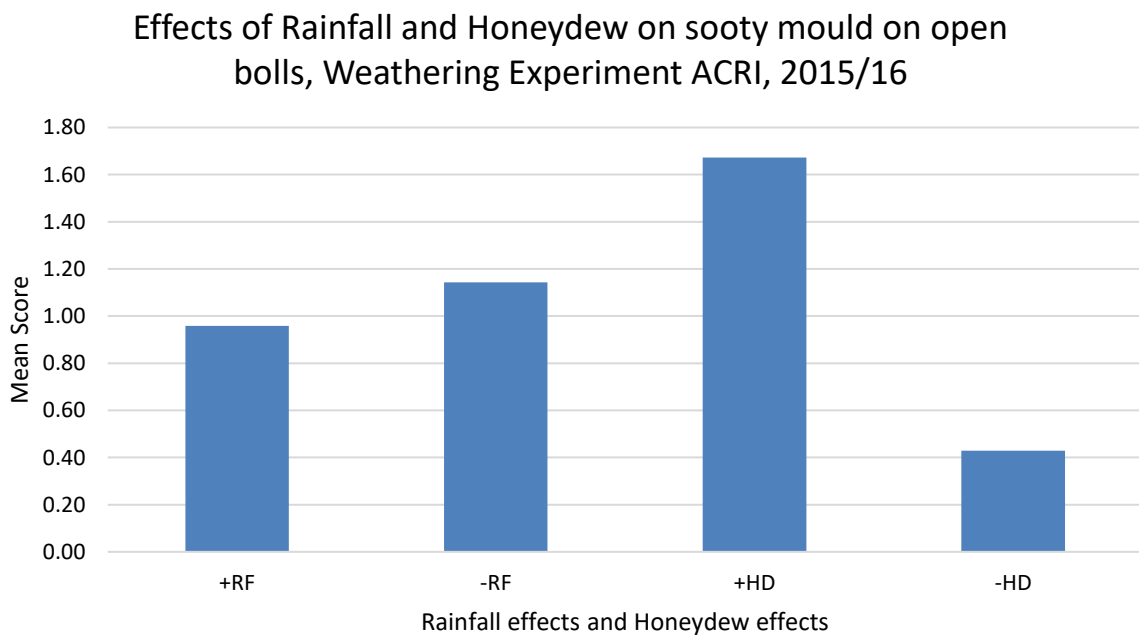


Figure 11: Effects of rainfall and honeydew on the sooty mould score of open bolls, 2015/16

Effects of Rainfall and Honeydew on sooty mould on open bolls, Weathering Experiment ACRI, 2016/17

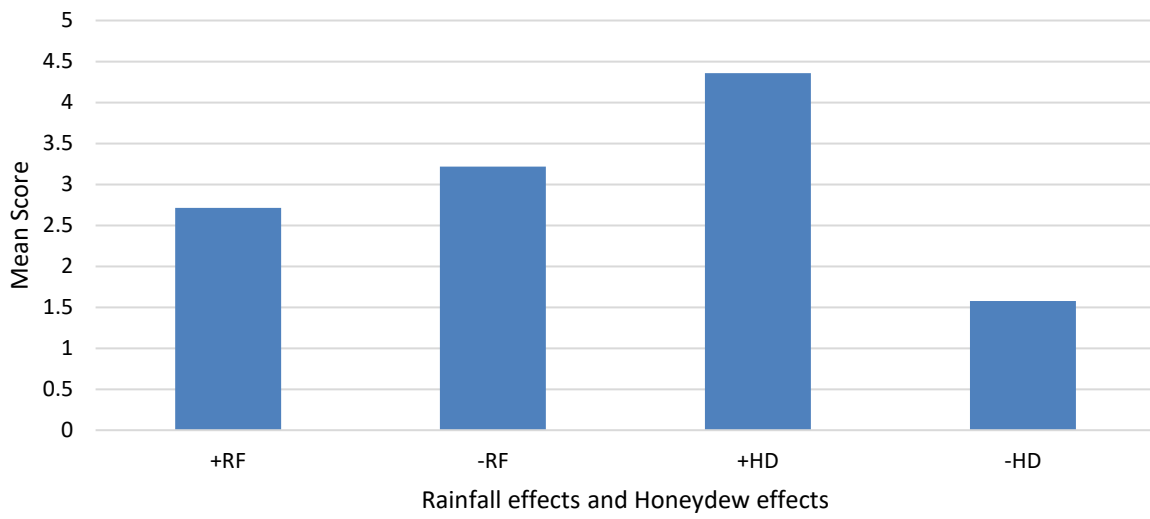


Figure 12: Effects of rainfall and honeydew on the sooty mould score of open bolls, 2016/17



Figure 13: Sooty mould contaminated bolls from different treatments – Rainfall 1= 10.4 mm. Treatments are from top to bottom: -RF-HD, +RF-HD, -RF+HD, +RF+HD



Figure 14: Sooty mould contaminated bolls from different treatments – Rainfall 2= 32.8 mm. Treatments are from top to bottom: -RF-HD, +RF-HD, -RF+HD, +RF+HD

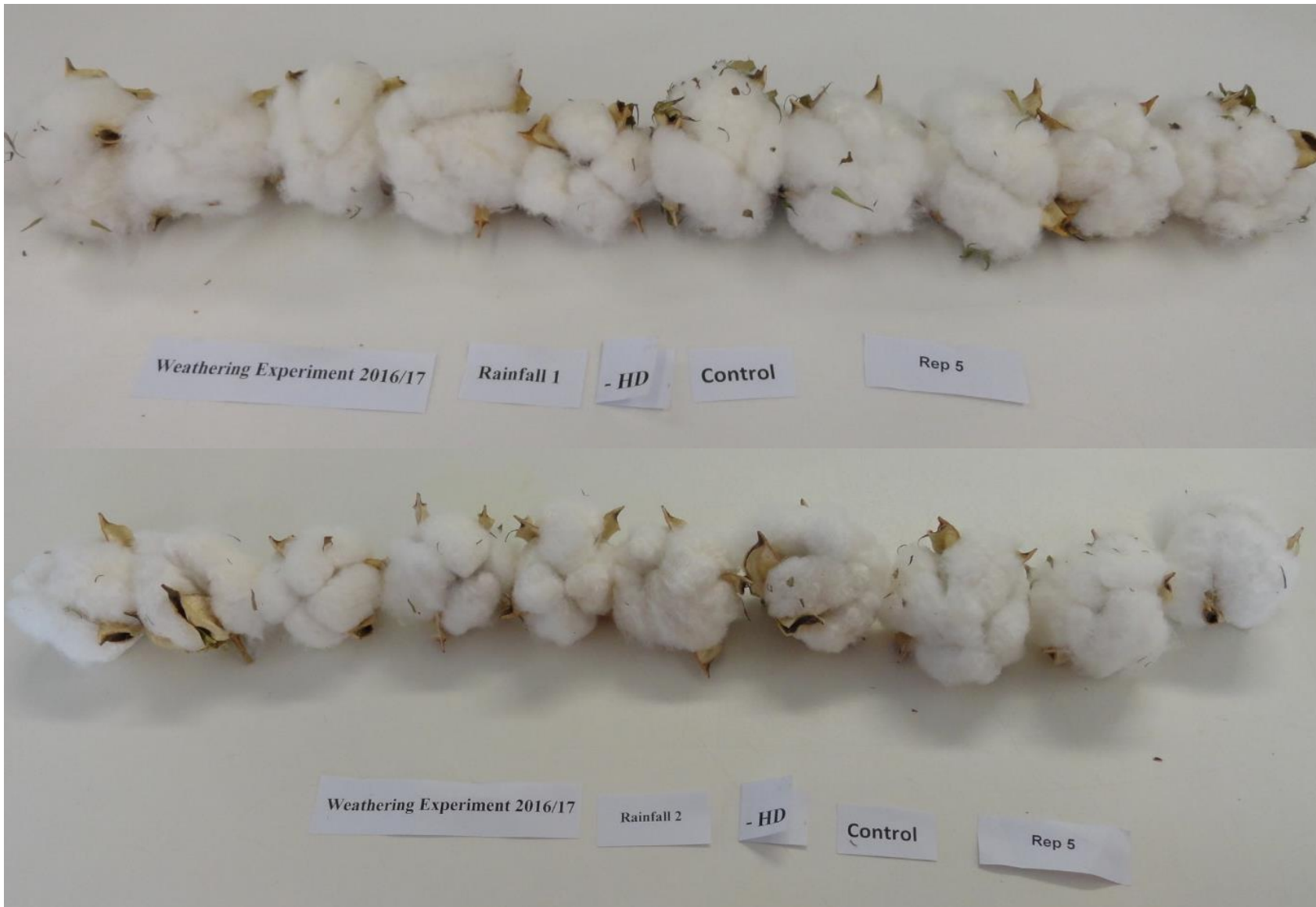


Figure 15 a: Weathering Experiment 2016/17. Control bolls – HD –RF Treatment, Rainfall 1= 106.4 mm (top) and Rainfall 2 = 10.4 mm (bottom)

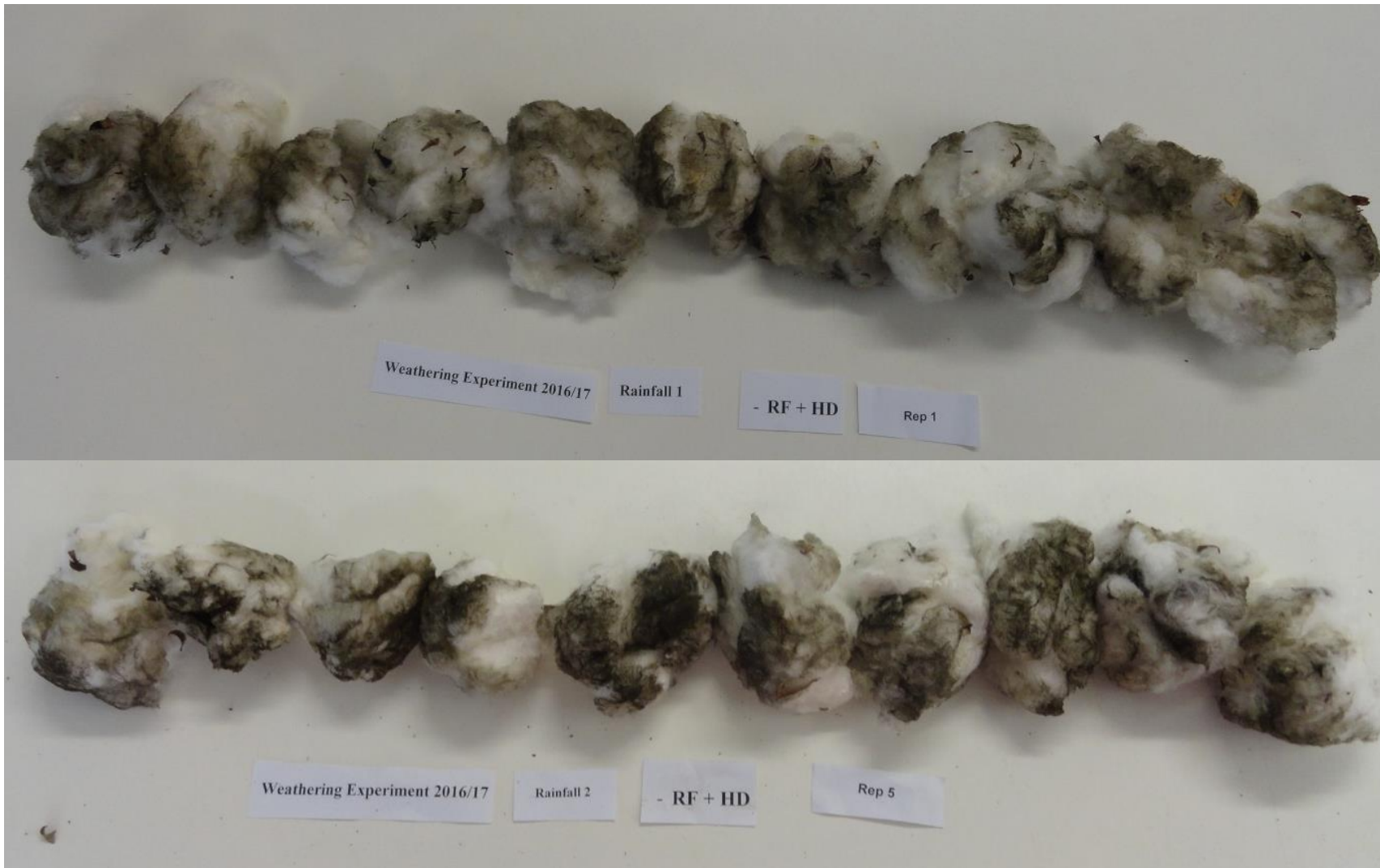


Figure 15 b: Weathering Experiment 2016/17. Sooty Mould bolls –RF +HD Treatment, Rainfall 1= 106.4 mm (top) and Rainfall 2 = 10.4 mm (bottom)

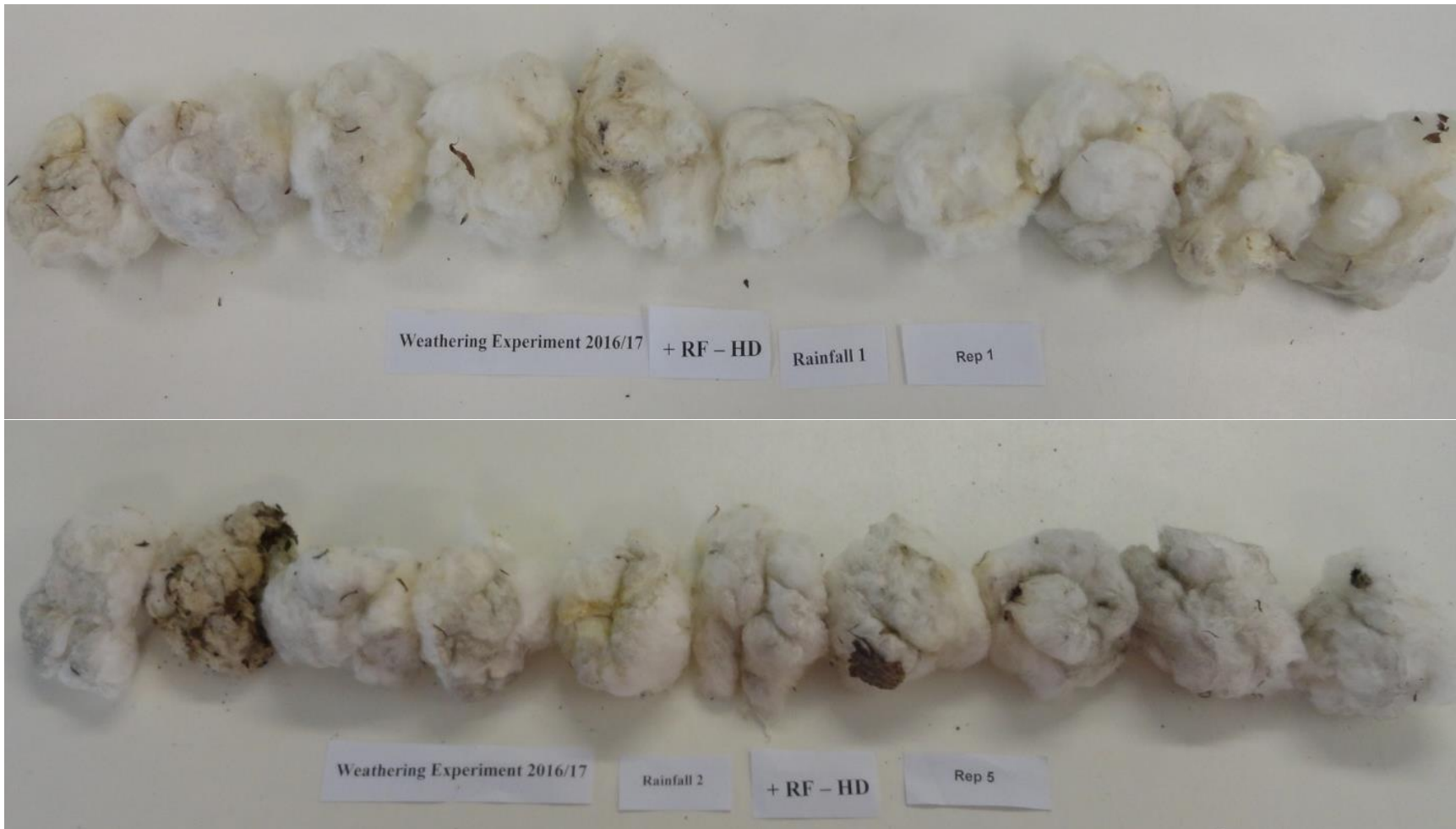


Figure 15 c: Weathering Experiment 2016/17. Bolls +RF -HD Treatment, Rainfall 1= 106.4 mm (top) and Rainfall 2 = 10.4 mm (bottom)

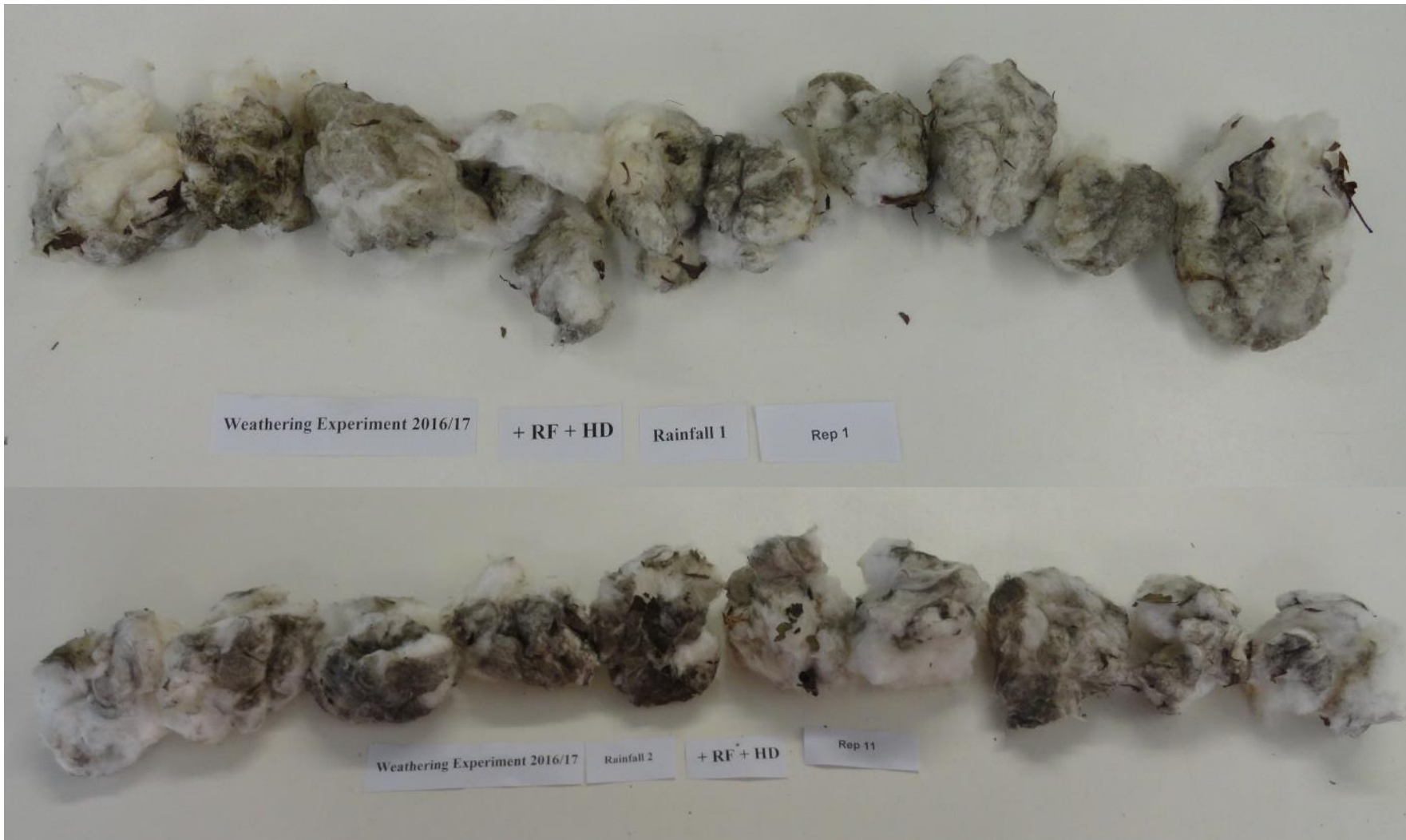


Figure 15 c: Weathering Experiment 2016/17. Sooty mould bolls +RF +HD Treatment, Rainfall 1= 106.4 mm (top) and Rainfall 2 = 10.4 mm (bottom)

2. To determine the impact of sooty mould on cotton colour

- a) Create sooty mould and develop a system that describes lint contamination

Sooty mould scale 2015/16 & 2017/18

To generate sooty mould for study, we used the humid atmosphere of greenhouse tents to create mould with the help of artificial honeydew but we also had the opportunity to collect sooty mould cotton from Croppa Creek in 2015/16. This cotton showed a range of greyish- blackish lint contamination and was used to establish a scale to rank and describe sooty mould levels in the absence of any instruments that could objectively measure it. There is no official classification system for sooty mould so how do we describe it? At best, descriptions of contamination levels are highly subjective and variable. Since sooty mould does not occur in cotton fields every year, we also made contact with consultants in different cotton growing areas to obtain their support and collaboration if they found any. We further collaborated with Sharna Holman in Emerald where the climate was more conducive to sooty mould growth and set up greenhouses so that she could produce contaminated bolls for us. This spread our risk of not being able to collect sufficient contaminated bolls for study and kept us in touch with industry demonstrating that science is working on solving the problems industry is concerned about.

Methods & Results

Cotton, both clean and with varying degrees of sooty mould contamination, was collected and sorted to produce a contamination scale from creamy white to dark grey or blackish. Bolls were presented with their mouldy side up and we tried to match them to paint cards (Fig. 16), however, this was highly subjective. The best, clean cotton is usually graded as 11 or 21 and equated to a rating of 0 while the most contaminated cotton would be graded as 51 or 61 and was rated 4. In between would be cotton graded 31 and 41, rated between 2 and 3. Penalties for colour discounts are most severe where the base grade drops from 31 to 41. The boxes were useful for three seasons after which we purchased a Hunterlab Miniscan EZ portable spectrophotometer (Fig. 17) to take objective cotton colour measurements. The instrument was part of a capital Expenditure Application (CSP1802) from savings made in the Cotton Colour Project CSP1703 and has been extremely useful in defining colour grades in the field. It will be discussed further in Section 5.



Figure 16: Sooty mould rating scale. Contaminated cotton rated 4 (left) and clean cotton rated 0 (right).

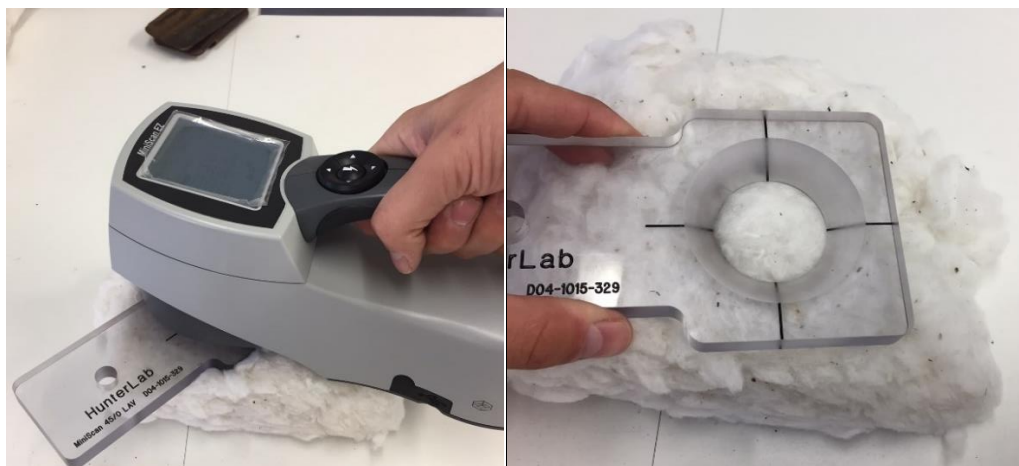


Figure 17: Hunterlab Miniscan EZ portable spectrophotometer

In 2017/18, we generated cotton with severe sooty mould infections in closed up greenhouses and were able to observe a different distribution of the mould over the boll surface. The strongest discolouration as seen in category 4 of the sooty mould scale (Fig 18a) shows spores sitting on the honeydew distributed over the *lint surface*. We noticed that sooty mould spread *through the lint* of the boll (Fig. 18 b). This may be a result of sugars melting and seeping into the lint in hot plastic greenhouses.



Figure 18: Sooty mould distribution on lint a) and in (b) lint

Conclusion

In the absence of objective methods to measure sooty mould, the greyscale grading boxed helped us to describe the degree of sooty mould contamination when we rated sooty mould in the Weathering and Mixing Experiments (Section 2e). For any other purposes, however, it was highly subjective and variable between different operators and we were glad to replace it with a suitable instrument.

- b) Investigate if sooty mould reduces the sugar content of honeydew contaminated cotton bolls

Sooty mould sugar deterioration 2017/18

Experiments to investigate the effect of sooty mould fungi on sugar levels of honeydew contaminated lint were set up in 2017/18 by spraying known quantities of honeydew on cotton bolls and inoculating bolls with sooty mould spores. Bolls were incubated for predetermined time periods before being washed to assess sugar levels.

Our arrangement with Drs. Anne Rae and Donna Glassop at QBP in St Lucia, Brisbane came to an unexpected end when the project that had used the machine concluded in 2017 (we were unaware of the end of the project). I enquired with Donna about the costs to keep the machine going as previously we had contributed to the cost of maintenance, chemicals and the required column (as part of CSP1401). Due to the high cost of refurbishing the HPLC (\$35,000 including a service, required parts and chemicals – but not including labour costs to process the samples), and the fact that - irrespective of whether it can remove sugar or not – sooty mould is undesirable in cotton due to its ability to discolour lint, we decided not to pursue measurement of sugar values in these samples.

- c) Test if production line processes contribute to the physical removal of mould spores and improve cotton colour

Hand pick vs machine pick and Pre- vs Post-ginning grades 2015/16 & 2016/17

During the 2015 harvest we were called to a property near Moree that had been contaminated with sooty mould due to late SLW infestation. While checking the crop we noticed that the harvester was shrouded in a dark cloud and that the nearby gin also puffed out dark clouds not typical of just dust or soil. We discussed whether these clouds could consist of sooty mould spores blown out of the contaminated crop by the mechanics of picking and ginning and decided to investigate if such processes could improve colour grades of contaminated crops.

Methods

Once bolls opened in mid-February 2015/16, sooty mould was generated in the field by spraying 3 rows x 6 m of cotton in 2 greenhouses with sugar solution (simulating whitefly honeydew) and keeping the greenhouse closed to maintain heat and moisture. Sooty mould developed on leaves and bolls with various spore loads. We handpicked 1 m from each tent and each row on the 08/06/2016, then machine picked each remaining 5 m of row separately on the 15/06/2016. Samples were ginned, subdivided into approx. 150 g lots for each tent (Reps 1-6) and classed, then pooled back into rows for testing in Geelong to determine fibre, yarn and dye characteristics (See Section 4b for results). The experiment was repeated using one tent in 2016/17.

Results & Discussion

In 2015/16, handpicked samples graded better (31 and 41) than machine picked samples (51) and were also cleaner than machine picked samples with regards to leaf material (Leaf Grade 3 vs Leaf Grade 5, Fig.19). However, leaf grade was a confounding factor as plants defoliated poorly inside tents due to lack of wind shaking them off the plants after defoliation (Fig. 20).

In 2016/17, handpicked samples also graded better (41) than machine picked samples (51) and greater care during defoliation resulted in an improved leaf grade (2, Figs. 21 & 22). The field and tents had more sooty mould contamination than the previous year which can be seen in the higher pre-gin grades (61 and 71).

Fibre Quality

Fibre quality as measured by HVI (staple, length, micronaire, uniformity, strength, SFI, Elongation) was not affected by picking method or sooty mould contamination (all $R^2 < 0.29$, data not shown)

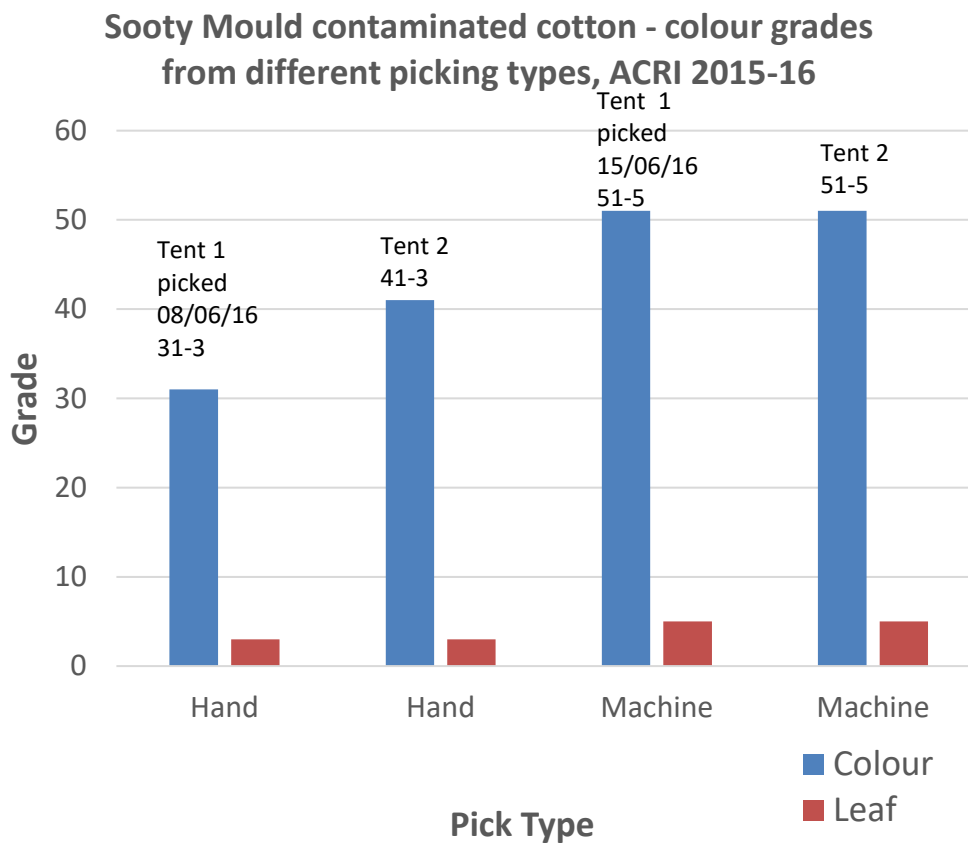


Figure 19: Sooty mould and leaf scores of cotton picked by hand and machine, ACRI, 2015/16



Figure 20: Defoliated tents with poor leaf drop due to protection from wind and consequential high leaf grade

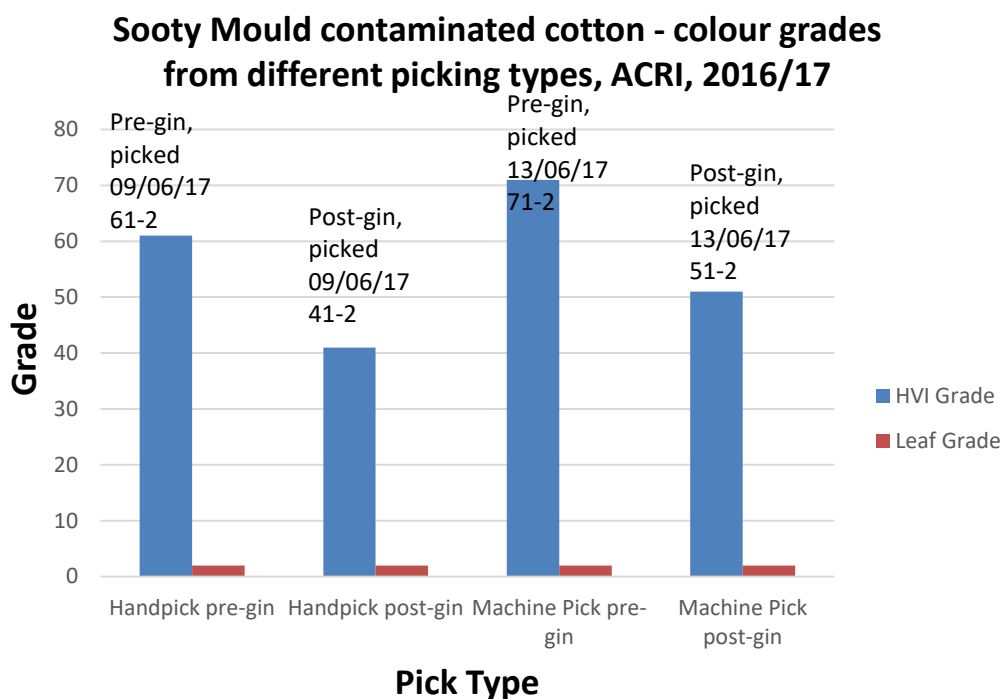


Figure 21: Sooty mould and leaf scores of cotton picked by hand and machine, ACRI, 2016/17



Figure 22: Ginned handpicked (left) and machine picked (right) cotton, ACRI, 2016/17

Conclusion

Contrary to our assumption that the physical action of machine picking can “clean up” cotton contaminated with sooty mould spores, the mixing of surface attached spores into the total lint actually worsens the discolouration problem by 1-2 grades. This occurs irrespective of the leaf grade. In contrast, the ginning process improved cotton colour by up to 2 grades even with heavy sooty mould contamination. This could be an advantage when there is light sooty mould contamination in a crop to ensure that ginned cotton makes base grade.

- d) Assess effect of sooty mould on lint colour and determine the proportion of contaminated lint required in the total pick to incur colour downgrades

Sooty mould mixing experiment 2017/18

We previously showed that colour grades of sooty mould contaminated cotton can improve during the ginning process. To quantify these effects we wanted to know more about the pre- and post-ginning effects of sooty mould and the proportion of the crop that had to be contaminated with mould before we would could detect colour downgrades with the HVI.

Methods

Large quantities of both clean (stored in the fridge from the previous season) and heavily SM contaminated cotton bolls (SM rating 4) were collected and mixed in various proportions ranging from 100% clean : 0 % mouldy to 100% mouldy : 0 % clean. The seed cotton of each portion was placed in the HVI for an initial pre-gin grade measurement. The cotton was combined and ginned, and then put it through the HVI again for a post-gin measurement.

Results & Discussion

The pre-gin grade for the clean cotton was 21-1 to 31-2, with the base grade of Australian cotton being 31-3. This showed that the grade of stored cotton may be recovered by ginning. For the mouldy cotton, the HVI refused to give a reading and interpreted the blackness as leaf material, hence there was no grade for the mouldy portion. The 100% clean cotton grade improved to 11-1 indicating that the inner parts of the boll were still in better condition than the outside. With 10% of sooty mould in the crop, grades were not strongly affected, however, with 20% sooty mould in the mix, grades dropped to 31-1 and 31-2 though this was still within base grade. With 30-50% sooty mould in the mix, there was a drop to 41 and past 60% contamination in the mix grades went to 51 to 61. It must be noted that these results are based on portions of heavily contaminated sooty mould bolls and that these results would be less severe where the sooty mould rating would only be 2 or 3.

Conclusion

Ginning improved the colour grade of stored cotton by mixing the better inner lint of bolls with the slightly discoloured outer portion of lint. Highly SM contaminated cotton however, confounded the HVI. Thirty to fifty percent of heavily contaminated sooty mould cotton in the pick effected a drop in colour grade from 31 to 41 which would result in quality discounts. At 40%, colour grade dropped to 51 which would result in even heavier discounts. It may be possible to extend this information into a “SM effect chart” by including less heavily contaminated bolls in the mixes.

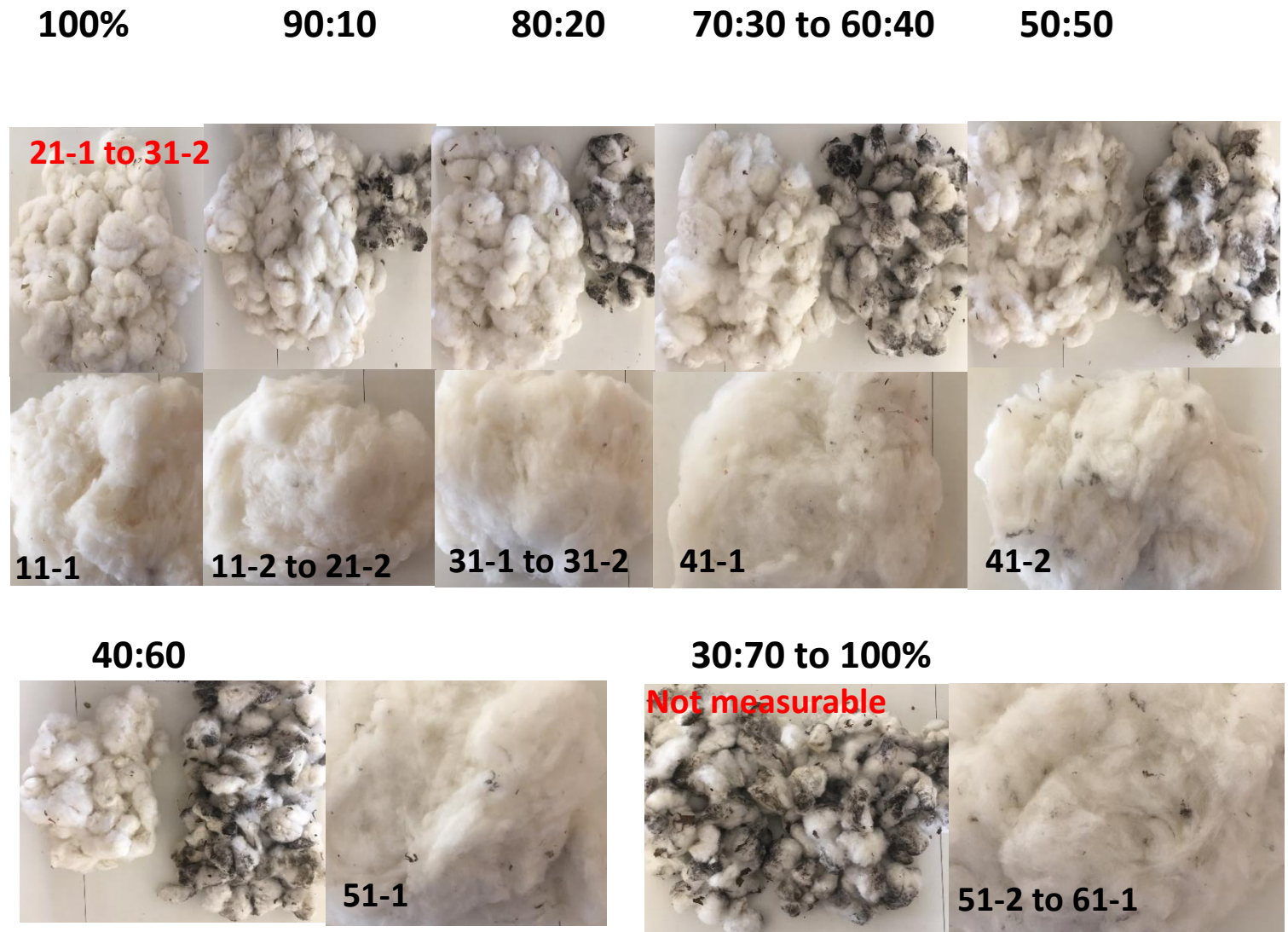


Figure 21: Pre- and post-ginning grades of clean and sooty mould contaminated cotton and post-ginning grades for various proportions of Clean : SM mixed cotton.

3. To discover novel management options for avoiding and mitigating colour downgrades

The avoidance of honeydew on open cotton bolls in the field is so far the most effective way of preventing sooty mould on lint. However, managing the insects that cause honeydew is not always simple and not always successful. In situations where SLW or aphids have not been satisfactorily managed, are present in the crop at boll opening, and weather conditions conspire to increase sooty mould contamination of the crop, mitigation of either honeydew or sooty mould would be highly desirable. At present though there are no agents or processes available – either preventive or curative - that could remedy sooty mould. For this reason we have begun to search for suitable solutions, either managerial or chemical, that would reduce the impact of sooty moulds on colour grades

- a) Test a range of fungicides to reduce sooty mould in the field

SM Fungicide Field Experiment 2016/17

As sooty moulds are fungi, we started searching for fungicides that would be effective against one of the main species that we had previously identified, *Rhizopus stolonifer*. Biologically, *R. stolonifer* is not a sooty mould and is commonly known as black bread mould (Zygomycota). It depends on sugar and starch, can grow under both acid and alkaline conditions at pH 2.2 to 9.6, between 15-30°C and is the most common decay fungus in the world. True sooty moulds belong to the Ascomycota and include *Alternaria*, *Cladosporium* and *Capnodium* spp.

There are no registered fungicides for sooty mould in cotton so we started with one that was readily available in the cotton industry: Dynasty, a seed treatment which contains low rates of the fungicides azoxystrobin, metalaxyl-M and fludioxonil. Azoxystrobin is a systemic, broad-spectrum fungicide that inhibits spore germination, however it is only 25% effective against *R. stolonifer*. Metalaxyl is also a systemic, effective against *Phytophthora* and *Pythium*. Fludioxonil is non-systemic and reduces mycelial growth by inhibiting transport associated phosphorylation of glucose. It is most active against *Rhizopus* at 120 g/L. The other chemical we had available was Amistar which contains a higher rate of azoxystrobin. Both fungicides were provided by CSD, Wee Waa.

Method

To generate sooty mould in the field, we sprayed 16 rows of cotton with honeydew. Mould growth was moderate as the weather after boll opening remained dry. Three rows were regarded as three replications with three 15 m long sections in each row marked for treatments (Control – no fungicide, Amistar @ 1L/ha, Dynasty @ 2 L/ha). When some mould growth became evident in the lower canopy, we picked 200 bolls/plot from the lower canopy and then thoroughly sprayed Dynasty and Amistar with a handsprayer on the crop to assess any curative effects. Twelve days later we picked another 200 bolls from each plot and assessed them for sooty mould contamination, using SM scoring boxes. Samples were photographed, ginned and graded.

Results & Discussion

Colour grades – The pre-ginning colour grades for all treatments ranged from 41-71 with a mean of 51. Post ginning they ranged from 21 to 41 with a mean of 31. The difference between pre and post gin grades was significant ($F < 0.001$, $p = 0.05$, $df (2, 35)$) hence ginning improved colour grade by diluting a low spore load.

Visually there was no noticeable remedial activity of the fungicides (Fig. 23). When analysing the score data, means for the Control, Amistar and Dynasty treatments were 3.17, 3.33 and 3.83, respectively. However, when analysing the scores, there was a significant difference between picking dates, 3.11 for the 04/05/17 and 3.78 for the 16/05/17. ($F = 0.032$, $p = 0.05$, df

(2, 17). Since scoring is very subjective, and the score still pertains to the same grading box, this difference is probably not practically meaningful.

We wanted to repeat fungicide field experiments in 2017/18, however, the season was so dry that we failed to generate sooty mould in the field, despite our best efforts. We attempted to spray honeydew and provide moisture by covering rows in the field with black plastic, but only succeeded in cooking the plants (Fig. 22) and for that reason continued with fungicide screens in the laboratory.



Figure 22: Trying to generate sooty mould but cooking the crop instead, [ACRI](#), 2017/18

Conclusion

The experiment showed no real differences in cotton colour between fungicide treatments and controls. This could have been due to:

- (i) the chemical compounds in the fungicides were not effective on the fungal species present,
- (ii) the active ingredients were present in too low concentrations, and/or
- (iii) the fungicide may act as a preventive rather than as a remedial treatment.

We intend to continue our investigations into fungicides in the future.



Figure 23: Fungicide treatment of sooty mould contaminated bolls, pre spray and 8 days post spray: Top – Controls, Middle – Dynasty, Bottom – Amistar

- b) Screen a range of fungicides and biological agents for their activity against sooty mould fungi in the laboratory

Fungicide Screens 2017/18 – preventive and remedial (petri dishes and lint)

To evaluate the efficacy – prophylactic and curative - of more antifungal substances against sooty mould (such as *Rhizopus stolonifer*), three laboratory experiments were set up:

- (i) to test inhibitory effects of a number of substances diluted in potato dextrose agar (PDA) media in petri dishes
- (ii) to test curative effects of a number of substances on sooty mould infected cotton bolls
- (iii) to test prophylactic effects of a number of substances on clean cotton bolls inoculated with sooty mould spores after treatment.

Treatments included four commercial insecticides, two plant-based compounds, a limonoid and an antifungal polymer. Details of each treatment are provided in Table 6.

Method

(i) PDA plates were prepared with the help of Duy Le (NSW DPI). Diluted test agents were incorporated into the media before setting. The experiment was set up in 4 replications. Inoculate was sourced from cotton bolls infected with sooty mould by vigorously swirling 12 bolls in 250 ml of deionised water to dislodge mould spores. This process has to be repeated about 10 times as spores are difficult to dislodge in water. Agar plates were inoculated with 1 ml of this spore solution which was swirled over the surface before incubation at 28°C for 24 hrs. Plates were checked every 24 hrs until fungal growth was detected. Each plate was photographed to keep a record of mould growth.

Results & Discussion

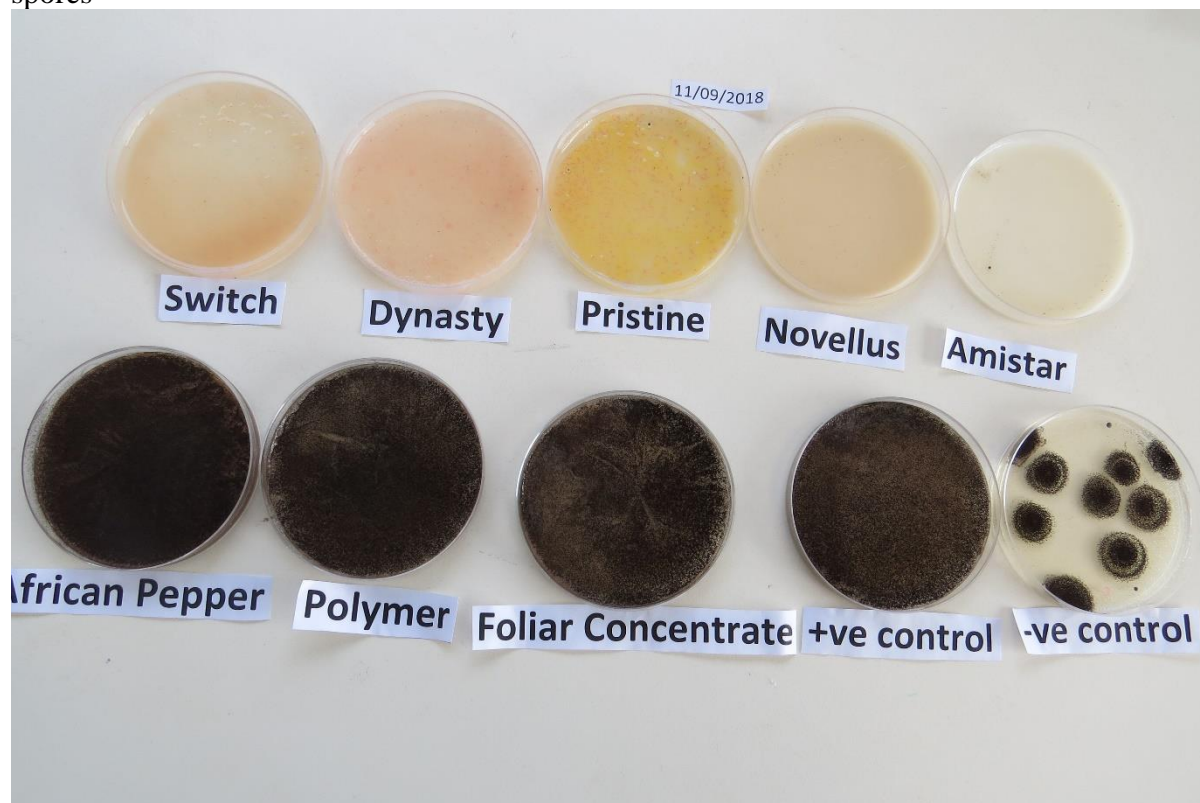
Figure 24 shows the effect of fungicides incorporated into media on the growth of sooty mould spores. All commercial fungicides inhibited sooty mould growth. Novellus limonoid and Amistar completely inhibited the growth of any fungi while Switch, Dynasty and Pristine also inhibited growth of SM spores but allowed pale moulds to develop. Nigerian Pepper had the highest density of black SM spores followed by antifungal polymer and AgriSea extract. The positive control (no trt + spores) allowed SM to produce spores and the negative control (no trt, no spores) was contaminated in the lab and produced spores at those points.

Nigerian pepper also contains limonoids and has reportedly been effective against fungi, including *Rhizopus stolonifer* though the water extract did not show any efficacy against SM fungi. The active ingredients (limonoids) are most likely not water soluble and require an alcoholic extraction. This would probably be more effective as the limonoids in Novellus prevented SMs from developing. Obtaining a thick and sticky ethanolic extract has been difficult and time consuming due to lack of equipment (rotary evaporator). We did manage to obtain the powdery water extraction quite easily and used it in this experiment. Sea weed extract was supplied by a New Zealand Company while Novellus, the limonoid product is used as a fungicide in the wine industry. The antifungal polymer is primarily used in the medical field to combat *Candida* infections and was included to test if it also had efficacy against other type of fungi.

Table 6: Antifungal treatments evaluated against sooty mould fungi

Chemical	Active Ingredient & reported efficacy against <i>Rhizopus stolonifer</i>	Rate	Concentration	Vol. of chemical required in 250 ml(g or ml)	Quantity for 100*conc. made up to 10ml for Duy
Dynasty Syngenta	Azoxystrobin 75 g/L Metalaxyl 37.5 g/L Fludioxonil 12.5 /L (low)	200 ml/100 kg of seed (assume 100 L)	500ul made up to 250ml with deionised water	0.5 ml (500ul)	2ml
Amistar Syngenta	Azoxystrobin 250 g/L (low)	800 ml /ha	2ml made up to 250ml with deionised water	2 ml	8ml
Switch Syngenta	Cyprodinil 375 g/kg Fludioxonil 250 g.kg (very high)	80g/ha	0.2g made up to 250ml with deionised water	0.2 g (200ug)	800mg
Pristine BASF	Boscalid 252 g/kg Pyraclostrobin 128 g/kg (high)	40 g /100 L	0.1g made up to 250ml with deionised water	0.1 g (100ug)	400mg
Novellus Eden Research plc	Limonoids: Eugenol 3.3% Geraniol 6.6% Thymol 6.6% (moderate)	4 L/ha	10ml made up to 250ml with deionised water	10 ml	40ml (so give pure)
<i>Aframomum melegueta</i> extract Nigerian Pepper	Limonoids (good – ethanolic extract, poor- water extract)	10mg/4 milligrams/ml	1g made up to 250ml with deionised water. Can add up to 5% ethanol to agar (12.5ml).	2.5g/1 g	10000mg/4000mg\ Water extract used
AgriSea Foliar seaweed concentrate AgriSea NZ Seaweed Ltd	Unknown	10ml/L	2.5ml made up to 250ml with deionised water	2.5ml	10ml (so pure)
Antifungal polymer (720mg) CSIRO	Unknown	128 microgram/ml	0.032 g made up to 250 ml with deionised water	0.032 g	128mg
Deionised water (+ve Control)	Nil		pure		
Deionised water (-ve Control)	Nil		pure		

Figure 24: Effect of fungicides incorporated into PDA media on the growth of sooty mould spores



Method

(ii) Sooty mould infected bolls (scored as 4+ according to the grading boxes described above) were collected from the field and sprayed with the fungicides listed in Table 5. Controls were sprayed with deionised water. Each boll was sprayed from each side and over the top, resulting in 5 puffs from a spray bottle that produced a fine mist. Each treatment was replicated 4 times. Bolls were placed into individual plastic containers lined with moist tissue paper and were incubated at 26°C for 1 week. Every time bolls appeared dry, they were misted with water and the tissue paper was re-wetted. Bolls were inspected 4 and 9 days after treatment to evaluate if sooty mould infection had improved.

Results & Discussion

Over 9 days, sooty mould infestation of control bolls did worsen, the woody bracts became softer and the whole boll appeared to exhibit a secondary infection by a whitish mould (see Appendix 3). This secondary mould was also seen in the Switch, Amistar, Dynasty, African Pepper and Pristine treatments but not for the Novellus, AgriSea Foliar Concentrate, or Polymer treatments. Sooty mould worsened in the Novellus, Dynasty, African Pepper and AgriSea Foliar Concentrate. Sooty mould did not worsen for the Amistar and Switch treatments but both these treatments were strongly affected by the secondary mould. None of the products tested had any curative action against sooty mould on cotton bolls.

Method

(iii) Clean cotton bolls (scored as 0 according to the grading boxes described above) were collected from the field and sprayed with a solution of fungal spores derived from field collected mouldy bolls. Each boll was sprayed from each side and over the top, resulting in 5 puffs from a spray bottle that produced a fine mist. After drying, bolls were sprayed with the fungicides listed in Table 5. Each treatment was replicated 4 times. Negative controls were sprayed with deionised water only while positive controls were sprayed with fungal spores and deionised water. Bolls were placed into individual plastic containers lined with moist tissue paper and were incubated at 26°C for 1 week. Every time bolls appeared dry, they were misted

with water and the tissue paper was re-wetted. Bolls were inspected 4 and 9 days after treatment to evaluate if sooty mould infection had improved.

Results & Discussion

None of the treatments sprayed onto open cotton bolls promoted the growth of sooty mould fungi on lint (see Appendix 4) though the moisture provided on tissue paper promoted black spore growth on the bracts in the following treatments: +ve control, -ve control, Novellus, Dynasty, African Pepper, AgriSea Foliar Concentrate, Antifungal Polymer and Pristine. The following also exhibited the white mould growth: +ve control, -ve control, Novellus, Switch, Amistar, Dynasty and Antifungal Polymer. Preventive effects were shown by Switch and Amistar as these did not produce black spores on the bracts.

Conclusion

All commercial fungicides inhibited the growth of black spore producing mould on agar plates. Novellus and Amistar. Switch, Dynasty and Pristine allowed for the growth of a pale moulds, however, this should not be consequential for cotton lint in the field. The plant-based and unconventional treatments variously promoted the growth of sooty mould spores though the ethanolic extract of African Pepper is expected to be more effective. None of the treatments gave indication of curative effects on developed sooty moulds though Amistar and Switch inhibited further mould development. These inhibitory or preventive effects by Amistar and Switch were again shown in the third experiment carried out on clean bolls. These bolls were treated with fungal spores, however, they were free of honeydew. While this again proved that sooty mould spores require a sugary substrate (honeydew) to grow on lint, it also exposed a flaw in the experiment: we should have sprayed the lint with artificial honeydew prior to the experiment to evaluate preventive effects of fungicides *on lint*. Mould spores, normally present on vegetative material, still managed to develop where there was a starch source (on the bracts) and provided an indication of the preventive effects of Amistar and Switch. Checking back in Table 6, these treatment effects further confound the experiment since reported action of Amistar against *Rhizopus stolonifer* is low while that of Switch is very high. In 2011, we identified *Rhizopus stolonifer* and *Cladosporium cladosporioides* as the two main fungal species with traces of other fungi in the samples (Appendix 5). Both species have dark spores, with those of *C. cladosporioides* ranging between dark green to black. Our unidentified species were black hence there is a possibility that our mould species could be *C. cladosporioides*, also common in the environment, instead of *R. stolonifera*. This would explain why Amistar was very effective in the PDA plates and boll experiments since azoxystrobin controls *C. cladosporioides*. Cyprodinil and fludioxonil (Switch) are also effective against *C. cladosporioides* supporting the speculation that this species was present on cotton lint. Pristine (boscalid and pyraclostrobin) is also reported to effectively inhibit *C. cladosporioides*, which was seen on PDA plates but was not obvious from the boll experiments. We will organise to identify sooty mould samples again for further experiments.

- c) Evaluate the effect of early defoliation on sugar concentration of bolls and sooty mould development

Not accomplished – we were unable to carry out defoliation experiments as only shared field space was available and it was not possible to co-ordinate with other experiments that would not be impacted by this procedure.

- d) Investigate the role of water repellents

Since moisture contributes to the development of sooty mould, we wanted to test the effect of a water repellent on its development. We used 3M – Scotchgard™ Heavy Duty Water Shield to protect cotton bolls from moisture, hoping that this would prevent the formation of sooty mould.

Method

The experiment was set up for two treatments (-Water repellent (Control) and +Water repellent) in 4 replications per treatment with 150 open bolls per replication. In total 600 bolls were sprayed with 3M Scotchgard™ while the other 600 were left untreated. Each boll received 5 puffs of repellent, one from each direction and one over the top of the boll. Bolls were free of honeydew and had been stored in a cool room. All bolls were exposed by pinning them onto the fence line in Block 18 at ACRI on the 08/06/2017. Three days later, they received 1mm of rainfall. On the 27/06/2017 the bolls received another application of 3M Scotchgard™ while still in the field. A further 25 mm of rain fell on the 28-29/06/2017. Bolls were collected on the 13/07/2017 and stored in the freezer before being ginned at ACRI. Bolls were tested with the HVI at ACRI (Myall Vale – MV) before and after ginning. Ginned samples were subsequently classed at ACS in Wee Waa.

Results & Discussion

The exposed bolls started to show sooty mould infestation after receiving 26 mm of rainfall irrespective of their treatment. Hence, the 3M Scotchgard™ water repellent treatment did not prevent mould formation. There was a significant difference for both reflectance (Rd) and yellowness (b+) before and after ginning across both treatments (Table 7). The post-ginning colour grade colour grade (31-1) had improved from the pre-ginning colour grade (41-1) indicating that during a shorter exposure to weathering only the lint at the boll surface may experience colour changes. This effect has also been observed in other experiments in this project. The effects of the water repellent, when compared to the control, were not statistically significant for Rd and b+ assessments with both the Myall Vale HVI and the ACS HVI, but numerically they produced a change by one colour grade. Cotton bolls treated with water repellent graded worse than the control bolls (Table 8).

Table 7: Pre- and post-ginning effects – water repellent experiment ACRI, 2016/17

Timing	Rd	b+	Colour Grade
Pre-ginning	82.47	4.8	41-1
Post-ginning	83.63	6.3	31-1
F (P=0.05)	0.021	<0.001	
LSD	0.94	0.43	
df	(1, 15)		

Table 8: Effect of water repellent on colour grade of exposed cotton, ACRI, 2016/17

Treatment	Rd (MV)	b+ (MV)	Colour Grade	Rd (MV)	b+ (MV)	Colour Grade
Control	83.98	6.5	21-1	80.8	6.2	31-1
Water repellent	83.28	6.13	31-1	79.7	5.98	41-1
F (P=0.05)	0.08	0.276		0.281	0.266	
LSD	n.s.	n.s.		n.s.	n.s.	
df	(1, 7)			(1, 7)		

Conclusion

Water repellent did not prevent the occurrence of sooty mould on cotton bolls exposed for 5 weeks in the field receiving 26 mm of rainfall. Considering that we have shown that sugar is required for sooty mould formation, we may assume that the stored bolls contained either residual honeydew sugars or maturity related sugars. The inherent properties of the water repellent also affected lint grading with treated lint having worse grades than control lint.

e) Investigate the role of yeast in sugar degradation

The fungi identified from cotton lint (*Cladosporium cladosporioides* and *Rhizopus stolonifera*) utilise sugar as a metabolite and in the process produce cellulases that may affect cotton fibre which is 99% cellulose. They also produce black spores which discolour cotton lint. We considered other fungi that will primarily use sugars as substrates and *not* produce black spores and cellulases (or pectinases). A different type of fungus, yeasts may be an alternative. The budding yeasts ("true yeasts") are classified in the Ascomycota, order Saccharomycetales, and are chemoorganotrophs, that use organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained mostly from hexose sugars, such as glucose and fructose, or disaccharides such as sucrose and maltose. Some species can metabolize pentose sugars such as ribose, alcohols, and organic acids. Honeydew consists of glucose, fructose and sucrose (which will split into the former) as well as melezitose and trehalulose. When exposed to high temperatures (200°C), melezitose degrades into mostly glucose and a small amount of turanose with about 10% of melezitose remaining. Trehalulose degrades into a small amount of glucose and non-carbohydrate products, a reaction enhanced by catalysts. Hence, yeasts should be able to metabolise at least some sugars in honeydew.

Yeast Experiments 2016/17 and 2017/18

To investigate the ability of common baker's yeast (*S. cerevisiae*) to metabolise all the sugars commonly found in insect honeydew we set up a preliminary experiment. All sugars were made into separate solutions (glucose, fructose, sucrose, melezitose and trehalulose) with water as the control.

Methods

In 2016/17, we allowed for 4 treatment reactions: R1 = sugar solution only, no yeast (Control), R2 = Sugar + Yeast 10 min reaction, R3 = Sugar + Yeast 20 min reaction, R4 = Sugar + Yeast 50 min reaction (16 samples). The experiment was carried out using falcon tubes in a hot water bath at 37°C. Three grams of each sugar were dissolved in 30 ml of water and 1 g of baker's yeast was added to each sample. Each tube was shaken vigorously and allowed to froth for the determined time intervals. At 20 minutes R 4 was shaken again to remix the solution, then again at 30 and 40 minutes, assuming a reaction end point at 50 minutes. The degree of frothing for each sugar at each time was assessed as a sign of activity and scored (R = reaction, take photos). Froth Score: 0 = no froth, 1 = signs of froth, 2 = some froth, 3 = good froth, 4 significant froth. After scoring, each sample was immersed into boiling water and heated to 62°C for 3 minutes to kill the yeast. Samples were poured through a filter funnel to separate the yeast, then pushed through a syringe nylon filter and divided into a falcon tube for sugar testing. (This however, did not occur due to the samples spoiling during the QBP power outage). This experiment was repeated in 2017/18, when brewer's yeast was also included in the assessment, and the reaction time was assessed at 30 minutes and extended to 90 minutes.

Results & Discussion

In 2016/17, fructose was rapidly metabolised by baker's yeast (Fig. 25), followed by glucose and sucrose (Table 9). These three sugars were also metabolised readily in 2017/18 (Fig. 26). The yeast metabolised trehalulose much slower than these sugars, and never as vigorously as fructose or glucose. Melezitose was either not utilised by the yeast, or the reaction is so slow that it may take hours of incubation (Fig. 27) though the reaction was somewhat stronger in 2017/18 (Fig 28). Considering that whitefly honeydew contains only a small amount of melezitose, yeast could potentially metabolise most of the sugars in that type of honeydew. The proportion of melezitose in aphid honeydew is usually less than 15% (except for Hendrix *et al.* (1992) – 38.3% melezitose) and the remainder consists of glucose, fructose and sucrose

and a very small amount of trehalulose, all of which can be used by baker's yeast, hence most of the sugars in the honeydew could be used. Brewers's yeast did not metabolise any sugars.

Table 9: Froth scores of individual sugars metabolised by baker's yeast at different reaction times, 2016/17 and 2017/18 (figures in brackets)

Sugar	Control	10 min	20 min	30 min	50 min	90 min
None	0 (0)	0 (0)	0 (0)	(0)	0 (0)	(0)
Glucose	0 (0)	3 (4)	4 (4)	(4)	2.5 (3.3)	(4)
Fructose	0 (0)	4 (4)	2.5 (3.3)	(4)	2.5 (3)	(3.7)
Sucrose	0 (0)	3 (4)	2.5 (3.3)	(4)	2.5 (3)	(3.7)
Melezitose	0 (0)	0.5 (1)	0.5 (1)	(2)	0.5 (2)	(2)
Trehalulose	0 (0)	2 (3)	2.5 (2)	(2)	2.5 (2)	(3)

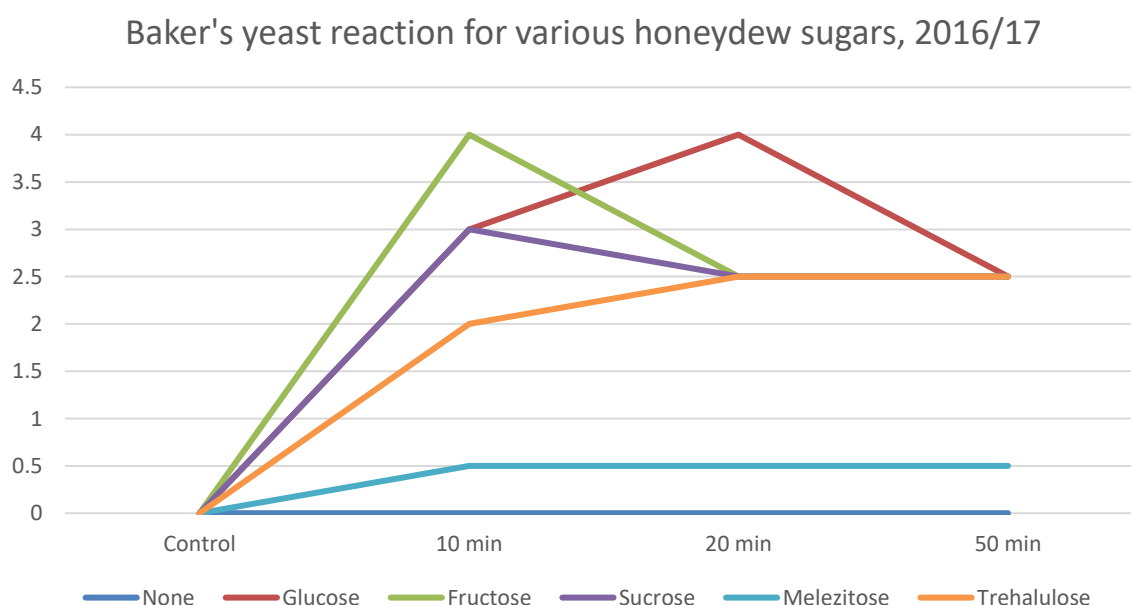


Figure 25: Froth score reactions of various honeydew sugars over time, 2016/17, Note that after 20 minutes fructose, sucrose and trehalulose reactions are identical

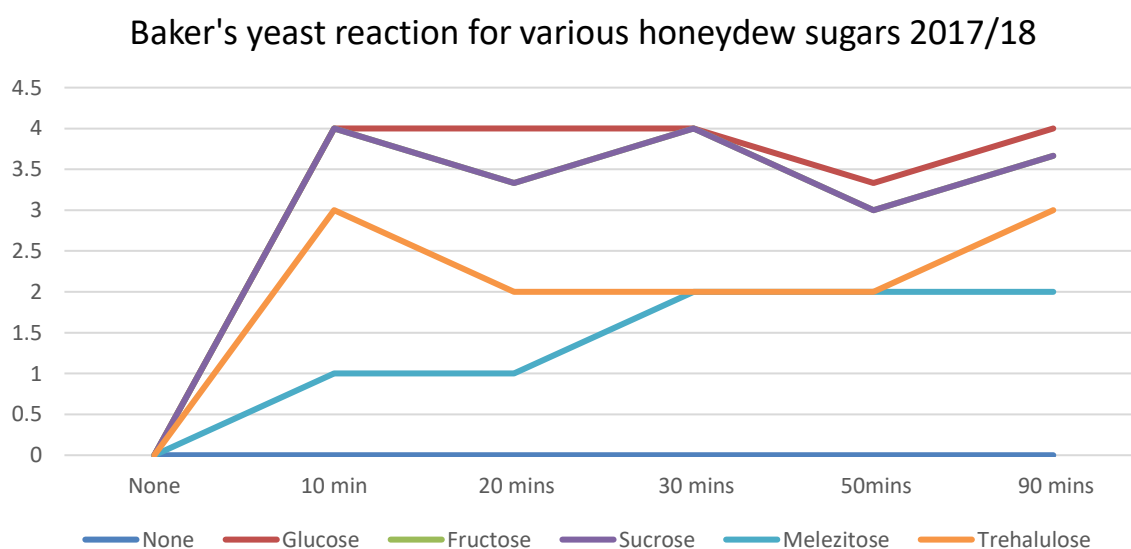
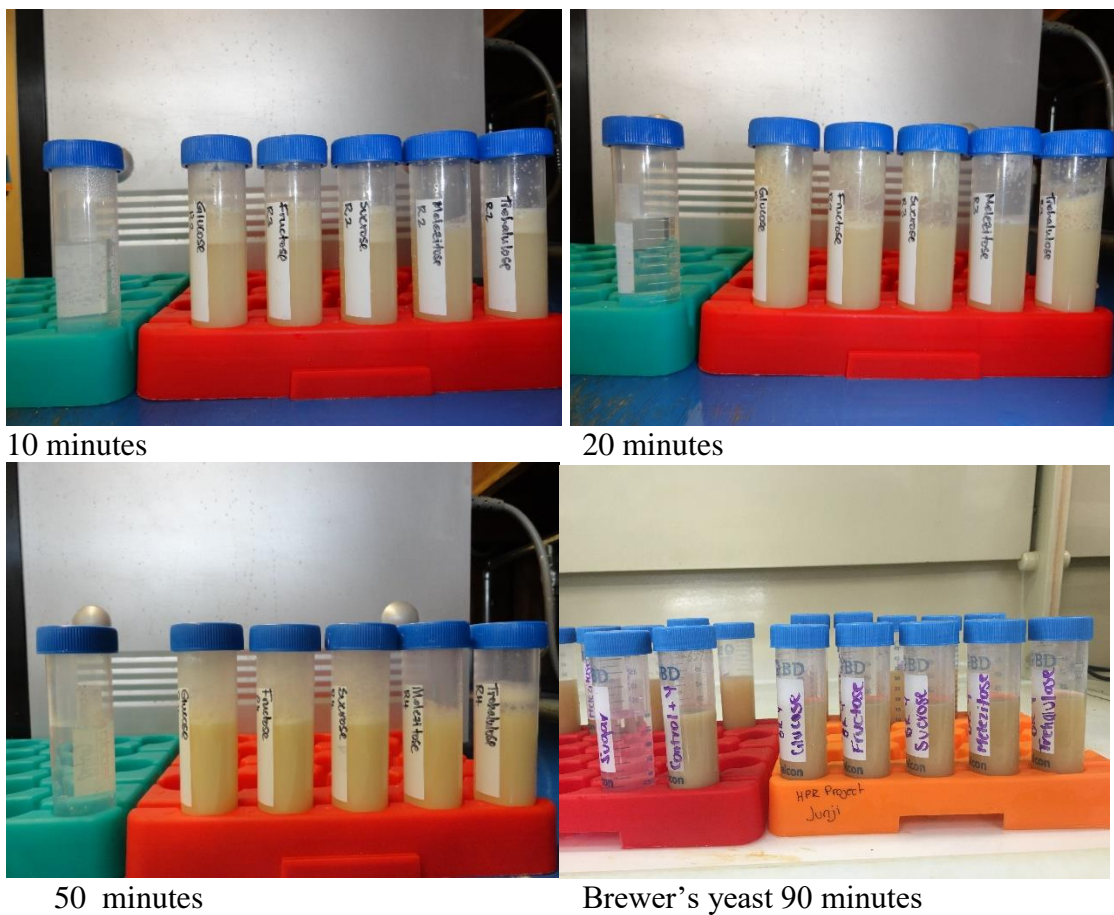


Figure 26: Froth score reactions of various honeydew sugars over time, 2017/18. Note that fructose and sucrose reactions are identical



10 minutes

20 minutes

50 minutes

Brewer's yeast 90 minutes

Figure 27: Baker's yeast reaction on various sugars (water Control on left) and Brewer's yeast (bottom right)

Conclusion

Baker's yeast readily metabolises glucose, fructose and sucrose in honeydew as well as trehalulose at a slower rate. Fructose and glucose are breakdown products of trehalulose. Melezitose is metabolised to a limited degree. There may be potential application of yeast in the breakdown of honeydew, however, the yeast itself could be a contaminant of lint. Brewer's yeast did not metabolise sugars.

Objective 4: To establish whether a relationship between colour grade and yarn quality exists

Ginned and classed samples from the Colour Post Rain (CPR) and Hand vs Machine Pick (Sooty Mould - SM) experiments were pooled to the same colour grade within a treatment to be able to provide approximately 500 g samples for spinning. Samples were spun by Dr. Rob Long at the CSIRO Fibre Unit in Geelong and the resulting yarn was tested, then knitted into fabric for dyeing.

- a) Measure the effect of cumulative rainfall on yarn/fabric quality of downgraded lint - CPR

Methods

The below method was supplied by Dr. Rob Long and was used for both the CPR and SM experiments.

General Spinning Procedure

- Using the new miniature spinning plant, spin 20tex yarns with a standard knit twist. Two replicate spinning lots per sample bag.
- Therefore for each spinning lot → opener → card twice as recommended → miniature draw frame → roving → spinning 20 tex yarn knit twist.

Preparation Processing

- Miniature processing as above
- Drawn sliver to speed frame to make appropriate roving (700tex, 42tpm)?
- Roving on miniature ring spinner

Spinning Processing

- Spinner 2, 8000 rpm
- Yarn to be produced at 20 tex yarn
- Twist factor 3.7 α
- Spin out roving onto two bobbins so there is plenty of yarn for all testing plus fabric

Textile Testing Lab

- Twist
- Tex
- Evenness (including standard 3DOM) 400m per minute for 0.5 minutes
- Tensorapid (100 tests)
- Repeat tex, twist, evenness and tensorapid 2 times (2 replicate tests) sequentially; ensure each tex result is entered in tensorapid instrument for each tensorapid run. This is to capture variation along the yarn package. NOTE: this is two reps which is different from other jobs, as we need to preserve yarn for fabric manufacturing

Winding

- Spun yarns will be steamed first, waxed and wound without clearing (open up clearers).

Knitting and Dyeing

- Yarns will be knitted into a sock then dyed
- Cotton fabric was caustically scoured and dyed with Cibacron Blue LS-3R HC

Yarn parameters measured

- Count – Number which indicates the mass per unit length or the length per unit mass of yarn.
- Twist – Twist is the spiral arrangement of the fibres around the axis of the yarn. The number of twists is referred to as turns per inch.
- Unevenness (U%) – Variation in the linear density of a continuous strand or of a portion of a strand.
- Nep 200% - Any small entanglement of textile fibers that cannot be unravelled in 1000 m of yarn, indicates the cross-section at the nep is 240% of the mean cross-section of the yarn or more.
- Elongation % - Maximum elongation before the yarn breaks indicated in %.
- 2DO (mm) – Mean value of the yarn's two-dimensional diameter (mm) over the entire length of the yarn
- B Force (cN) – Mean breaking strength of yarn (centiNewton)
- Thin 50% - A thin place of -50% mean the cross-section of the yarn at the thick place is only 50% of its mean cross section or less.

- Thick 50% - A thick place of +50% means the cross-section of the yarn at the thick place is 150% of its mean cross section or more.
- Tenacity (cN/tex) - Measure of strength of a yarn, usually defined as the ultimate (breaking) force of the yarn (in gram-force units) divided by tex (linear density – g/km)
- B Work (cN.cm) – work to break

Fabric parameters measured

Delta E – this is the single number metric of difference or distance between two colours based on the belief that a dE of 1.0 is the smallest colour difference the human eye can see. Hence, a dE of >1 is noticeable, however, an acceptable tolerance limit beyond 1 may be set in the range of >1-100. The dE76 formula developed in 1976 was used to calculate Delta E based on the CIELAB colour space L*a*b measurements”

$$\Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

Delta E	Perception
<= 1.0	Not perceptible by human eyes.
1 - 2	Perceptible through close observation.
2 - 10	Perceptible at a glance.
11 - 49	Colours are more similar than opposite
100	Colours are exact opposite

Photography of raw knitted fabrics

Photos were taken with a Nikon D80 in R. Long’s office na2.113 under normal room fluoro lighting. The camera was used in manual mode with custom white balance determined via three sheets of A3 white photo copy paper. An 18% Grey card was used to determine the correct exposure and in each photograph a name identifier tag and QPcard 101 with 3 neutral patches was included. The Lab for each patch is white L93 a0 b0, grey L4 a0 b0 , black L34 a0 b0 .

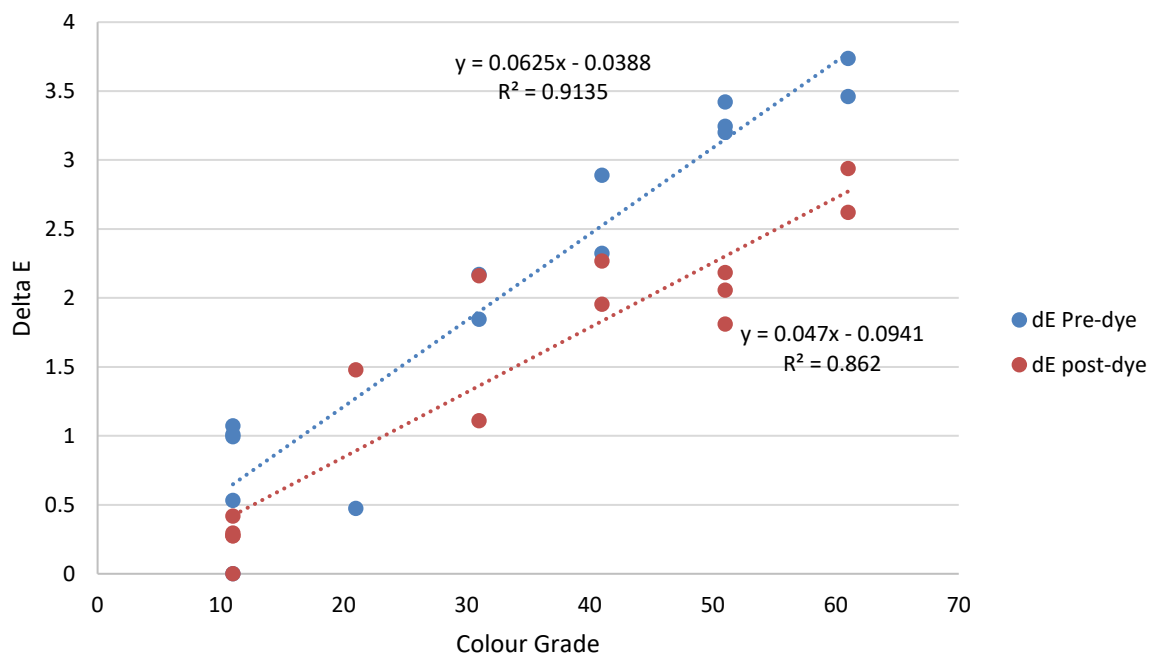
Results

Cotton harvested from the CPR experiment was spun into yarn and tested at CSIRO Geelong. Since samples had been pooled they could not be analysed by ANOVA but were regressed against rainfall data (Appendix 6). Yarn parameters, as outlined above, were not affected by weathering and increasing amounts of rainfall as the relationships for count, twist, unevenness, neps, elongation, break force, thin and thick areas, tenacity and break work all had R² values below 0.32 (R² = coefficient of determination, 1 indicating a very close relationship).

Yarn was then knitted into fabric and dyed with a standard blue colour to detect if weathering and rainfall affected fibre surface properties that would interfere with dye absorption. Appendix 7 summarises Delta E values from the control which was unexposed cotton graded to 11. Exposure of up to 9.5 weeks with 43.8 mm of rainfall did not visibly affect raw fabric colour and appearance. Beyond 11 weeks of exposure and 71 mm of rainfall raw fabric colour became perceptibly different with a Delta E value of 1.84, and increasing to 3.73 at 21.5 weeks of exposure and 203 mm rainfall. Once the fabric had been dyed, the perception in colour

changes was less pronounced but nevertheless present, becoming apparent at 9.5 weeks of exposure and 43.8 mm rainfall (Delta E = 1.47). Delta E varied between 1.11 and 2.93 for dyed fabric from cotton exposed between 11 and 21.5 weeks (71-203 mm rainfall), with more variability in the relationship. This is illustrated in Figure 28 where colour grades 11- 61 represent increasing exposure and rainfall. The relationship between colour grade and perception of colour is very strong for raw fabric ($R^2=0.91$) but somewhat more variable for dyed fabric ($R^2=0.86$).

Figure 28: Relationships between colour grade and perception of fabric colour, pre- and post-dye, for cotton exposed to weathering and rainfall, CPR Experiment, ACRI 2015/16. A Delta E of 1 indicated that a variation in colour is perceivable.



- b) Measure the effect of sooty mould affected and downgraded cotton on yarn/fabric quality (SM)

Methods

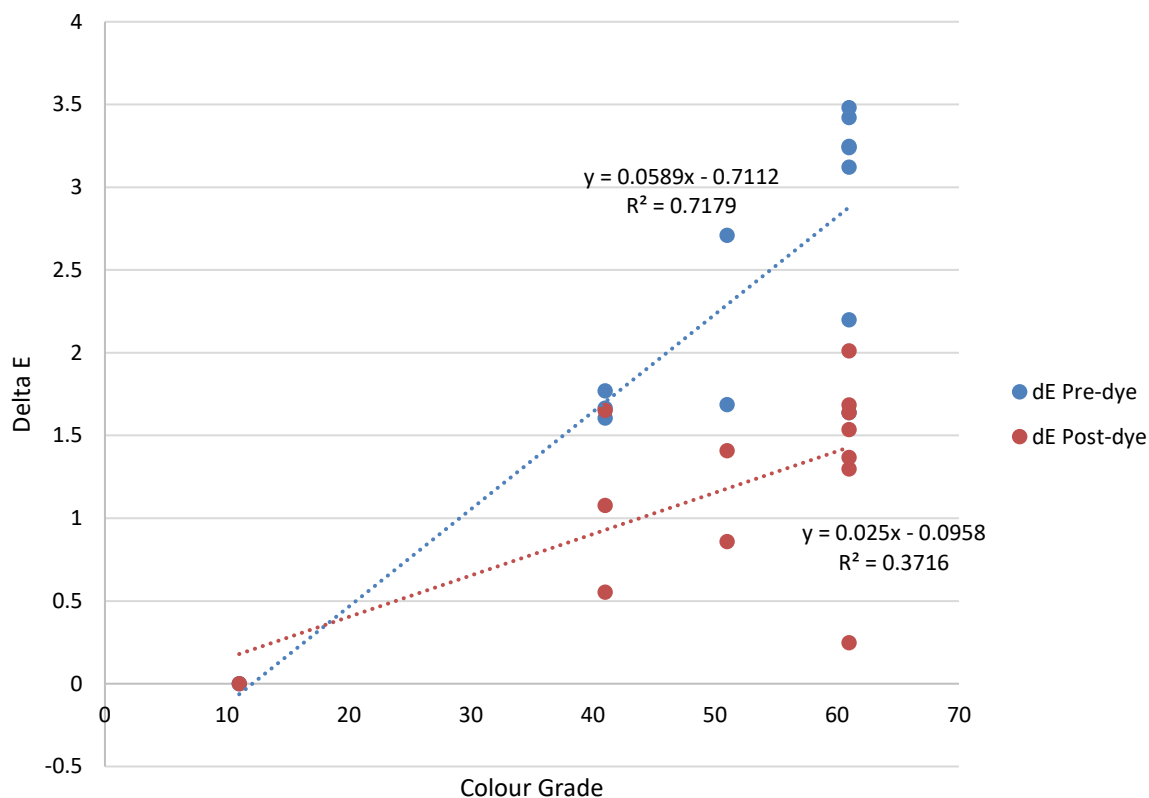
The methodology employed was identical to that described above.

Results

The quality of yarn spun from cotton infested with sooty mould was not negatively affected (Appendix 8) with respect to count, twist, unevenness, neps, elongation, break force, thin and thick areas, tenacity and break work with R^2 values not exceeding 0.28.

The Delta E values for raw fabric made from hand-picked sooty mould cotton graded at 41-61 ranged from 1.6 to 2.7 while those for fabric made from machine picked sooty mould cotton graded at 61 ranged from 2.2 to 3.48 (Appendix 9). However, the relationship between grade and perception of colour for sooty mould affected cotton ($R^2= 0.71$, Fig. 29) was less pronounced than that for rain affected cotton (Fig. 28). For dyed fabric, the Delta E values were highly variable ($R^2= 0.37$, Fig. 29) ranging from 0.5 to 1.65 for fabric from hand-picked sooty mould cotton to 0.24 to 2.01 for fabric from machine picked sooty mould cotton.

Figure 29: Relationships between colour grade and perception of fabric colour, pre- and post-dye, for cotton infested with sooty mould, SM Picking Experiment, ACRI 2015/16



Discussion

Exposure to weathering and cumulative rainfall that caused the deterioration of cotton colour, did not affect yarn parameters. This was to be expected, given that fibre quality parameters were also unaffected. When lint colour grades changed from 11 and 21 to 31, variations in raw fabric colour became perceptible with Delta E values greater than 1. By the time colour grade had deteriorated to 61, Delta E was greater than 3. After dyeing, the relationship between lint colour grade and perception of fabric colour was less strong but remained distinct. While variation in dye absorption of cotton has mainly been ascribed to a combination of fibre maturity and fineness (micronaire) and cotton colour, natural waxes on the surface of cotton fibres and within the primary and secondary walls may also play a role (Gordon *et al.*, 2002, Gordon *et al.*, 2004). Ashraf *et al.* (2014) found that yarns made from cotton with high reflectance values (Rd) and lower yellowness values (+b) give lighter colour depth after dyeing and vice versa. This would describe cotton graded 11-31.

Sooty mould did not affect yarn parameters either but the relationship between cotton colour grade and perception of fabric colour was less distinct than for rain affected cotton. At colour grades of 41, Delta E values were < 2 compared to Delta E values of 2-3 for rain affected 41 graded cotton. Post-dye, the Delta E values for cotton graded at 61 were also much lower in sooty mould cotton. This shows that the relationship between cotton colour and perception of fabric colour is not consistent. The cotton that was affected by sooty mould, was not exposed to rainfall as it was generated in protected conditions within a tent and did not experience the same weathering as the rain affected CPR cotton samples. This would suggest that the mechanisms of cotton discolouration with respect to dyeability can differ. For example, weathered cotton appears greyish which may be due to loss of lustre and reflectance or surface waxes. High lustre in cotton is associated with circularity of cross section (Adderley, 1924) and perhaps cotton that is repeatedly wetted and dried may change shape. Sooty mould cotton is believed to owe its greyish appearance to contamination by black spores, and possibly a

stripping of surface waxes and enzyme action by fungal microorganisms. Irrespective of the reason of cotton discolouration, it can visibly alter the dyeing property of fabric.

Conclusion

Yarn derived from cotton that had been downgraded for colour did not show any quality parameter defects. Differences in fabric colour perception for downgraded cotton were more distinct in raw fabric but became less pronounced after dyeing. When comparing rain exposed cotton and sooty mould affected cotton, the same colour grades did not produce the same degree of colour difference and the relationship was weaker for sooty mould cotton. This indicated different mechanisms of colour degradation which were likely due to lack of exposure to rain for sooty mould cotton. While we were able to show that colour downgrades did not significantly affect yarn quality parameters, we also demonstrated that fabric knitted from downgraded cotton affected the way it held dye. Further investigations into the physical effects of weathering and sooty moulds on fibre surface properties would help to explain the mechanisms at work.

c) Assess the effects of sooty mould on fibre surface properties

SEM Scans (SM)

Since fungi such as *Rhizopus stolonifer* are able to produce cellulolytic enzymes (cellulases) while metabolising sugars, there is a potential risk that they may damage and weaken cotton fibres. The surfaces of sooty mould contaminated cotton lint and clean lint were examined under the Scanning Electron Microscope at the Australian National University (ANC). We were uncertain about effects but imagined some form of surface pitting from cellulolytic action. Samples were prepared by ANC staff and examined at different magnifications. Figure 30 shows magnified sections of cotton lint. Fungal spores (possibly *Cladosporium cladosporioides*) are clearly visible in Figures 30A and 30B, with mycelia and hyphae visible in Figures 30C and 30D. The typical flattened shape and twist of the hollowed fibre can be seen in Figures 30D and 30H. Figures 30F to 30H depict clean control fibres. While the fibre surfaces of both sooty mould contaminated and clean cotton are highly textured, there is no obvious surface pitting or distinguishable surface degradation apparent in the contaminated fibre samples. While SEM allows for high resolution images, the technique could be inadequate for detecting fibre changes as a result of cellulolytic activity. If cellulases from sooty moulds do weaken fibres, we may be able to detect these changes in fibre strength tests.

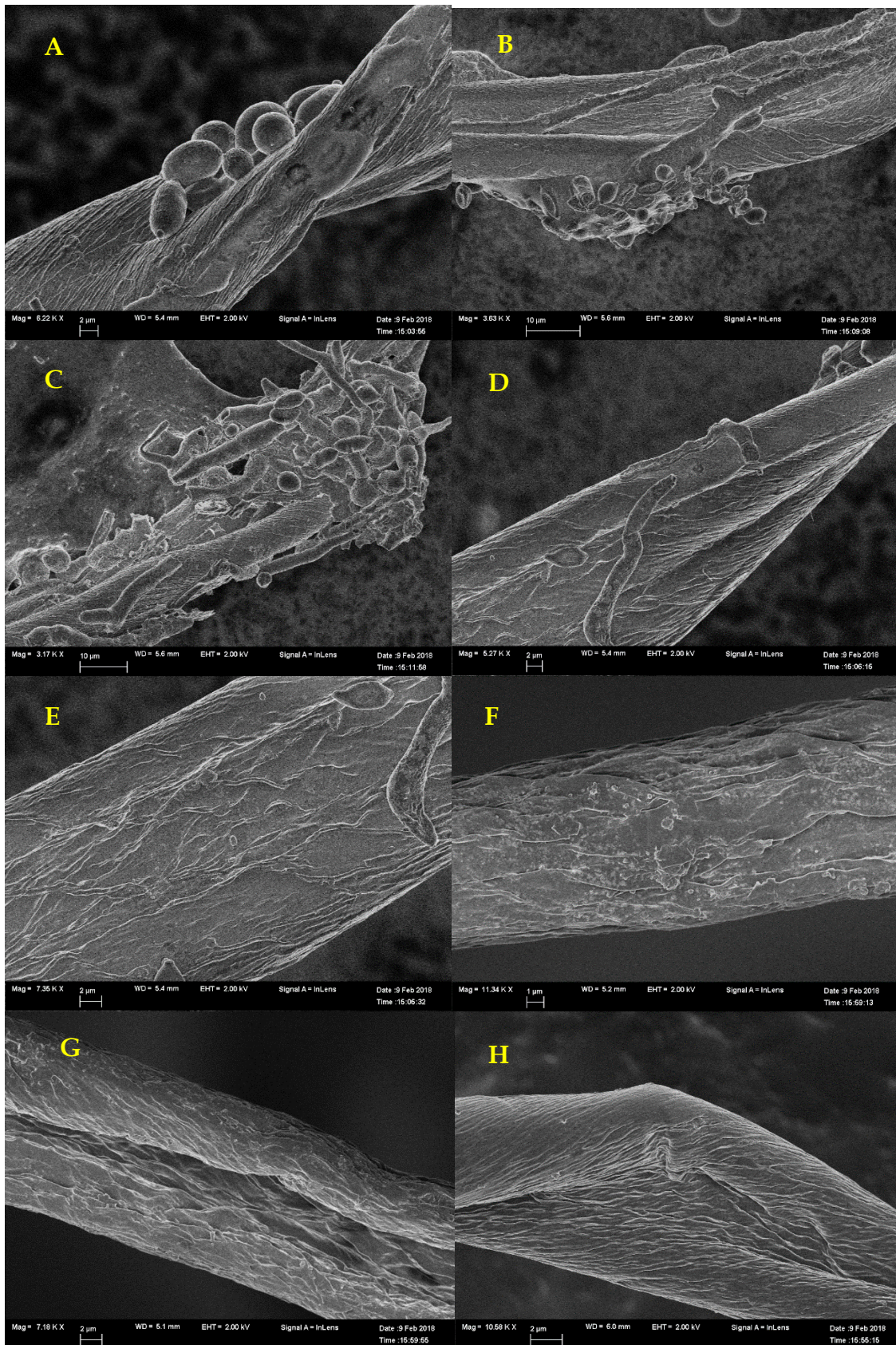


Figure 30: SEM images of cotton fibre: A-E) Sooty mould contaminated cotton fibres with fungal spores; F-H) Uncontaminated cotton fibres.

d) Investigate the effect of cellulases on fibre surface properties
Not accomplished – we have yet to identify cellulases produced by various sooty mould fungi but have so far not managed to isolate different fungal species for this work.

e) Assess the extent to which discoloured, graded cotton can be bleached back to a higher grade

There is a belief in the cotton industry that clean cotton that has suffered colour degradation in the field may be “bleached” back one grade by exposure to moisture and sunlight. In discussions with Andrew Baxter from ACS, Wee Waa, Andrew voiced his opinion that since growing Bollgard II cotton, natural bleaching may only achieve half a grade improvement due to dense canopies. We wanted to assess whether bleaching due to exposure occurred at all.

Methods

We procured graded cotton (41-1) from Andrew Baxter which we divided into eighty 15*15 cm squares. Prior to treatment, cotton colour (Rd and b+) was assessed using the handheld spectrophotometer and by HVI at Myall Vale. Measured spectrophotometer values were the average of three measurements. Forty squares were kept in a dark room as controls while the other 40 were exposed to sunlight with periodic wetting to see if this would produce any bleaching effects (Fig. 31). After 3 days of exposure with daily wetting using a sprinkler, samples were re-assessed. Values for Rd and b+ were analysed by ANOVA.



Figure 31: Graded cotton pads laid out for bleaching

Results and Discussion

Mean values for Rd and b+ of controls and bleached samples are shown in Table 10. There were significant differences in both Rd and b+ for pre- and post-exposure cotton samples in the bleaching treatment. Reflectance fell from 81.84 to 71.16 and yellowness from 5.32 to 4.48. When read against the HVI Colour chart, this represents a drop in colour grade from 41-1 to 51-2. In effect, the exposed cotton weathered to a worse colour grade through the process of

wetting and drying. This response is in line with our results from the CPR experiment in Section 1 and reinforces the detrimental effect of consecutive days of wetting.

Table 10: ANOVA for reflectance (Rd) and yellowness (b+) of bleached and unbleached cotton. (Genstat, 2013)

Rd	Pre-exposure	Post-Exposure
Control	81.84	81.84
Bleached	81.61	79.16*
F (P=0.05)		<0.001
LSD		0.501
df		(1, 156)
b+	Pre-exposure	Post-Exposure
Control	5.32	5.32
Bleached	5.22	4.48*
F (P=0.05)		<0.001
LSD		0.128
df		(1, 156)

Conclusion

It is unlikely that field bleaching in the form of repeated wetting and exposure to sunlight can effectively bleach cotton back to a more favourable grade. This may have implication for late crops that are exposed for extended times to dry sunny weather and cold dewy nights before harvest.

Outcomes

5. Describe how the project’s outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

The planned outcomes of this project included:

- 1) Clarifying the factors that cause discolouration of lint and helping growers to understand how these factors devalue their crop: Information from the weathering and colour post rain experiments clearly shows the relationships between honeydew, rainfall and sooty mould contamination of cotton fibre and provide quantitative effects on cotton colour.
- 2) Identifying the relationship between colour grade and yarn/fabric quality (dyeability) which may increase bargaining power to sell cotton: Yarn properties were not affected by colour downgrades. There was a strong relationship between the colour grades of weathered and rain exposed cotton and the colour shading of dyed fabric. This relationship was less strong for sooty mould affected cotton that was grown in covered conditions.
- 3) Understanding the effects of sooty moulds and *Alternaria* to enable growers to make decisions based on risk evaluation: We understand the field scenarios that increase the risk of sooty mould development. Growers are aware of these scenarios but may not always make the right decisions to avoid honeydew.
- 4) Understanding the relationship between sooty mould spore contamination and grade discounts which may give growers options to mix cotton: The mixing experiment has set up a

baseline for the degree of mould contamination that occurs in cotton bolls, and the proportion of such bolls required in the pick to detect a drop in colour grade.

5) Exploring the role of moulds on the degradation of honeydew to allow growers to assess risks of stickiness or greying: While we have anecdotal understanding of sooty mould feeding on honeydew sugars, we were unable to measure this effect in a more controlled experiment due to the lack of an analytical facility. However, the development of sooty moulds (assessed by scoring) was correlated with a reduction in sugar concentration on bolls in the absence of rainfall.

6) Understanding possible effects of fungi on fibre quality parameters: Sooty mould fungi did not negatively affect fibre quality parameters other than lint colour. Colour grade tends to change due to the presence of black spores rather than inherent changes to the fibre surface as happens during weathering.

7) Identification of effective fungicides against sooty mould and *Alternaria* to increase management options of moulds: We screened a range of fungicides with promising results but need to continue studying their impact and feasibility.

8) Explore the role of yeast in the degradation of honeydew to increase management options of moulds: The experiment with Baker's yeast showed the yeast's ability to metabolise most of the insect honeydew sugars well, except for melezitose, which occurs in aphid and mealybug honeydew.

9) Understanding of early or partial defoliation on canopy conditions, fungal development, crop maturity and quality to increase options for risk management near end of season: Not accomplished in this project.

10) Investigation of novel approaches to the preservation of lint quality: Applying 3M water repellent to bolls in the field did not prevent sooty mould fungi or improve colour grade but made it worse than that of control bolls.

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

No technical advances were achieved.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

There were several new methods developed as outlined in the main text of the report

c) required changes to the Intellectual Property register.

No changes to the IP register are required.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

This project has fulfilled the majority of its objectives and provided knowledge on the honeydew, weathering and fungal factors that affect cotton lint colour and are a challenge to the cotton industry. The project has:

a) thoroughly investigated the influences of extended field exposure and cumulative rainfall on bolls, quantifying the detrimental effects on cotton lint colour,

- b) defined the nature of rainfall events that are most likely to lead to colour changes,
- c) identified the differences between manual and HVI classing with respect to changes in colour grade,
- d) established that sooty mould development is strongly associated with the presence of honeydew sugars,
- e) found that moisture – while not essential to sooty mould growth on honeydew – promoted sooty mould spore development,
- f) observed a reduction in sugar concentration on bolls with increasing levels of sooty mould development,
- g) attempted to define sooty mould contamination though a scoring system is relatively subjective,
- h) established that sooty mould spores on cotton bolls carried through the harvester may contaminate the total pick and cause downgrades if present in sufficient numbers, however, the ginning process may recover lint colour by up to two grades,
- i) determined that between 30-50% of heavily contaminated sooty mould cotton in the pick can drop colour grade from base grade and incur penalties,
- j) tested a number of fungicides of which all commercial fungicides prevented growth of sooty mould spores on plates of PDA (although they are not registered for this purpose), while plant-based and novel fungicides performed poorly,
- k) shown that none of the fungicidal compounds tested had any curative activity on sooty mould though Amistar and Switch inhibited further development,
- l) shown that both Amistar and Switch exhibited preventive effects on fungal development,
- m) found no beneficial effect of water repellents on cotton colour grade,
- n) established that Baker's yeast (but not Brewer's yeast) metabolises honeydew sugars, except for melezitose,
- o) demonstrated a strong relationship between weathered cotton colour grade and dyeability of fabric
- p) shown that the relationship between sooty mould cotton colour grade and dyeability was less robust than that for weathered cotton
- q) not found any visual evidence of surface pitting or possible cellulolytic activity on cotton fibres from sooty mould enzymes using SEM technology and
- r) found that contrary to popular belief that exposure to sunlight and wetting can bleach back colour grade, the colour of exposed samples deteriorated further.

The research in this project has objectively investigated and quantified the relationships between the honeydew, weather, and fungal components of cotton lint discolouration. This information has added to the knowledge we have about cotton quality and has laid the foundation for further studies into the management of these components. Growers can use this information to make decisions about how long a crop can stay in the field or how much rainfall it can sustain before cotton colour starts to deteriorate. The sooty mould mixing experiment set a baseline for the proportion of heavily contaminated cotton bolls in the pick (the lower third or half of the crop) that would likely result in colour discounts, and growers can use this information as a guideline for management decisions. The research also re-enforces the critical effect of honeydew in mould formation and discolouration of lint, placing emphasis on sound whitefly management to prevent honeydew. Even residual amounts of honeydew will sustain sooty mould growth which, due to its persistence, cannot be remedied. Therefore, any future investigations should focus on its prevention. While there is a range of fungicides that can prevent sooty mould formation – and growers may call for use of these products - there would

be issues regarding registration, environmental impact, pre-harvest withholding periods, inherent effects on lint, penetration into the canopy (as there is with SLW insecticides) and application costs.

The take home messages are:

- To grow non-sticky and clean cotton, whitefly management needs to be spot on, which can be achieved using IPM principles.
- If someone manages their whiteflies poorly, has stickiness issues, and ends up with sooty mould – the penalty due to discoloured cotton *only affects the individual grower*.
- However, if someone's sticky cotton carries through the gin without being picked up until spinning, that individual may escape stickiness and colour discounts - but the penalty *may end up applying to the entire industry*.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

Any techniques developed during this project will serve us in the follow-up project CSP1901 “Reducing the impact of weather, insects and microbes on cotton colour”. We can also build on or modify methodologies to accomplish our objectives, e.g. pathology work to screen fungicides.

(b) for the future presentation and dissemination of the project outcomes.

Aspects of this work have already been published and presented at conferences and grower/consultant meetings. I am planning to produce two to three articles for The Australian Cotton Grower and Spotlight Magazine to summarise and extend the findings in this report. Information may also be presented at the CCA Meeting in June.

(c) for future research.

The research accomplished here provided the foundation for a 3-year follow up project, CSP 1901 “Reducing the impact of weather, insects and microbes on cotton colour”, that collaboratively investigates the options of field-based mitigation measures (with Dr. Stuart Gordon). Its focus is on management solutions to prevent lint discolouration and the screening of compounds that may mitigate colour effects. Components of the project include:

- 1) Assess crop management strategies that could lead to better whitefly management to avoid honeydew, e.g. planting density – to enable better chemical penetration into the canopy, defoliation – to reduce whitefly populations earlier, chemical pest management options.
- 2) Continue to screen fungicidal compounds both to prevent microbial action in the field and in modules, identifying optimal application opportunities.
- 3) Continue studies into the effects of sooty mould on sugar degradation
- 4) Assess mitigation treatment effects on cotton quality

**9. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)**

Conferences

1. Heimoana, S. (2018). Indirect whitefly/plant interactions: honeydew related factors affecting cotton lint quality and options for mitigation. In: International Whitefly Symposium, September 2018, Fremantle.
2. Heimoana, S. and Nott, M. (2018). Honeydew, sooty mould and fungicides. Three-minute thesis. In: 19th Australian Cotton Conference, August 2018, Broadbeach. Cotton Research and Development Corporation.

Extension

1. Heimoana, S. and Nott, M. (2018). The impact of sooty mould contamination on cotton downgrades. *The Australian Cotton Grower* 39(5):44-45.
2. Heimoana, S. and Wilson, L. (2017). How much rain is too much? The impact of rainfall on cotton colour grade. *The Australian Cotton Grower* 38(1):28-30.
3. Heimoana, S. and Wilson, L. (2015). Rain the best medicine for honeydew contamination. *The Australian Cotton Grower* 36(1):49-51.

Other extension activities

1. CottonInfo Webinar on sticky cotton, March 2019.
2. Heimoana, S. (2019). Information session on whitefly, honeydew and stickiness issues. CSIRO internal seminars, ACRI, Narrabri, February 2019.
3. Heimoana, S. (2016). Honeydew and sooty mould update on cotton. In: Cotton Consultants Australia Whitefly Seminar; December 2016; Moree. Cotton IPM Project.
4. Heimoana, S. (2015). IPM in cotton, it's a sticky business. CSIRO internal seminars, Queensland Bioscience Precinct, St. Lucia, August 2015.
5. Active participation in current sticky cotton issues, provision of data for CottonInfo newsletters.

B. Have you developed any online resources and what is the website address? No

Part 4 – Final Report Executive Summary

A two year project that investigated the influences of weathering, insect honeydew and fungal agents on cotton colour grades, yarn and fabric quality parameters provided a better understanding of these factors and the conditions under which they occur. Extended weathering in the field, particularly with successive rainfall events totalling 100 mm, was detrimental to cotton colour, reducing colour from base grade to 41 and beyond. In both years of the project this equated to 10 weeks in the field from the time of first open boll. Moist, overcast conditions for 4 -7 days were usually followed by a drop in colour grade. Short, sporadic rainfall events followed by sunshine did not cause colour degradation. The humidity they provided could, however, further the development of sooty mould fungi which grew on honeydew contaminated cotton. While sooty mould fungi can grow on the natural sugars of immature fibres in the absence of moisture, both the presence of insect honeydew and rainfall strongly promoted their development, reducing cotton colour from base grade to 41 to 71. The magnitude of the colour drop depended on the amount of black spores on the boll surface. Increasing development of sooty mould fungi was also correlated with a reduction in sugar concentration on bolls, providing some evidence of the ability of fungi to remove sugar from honeydew contaminated cotton. Sooty mould spores in cotton fields are rarely just blown out during harvesting. It was found that spores strongly adhere to open bolls and survive through the harvesting process to worsen the colour grade of the harvested lint. However, one or twocolour grades may be restored through the ginning process. Between 30-50% of heavily contaminated sooty mould cotton in the total pick can drop colour grade from base grade to incur penalties. Colour grade, while not showing effects on lint or yarn quality in these experiments, was strongly related to dyeability of fabrics. Cotton colour degraded through weathering and rainfall affected dyeability more than colour degraded through sooty mould fungi and this was most likely associated with the lower moisture levels received by the sooty mould cotton. SEM scans did not show obvious surface changes due to sooty mould activity.

At this stage options for mitigation of the degradation of colour grade are not available. There is no mitigation for rainfall and growers already aim to harvest their crop as timely as possible to avoid weathering and colour degradation. Agronomic and management practices are being investigated in a continuing project. As colour degradation due to sooty mould development largely depends on the presence of sugars in the form of insect honeydew, the most important goal a grower should have is to avoid insect pests such as whiteflies, aphids and mealybugs. This can be achieved through sound, IPM guided crop management using IPM compatible insecticides to control early season pests such as mirids in order to preserve beneficials that later on help with management of Silver leaf whitefly (SLW). Should conditions conspire, and a crop is sticky without rainfall in sight, a range of scenarios may be managed with suitable knockdown insecticides which may reduce SLW numbers until defoliation is complete. On the other hand, if rainfall is in sight, sticky cotton can benefit from this but incurs the risk of sooty mould development. Fungicides tested in this project have the potential to prevent sooty mould development, however, none are currently registered for use on cotton (other than as seed treatment). If registration becomes an option in the future, many parameters would have to be considered including environmental impact, pre-harvest withholding periods, inherent effects on lint, application efficiency and cost. Further research into mitigation options are being addressed in CSP 1901“Reducing the impact of weather, insects and microbes on cotton colour”, a collaboration between Dr Simone Heimoana (Simone.Heimoana@csiro.au) and Dr Stuart Gordon (Stuart.Gordon@csiro.au). Management of honeydew related cotton discolouration is up to the individual grower and while sooty mould tends to affect the individual grower with colour discounts, sticky cotton has the potential to penalise the entire industry.

Part 5 – Acknowledgements

I would like to acknowledge the dedication and input of our research team who has spent long hours in sticky fields and pinning bolls into canopies. Also, thanks to Matt Nott for his efforts in measuring cotton colour.



The current IPM Team:

Simone Heimoana, Tanya Smith, Ammie Foster, Dee Hamilton, Tianne Parker, Matt Nott and Lewis Wilson (retired)

We also want to acknowledge the efforts of staff who temporarily supported us each season: Zane Stahn, Blake Hilderson, Josh Baker, Mark Laird, Giulio Heimoana and others.

We thank our collaborators for providing analytical services which we could not access locally: Dr. Michael O'Shea (BSES, Brisbane), Dr. Anne Rae and Dr. Donna Glassop (CSIRO, QBP, St. Lucia), Dr. Michael Priest (NSW DPI), Dr. Rob Long and the Cotton Fibre Team at CSIRO, Geelong, Dr. Filomena Pettolino and Dr. Rosemary White, CSIRO, Canberra, ANU Microscopy Unit and Andrew Baxter, Australian Cotton Classing Services, Wee Waa.

For financial support we are grateful to the Cotton Research and Development Corporation for sponsorship of this project.

References

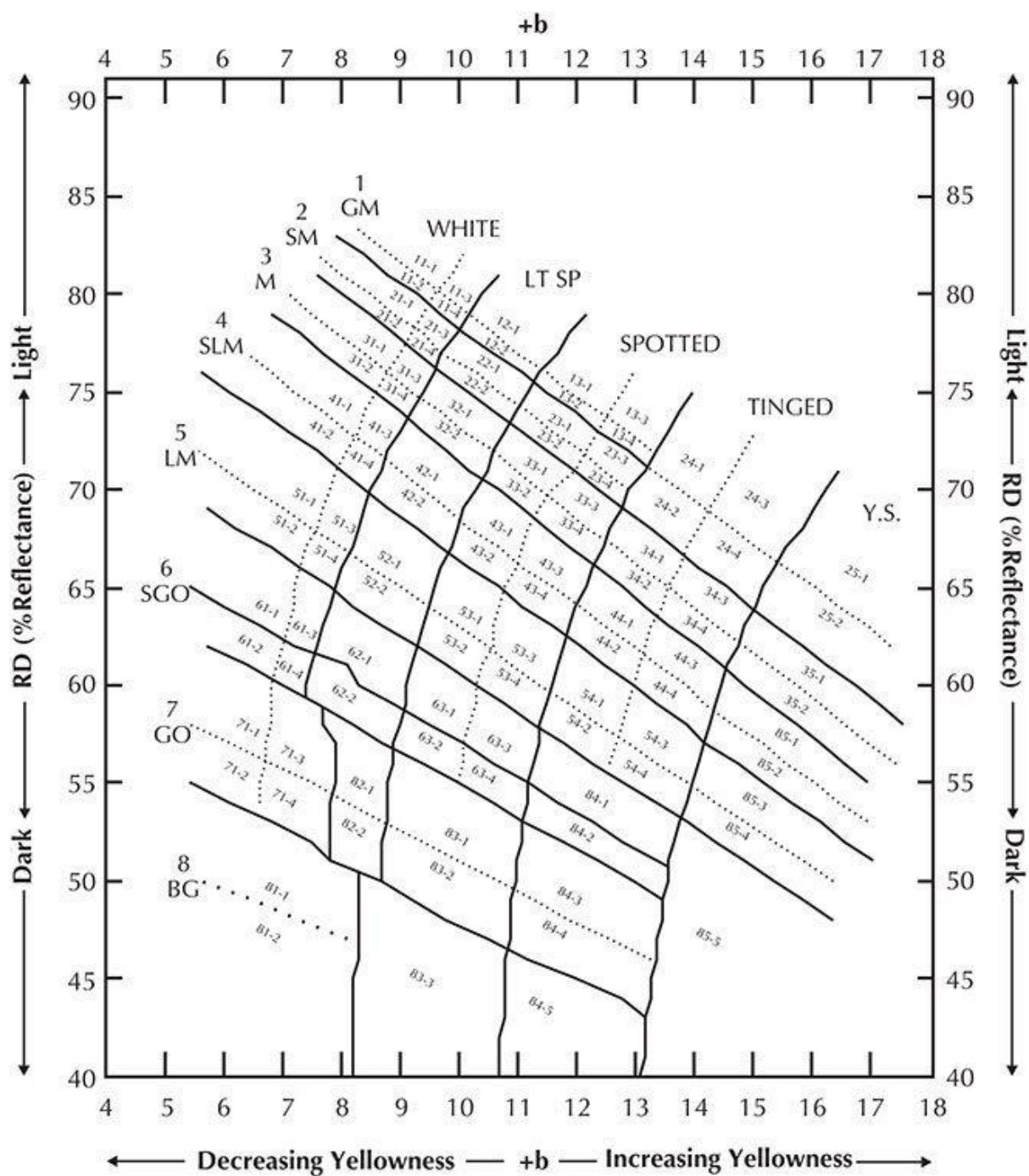
Adderley, A. (1924). The physical causes of the lustre in cotton. *Journal of the Textile Institute Transactions*, 15(4), T195-T206.

Ashraf, U., Hussain, T. and Jabbar A. (2014). Effect of cotton fiber and yarn characteristics on colour variation in woven fabric dyed with vat dyes. *The Journal of the Textile Institute*, 105(12), 1287-1292.

Gordon, S., Church, J. and Evans, D. (2002). Cotton wax in Australian cotton. *The Australian Cottongrower*, 23(5):16-18.

Gordon, S, van der Sluijs, M. and Prins, M. (2004). Quality issues for Australian Cotton from a mill perspective. CSIRO.

APPENDIX 1: Cotton HVI Colour Chart and fibre properties



Micronaire – fibre thickness ranges

≥ 5.3	G7
5.0 – 5.2	G6
3.5 – 4.9	G5
3.3 – 3.4	G4
3.0 – 3.2	G3
2.7 – 2.9	G2
2.5 – 2.6	G1
≤ 2.4	G0

Strength is expressed in terms of grams force per tex with the following classifications:

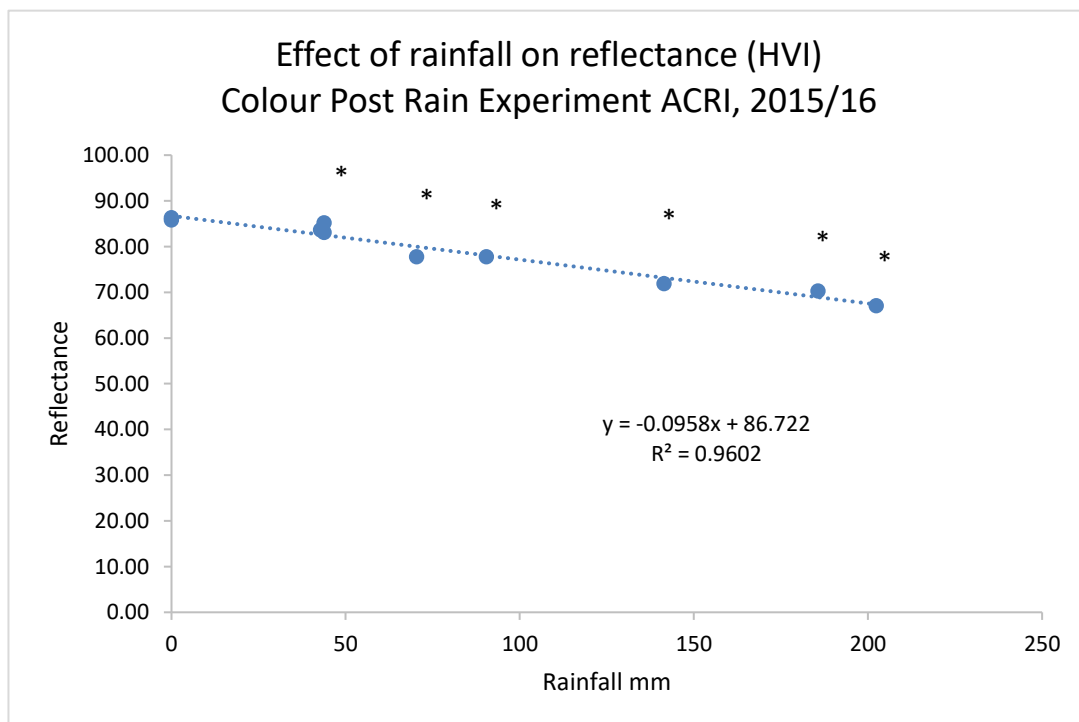
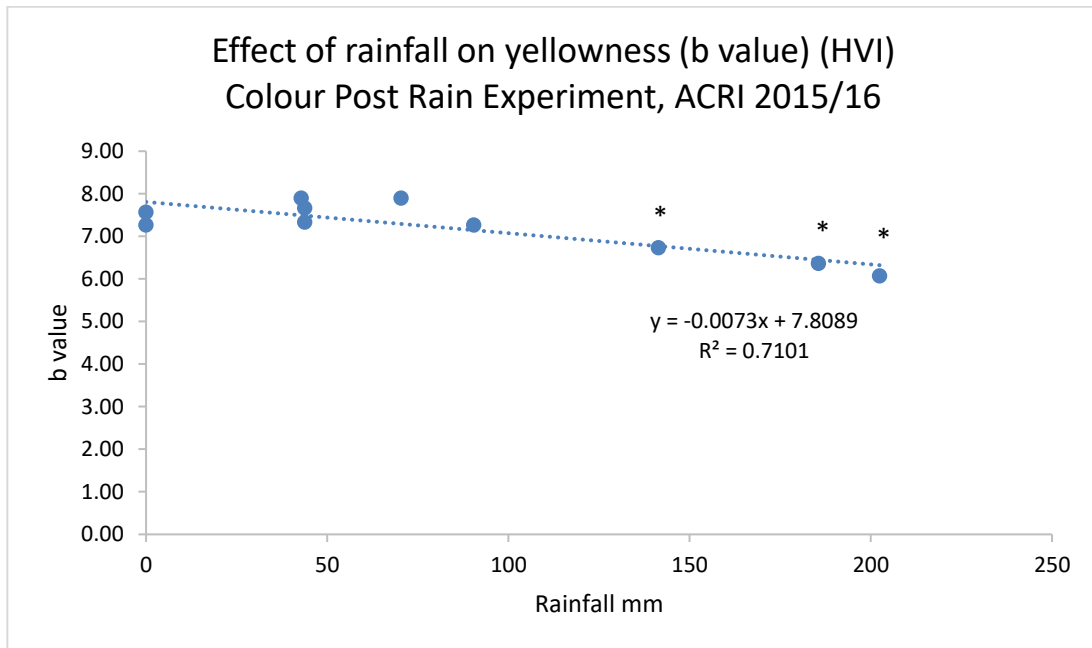
- ≤ 23 , weak;
- 24 - 25, intermediate;
- 26 - 28, average;
- 29 - 30, strong (most current Australian varieties); and,
- ≥ 31 , very strong.

UPLAND LENGTH CONVERSION CHART

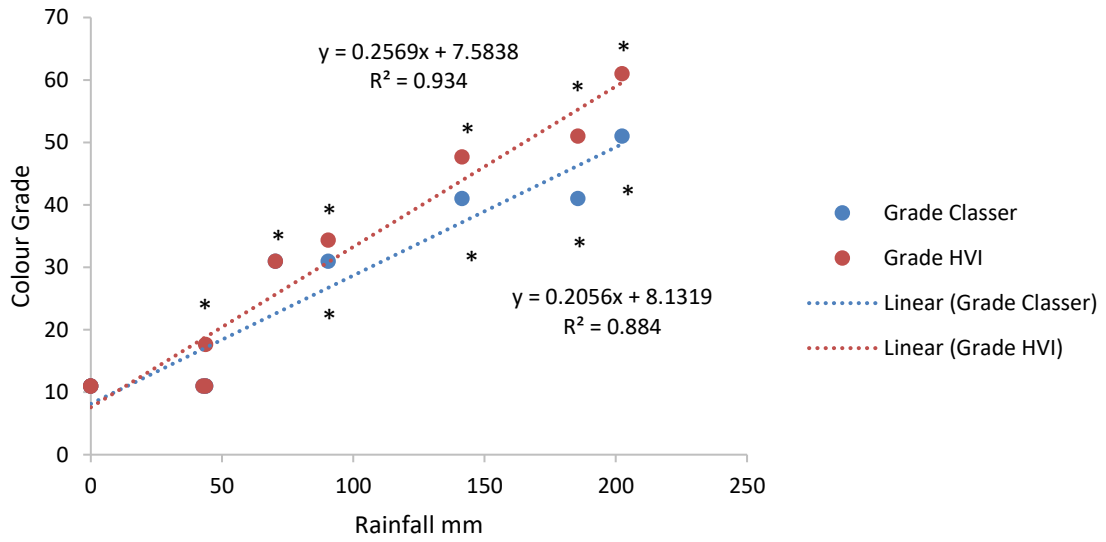
Length (32nds)	Length (inches)	Length (32nds)	Length (inches)
24	0.79 & shorter	36	1.11 – 1.13
26	0.80 – 0.85	37	1.14 – 1.17
28	0.86 – 0.89	38	1.18 – 1.20
29	0.90 – 0.92	39	1.21 – 1.23
30	0.93 – 0.95	40	1.24 – 1.26
31	0.96 – 0.98	41	1.27 – 1.29
32	0.99 – 1.01	42	1.30 – 1.32
33	1.02 – 1.04	43	1.33 – 1.35
34	1.05 – 1.07	44 & +	1.36 & +
35	1.08 – 1.10		

APPENDIX 2: Colour Post Rain Experiment 2015/16 Quality Parameters

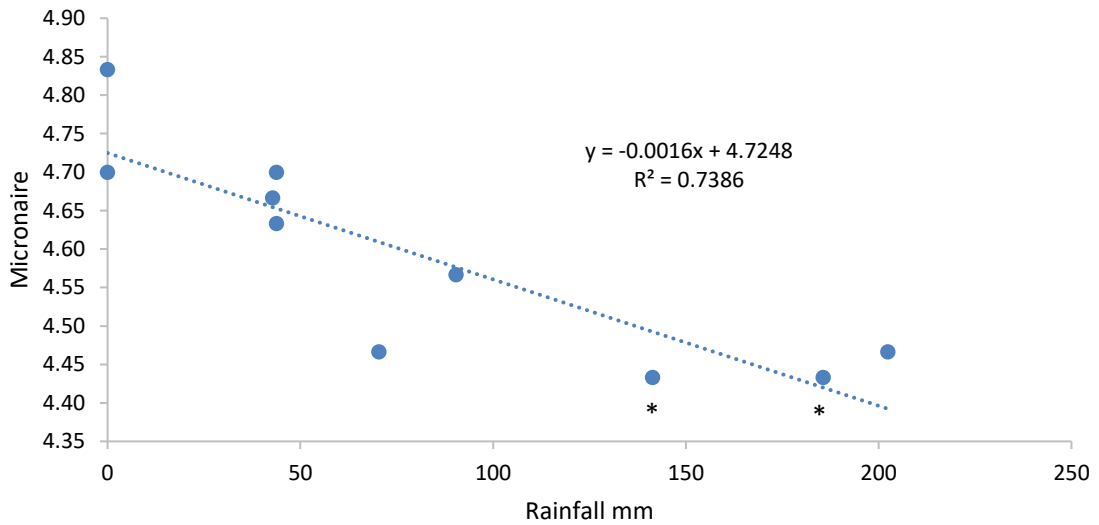
HVI Fibre Test Results - Australian Cotton Classing Services, Wee Waa

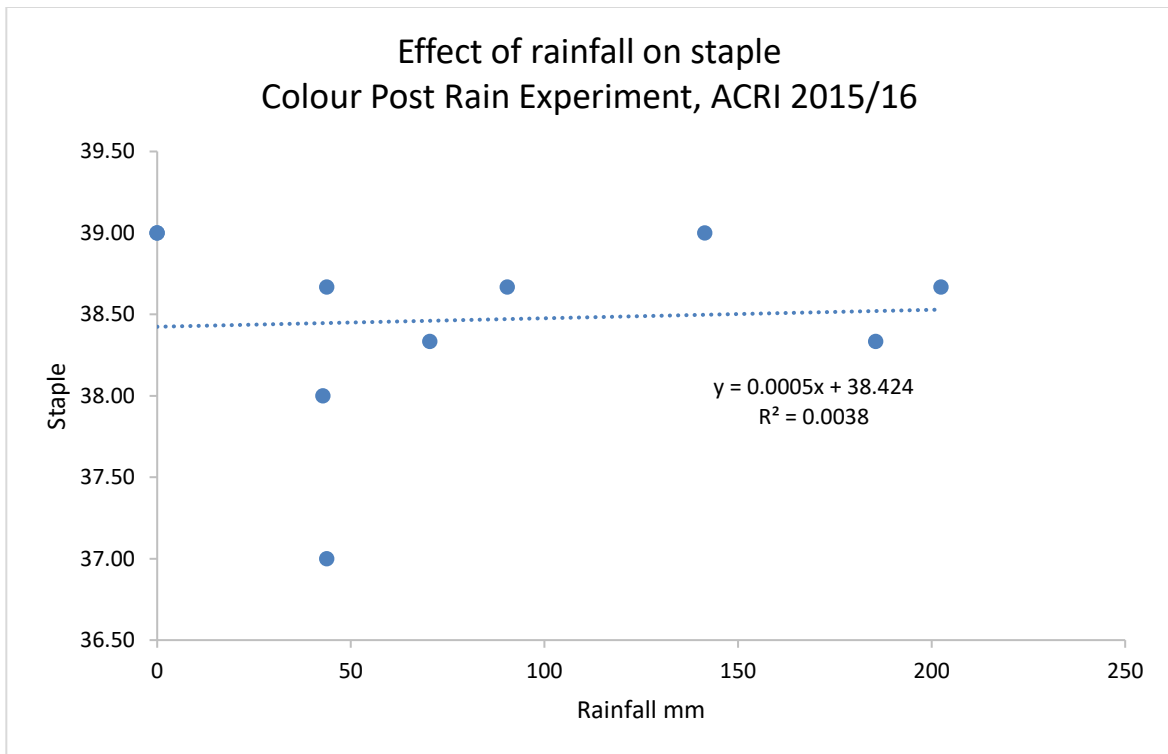
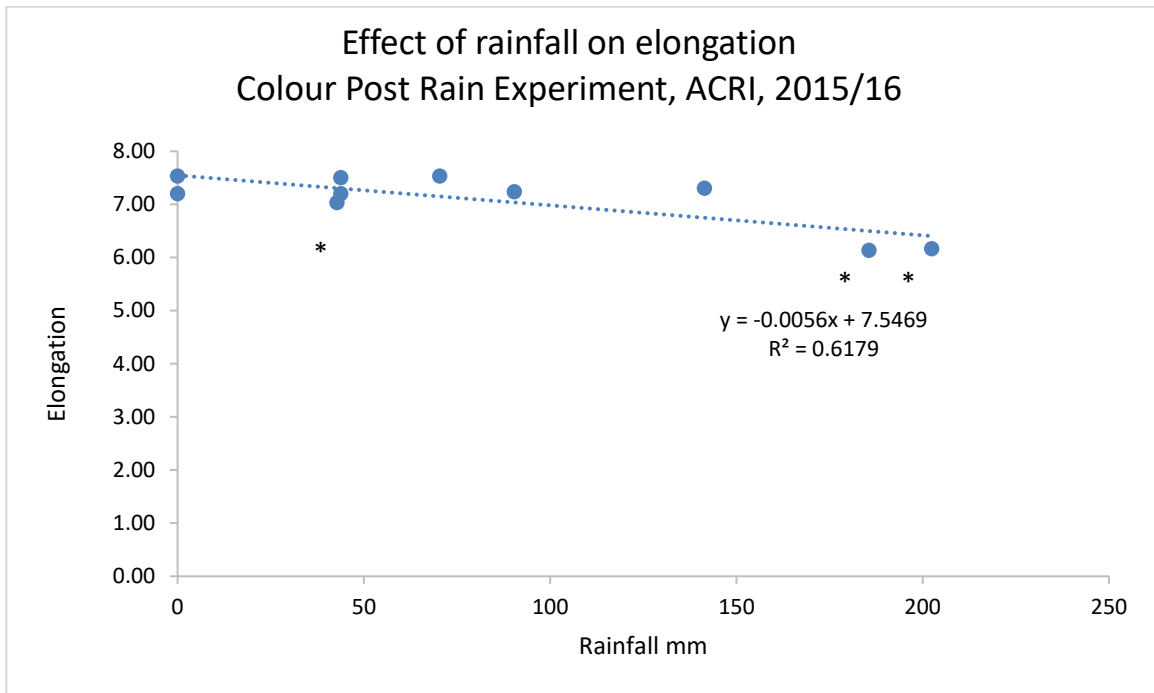


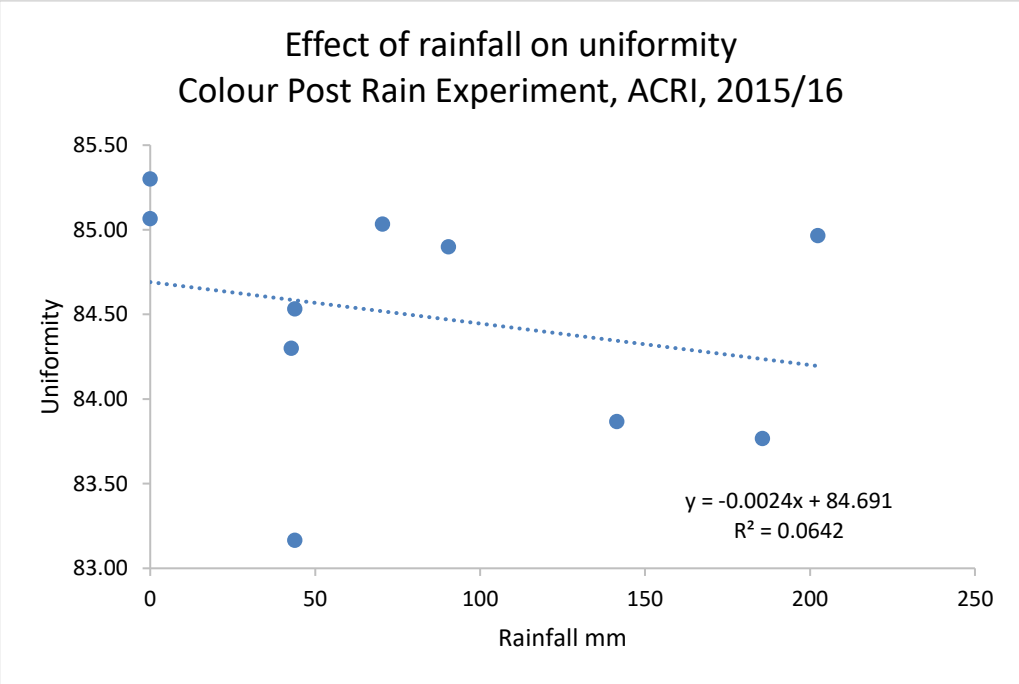
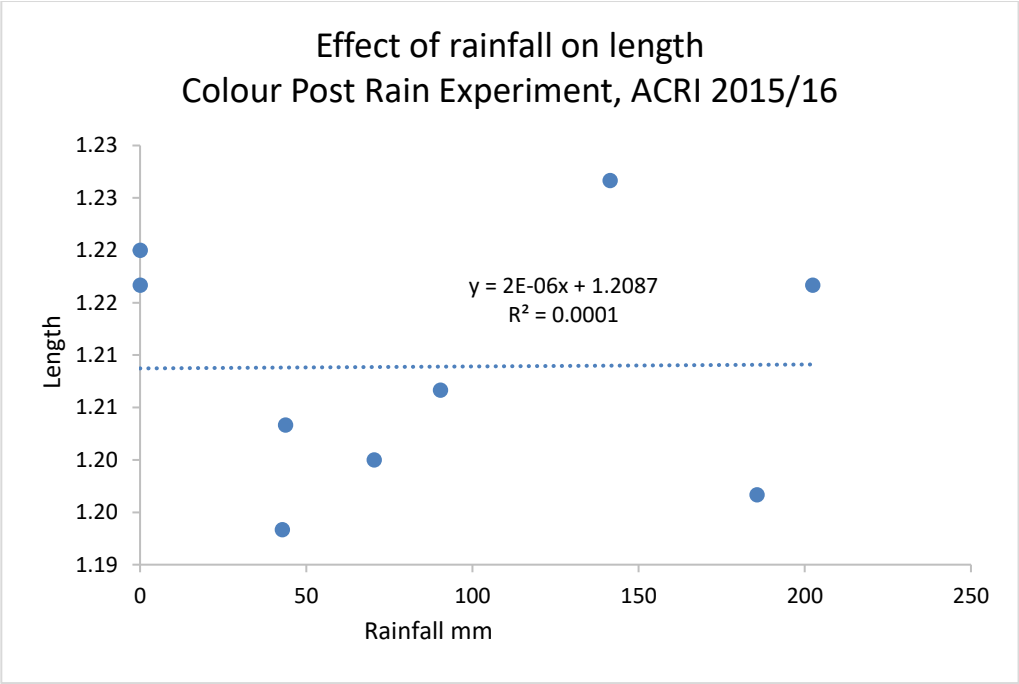
Effect of rainfall on colour grade Colour Post Rain Experiment, ACRI 2015/16



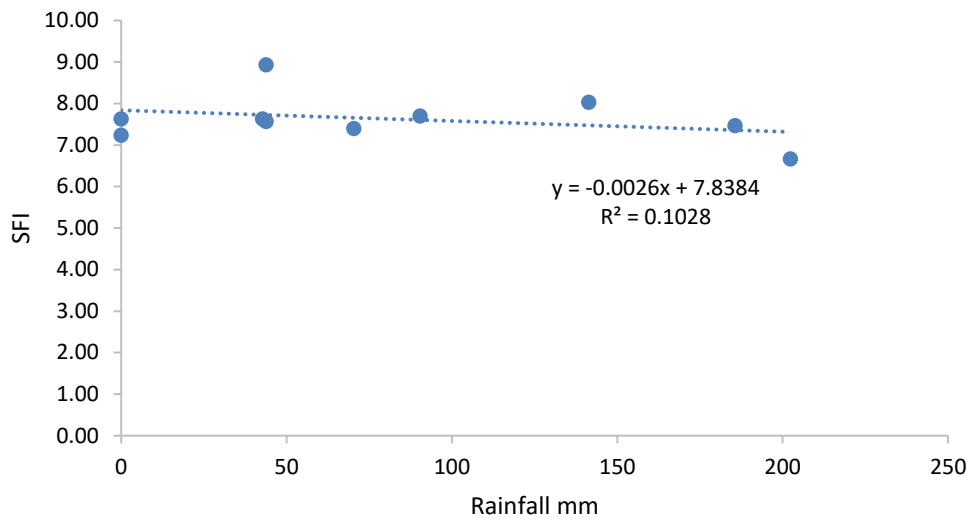
Effect of rainfall on micronaire Colour Post Rain Experiment, ACRI 2015/16







Effect of rainfall on SFI
Colour Post Rain Experiment, ACRI 2015/16



APPENDIX 3: Fungicide Screen on sooty mould infected bolls - Curative

Day 0 - pre-fungicide application (top), Day 4 - post fungicide application (middle) and Day 9 - post-fungicide application (bottom)

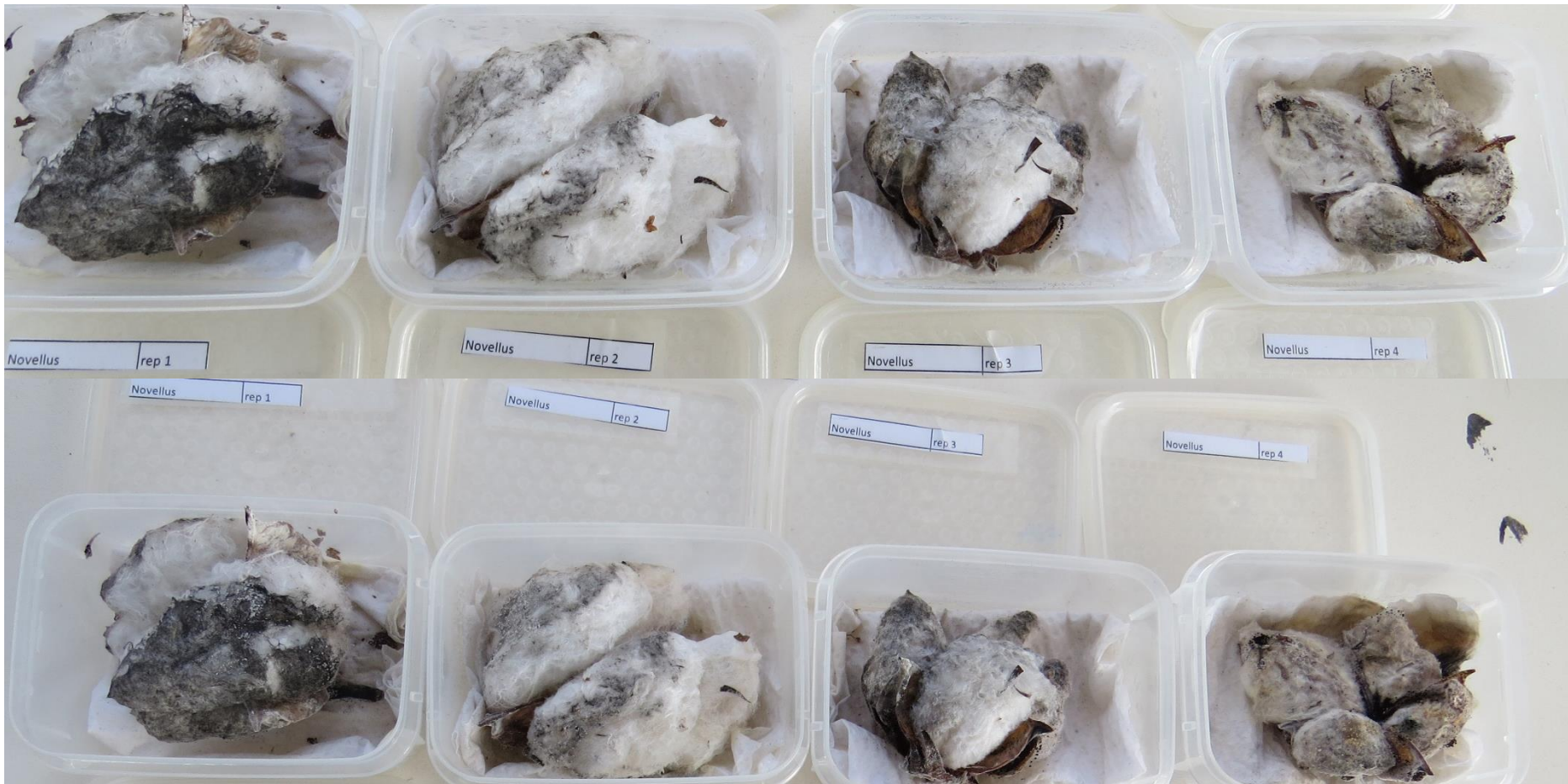
Control



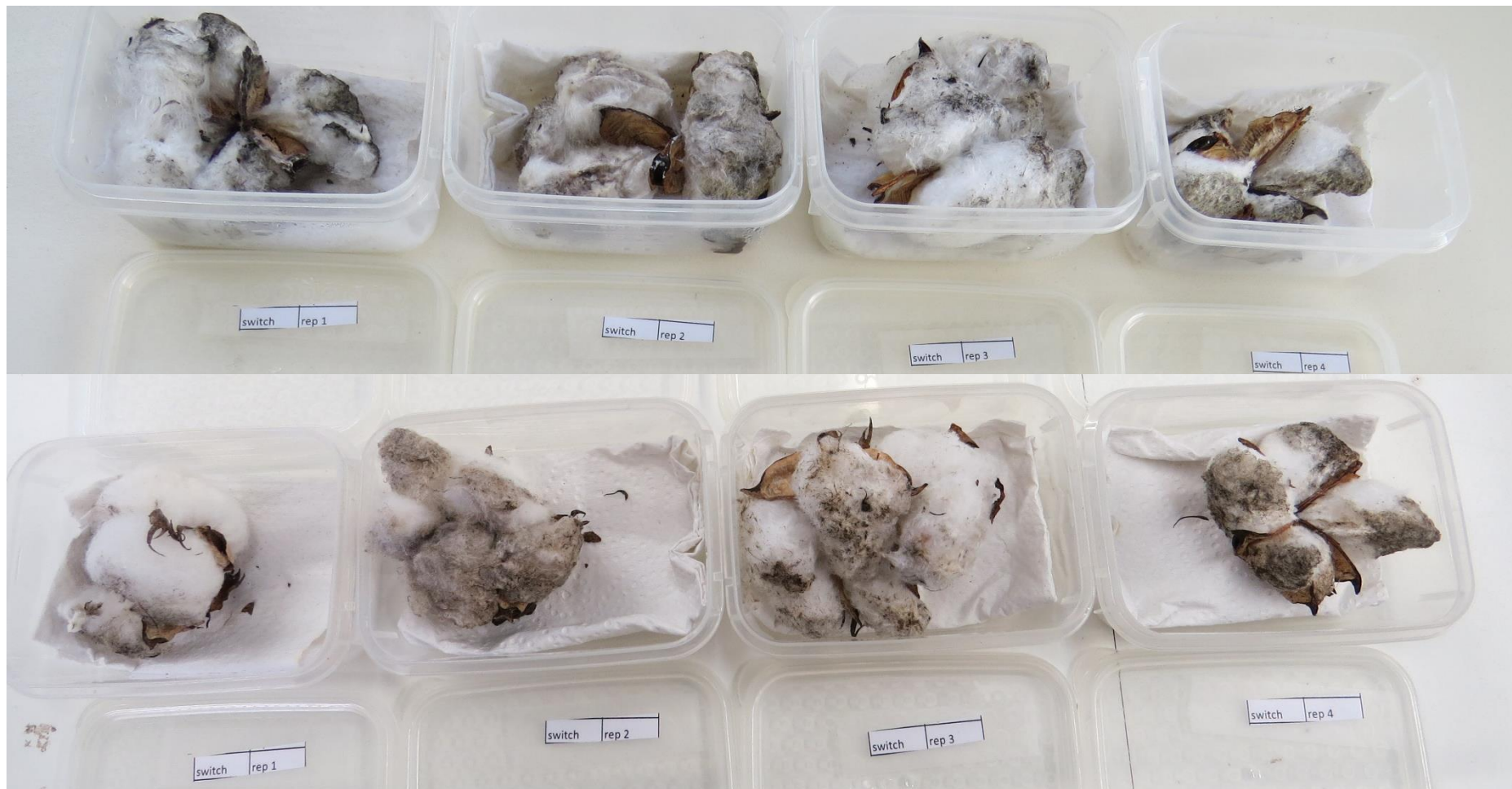


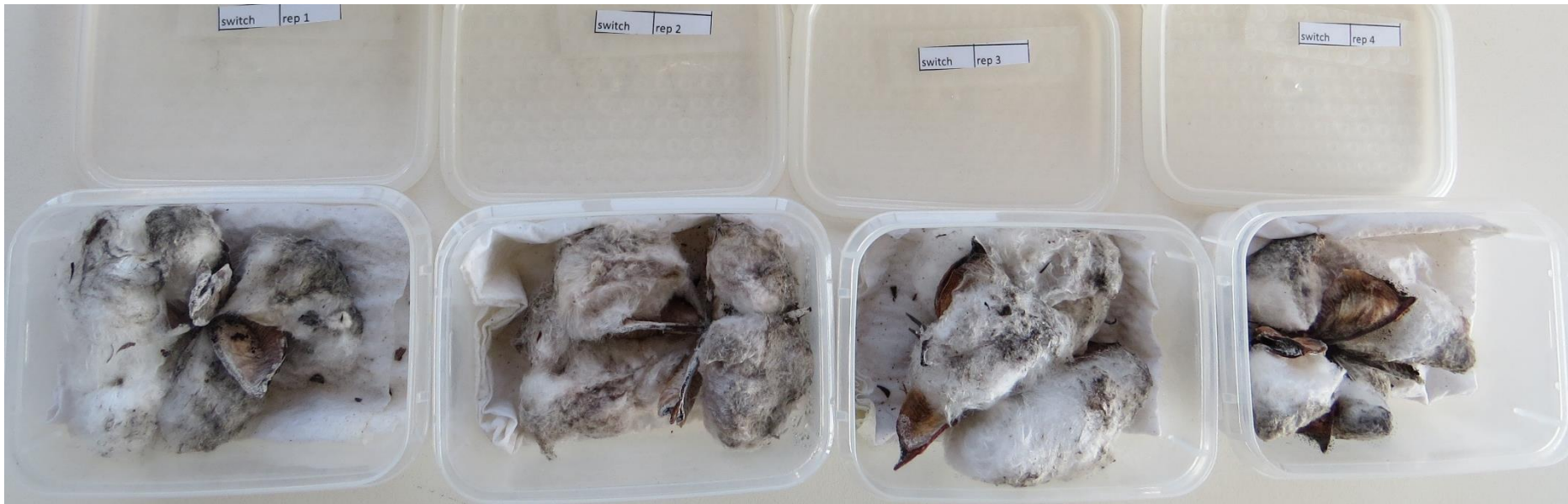
Novellus





Switch



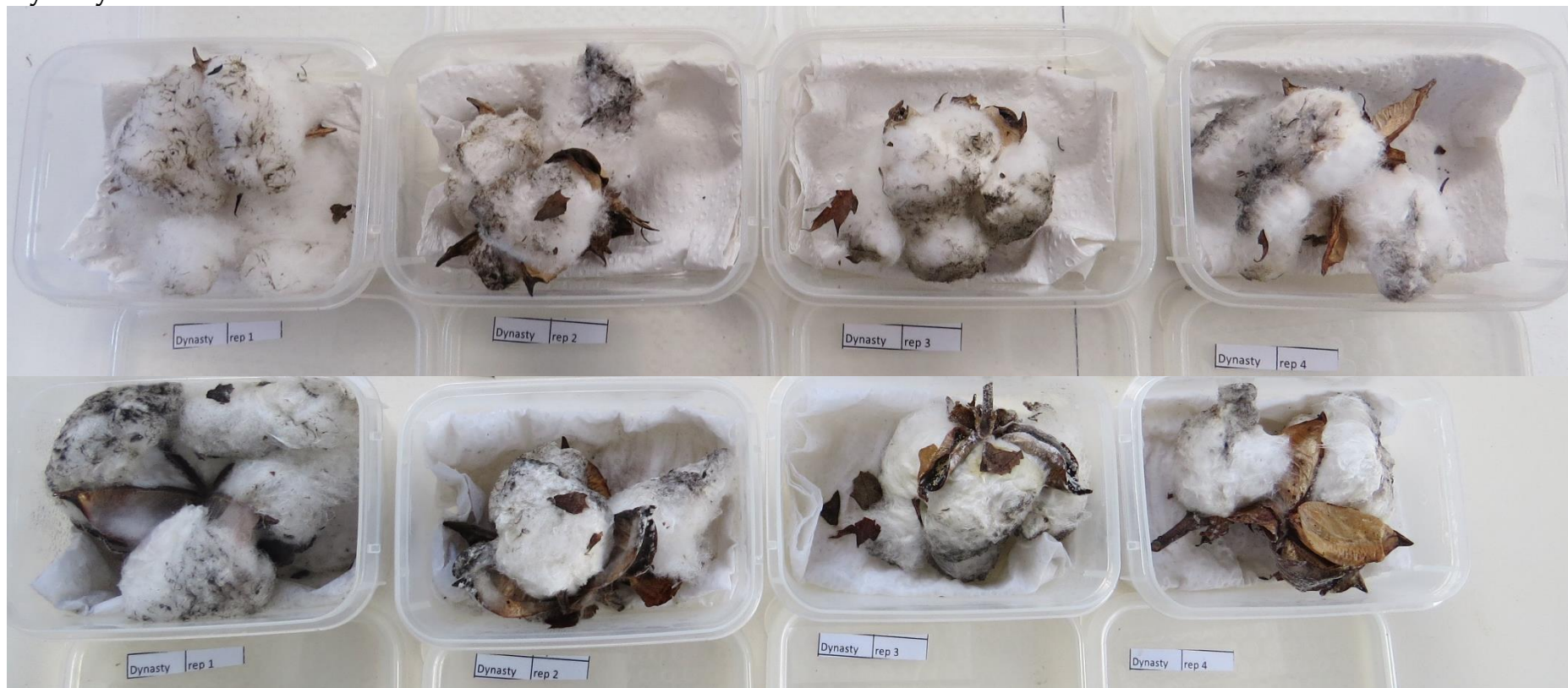


Amistar





Dynasty





African Pepper





Foliar Concentrate





Day 0 photos for antifungal polymer and Switch were corrupted and would not open - only Day 4 and Day 9 photos are shown

Antifungal Polymer





Pristine





APPENDIX 4: Fungicide Screen on sooty mould infected bolls - Preventive

Day 0 - pre-fungicide application (top), Day 4 - post fungicide application (middle) and Day 9 - post-fungicide application (bottom)

+ve Control





-ve Control





Novellus



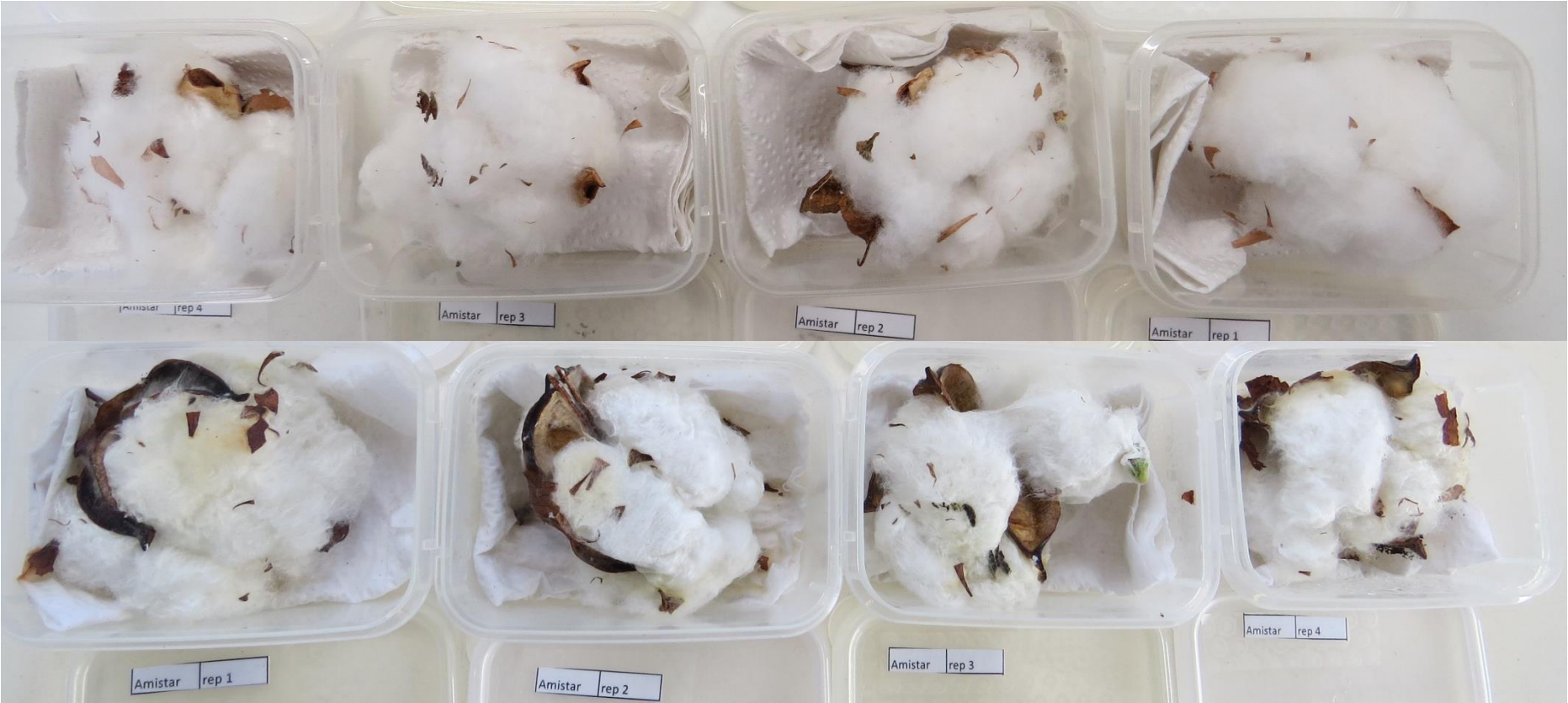


Switch





Amistar





Dynasty





African Pepper





Foliar Concentrate





Antifungal Polymer





Pristine





APPENDIX 5: Identification of sooty mould fungi

From: michael.priest@industry.nsw.gov.au [<mailto:michael.priest@industry.nsw.gov.au>]

Sent: Wednesday, 6 July 2011 11:11 AM

To: Heimoana, Simone (PI, Myall Vale)

Subject: Re: Sooty Mould samples

Simone,

Results are as follows. The fungi were isolated from visibly affected fibres which were cut into small lengths and placed onto Acidified Potato Dextrose Agar and incubated at 25 Degrees Celsius and examined after 3-5 days. The Sooty Mould & Honeydew samples were not plated but identified from the substrate.

B2- Microorganism checks

W1: Cladosporium cladosporioides, Rhizopus stolonifer

W2 Cladosporium cladosporioides, Rhizopus stolonifer and Fusarium chlamydosporum (trace only)

W3: Cladosporium cladosporioides, Rhizopus stolonifer

W4: Cladosporium cladosporioides, Rhizopus stolonifer

B18- Microorganism checks

Aphid 09/03/11: Rhizopus stolonifer

Aphid 16/03/11: Cladosporium cladosporioides, Rhizopus stolonifer, Fusarium chlamydosporum (trace)

Aphid 23/04/11: Cladosporium cladosporioides, Rhizopus stolonifer, Alternaria tenuissima (trace)

Aphid 30/03/11" Cladosporium cladosporioides, Rhizopus stolonifer, Fusarium chlamydosporum (trace)

Aphid 05/04/11: Cladosporium cladosporioides, Rhizopus stolonifer, Fusarium chlamydosporum (trace)

Honeydew & Sooty Mould

Rhizopus stolonifer, Cladosporium cladosporioides, Aspergillus flavipes, Eurotium chevalieri

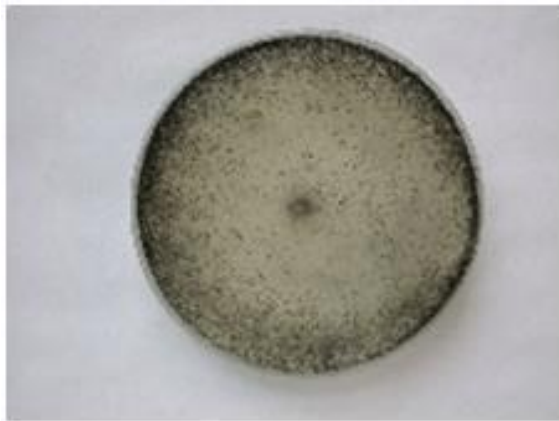
Michael

Dr. Michael Priest
Curator Plant Pathology Herbarium (DAR)
Industry and Investment NSW
Orange Agricultural Institute
Locked Bag 6006, 1447 Forest Road
Orange NSW 2800, Australia
Ph: +61 2 6391 3985 Fax: +61 2 6391 3899
email: michael.priest@industry.nsw.gov.au

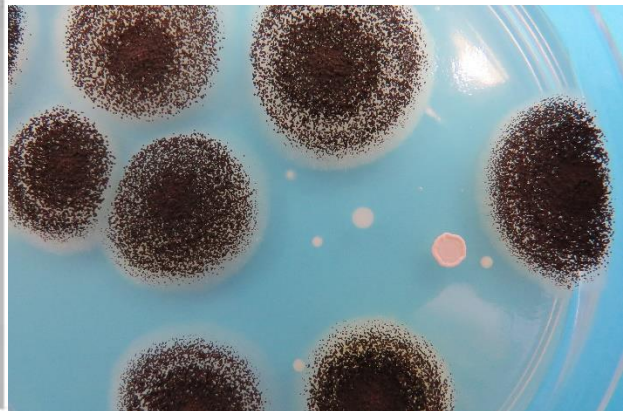
APPENDIX 5 Cont.: Dark spores of fungal species identified from cotton lint



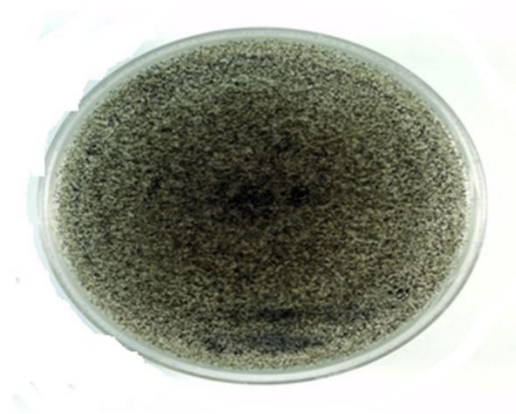
Cladosporium cladosporioides on PDA (dark green to black)



Rhizopus stolonifer on PDA (black spores)



Unidentified fungus on PDA



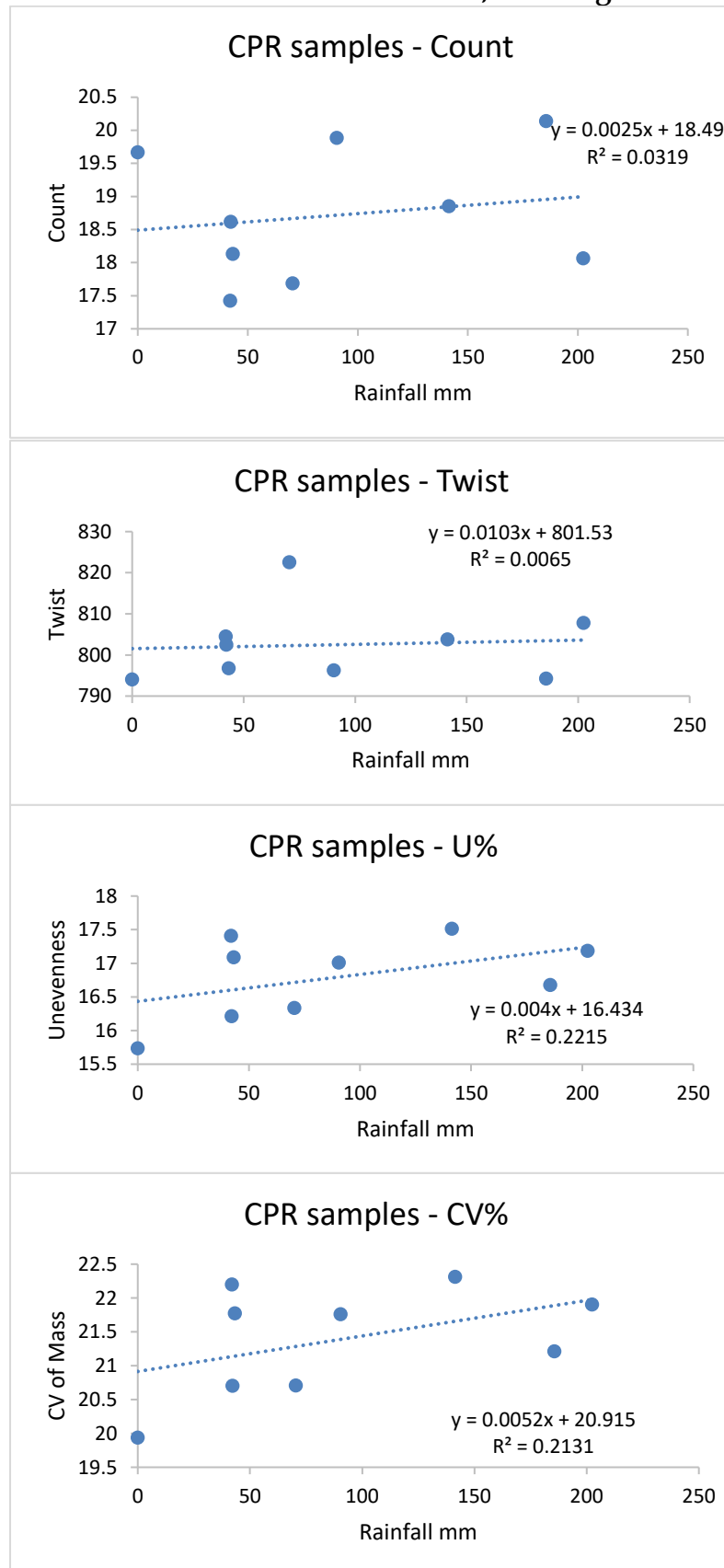
Aspergillus niger on PDA (black spores)

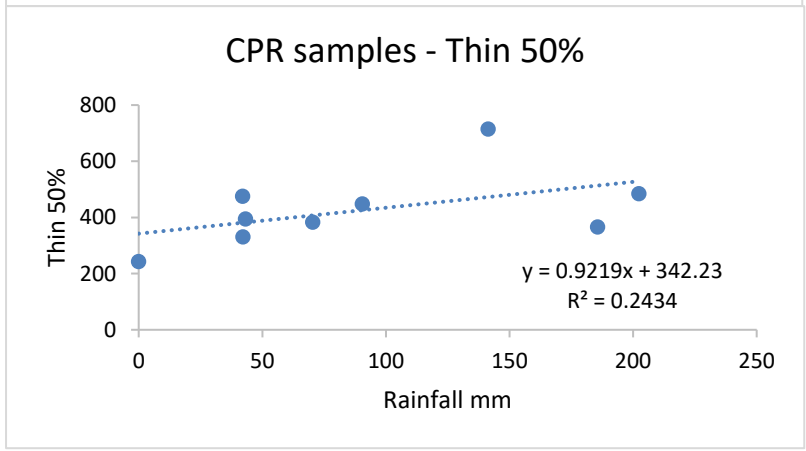
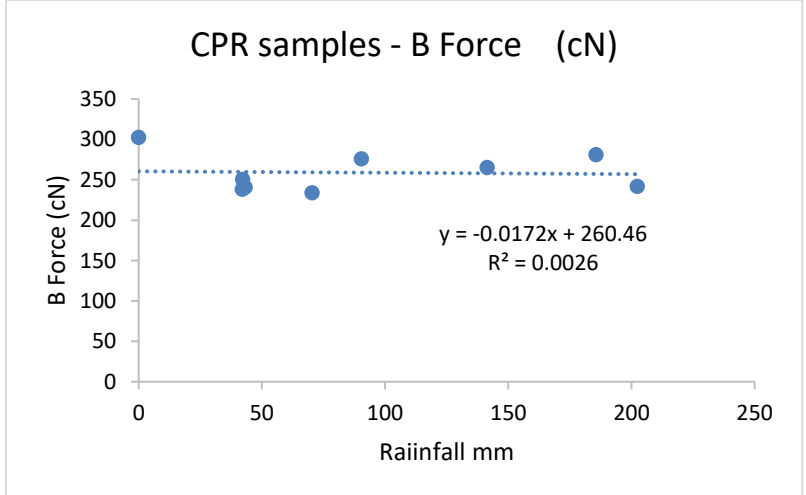
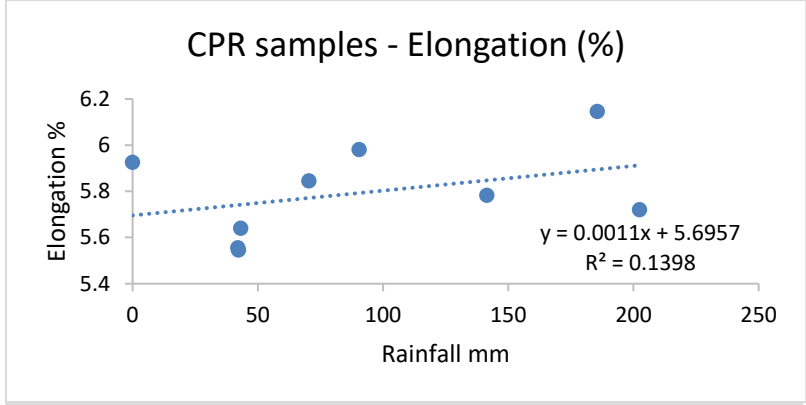
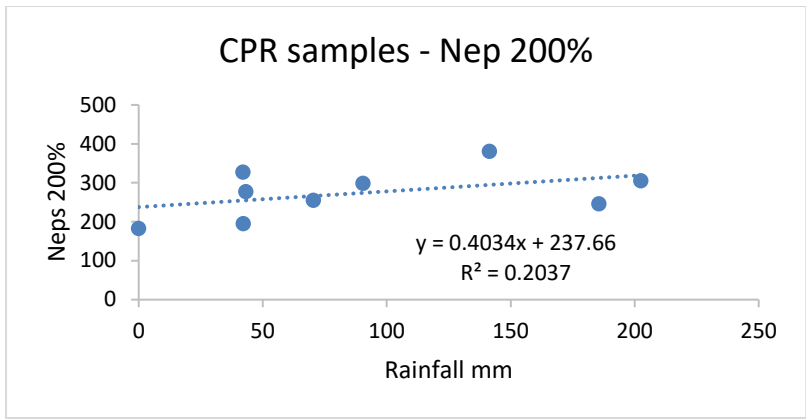


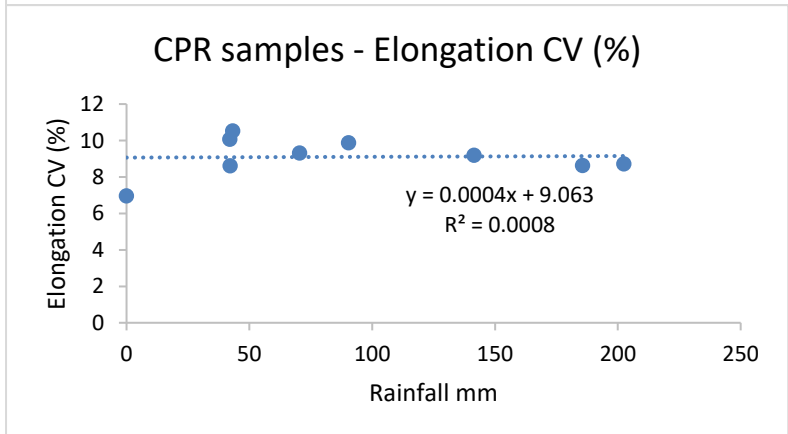
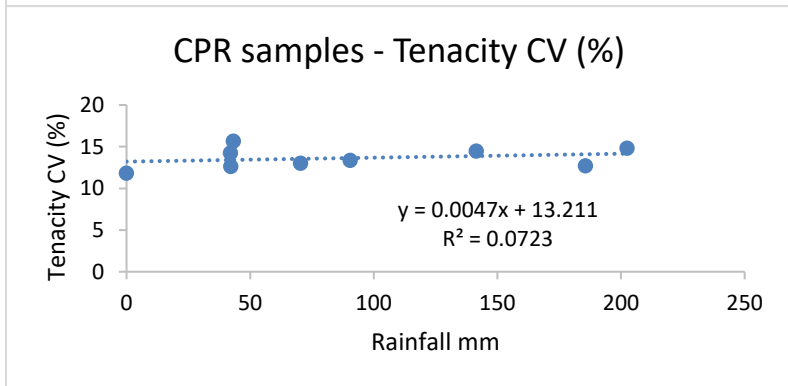
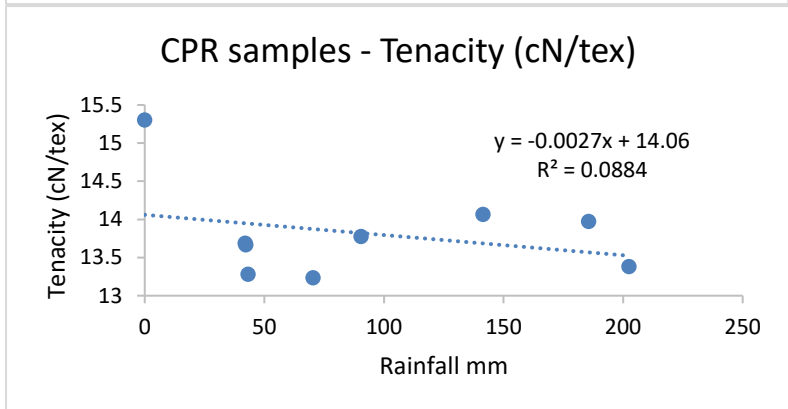
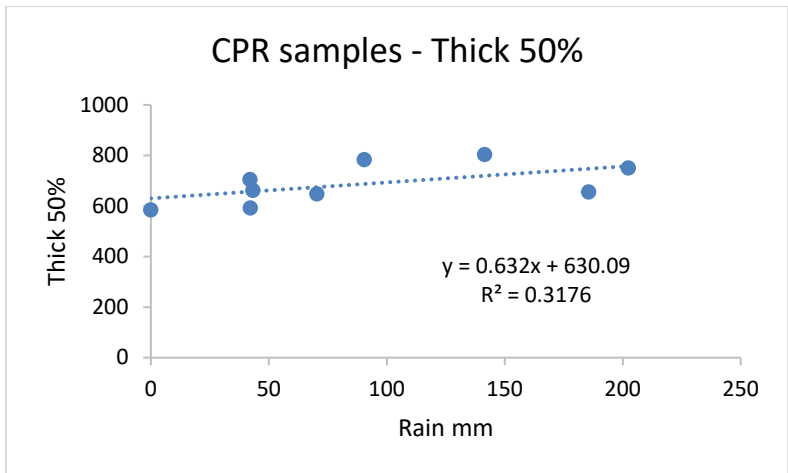
Unidentified fungus on PDA

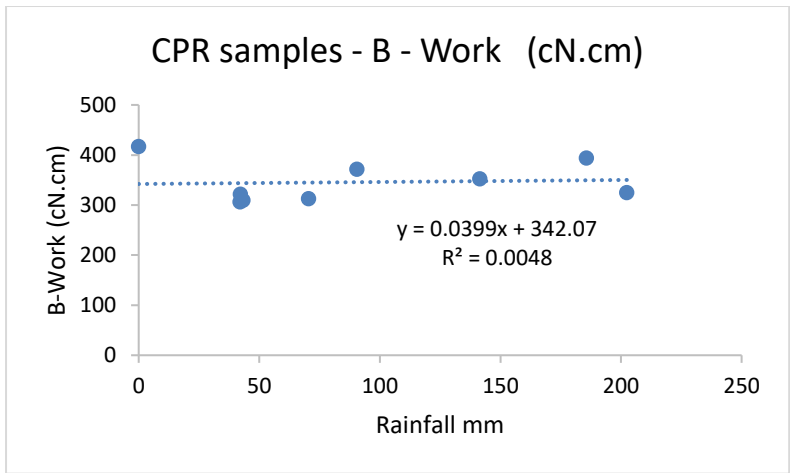
APPENDIX 6: CPR Yarn Test Results

CSIRO Material Science Fibre Unit, Geelong

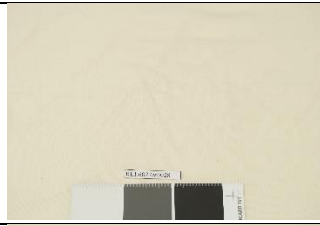
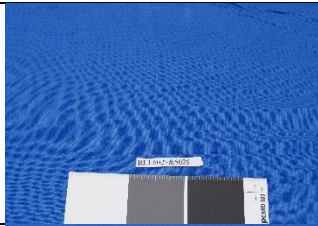
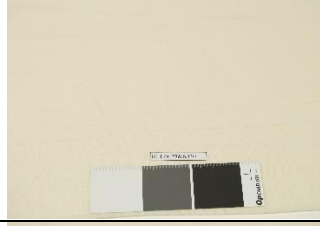
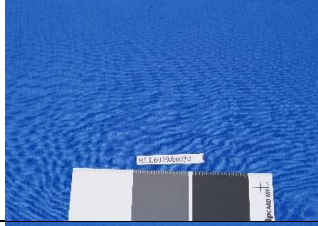

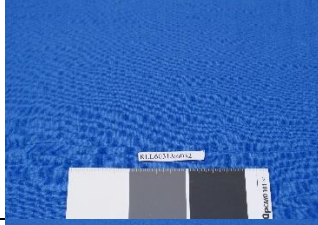

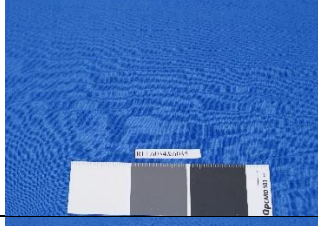


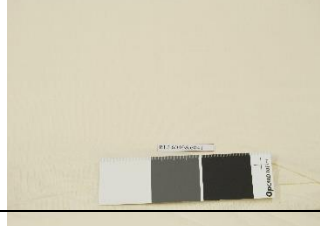
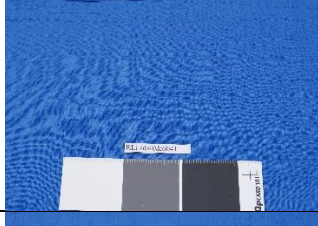

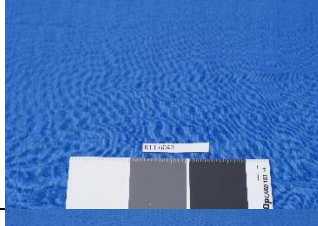





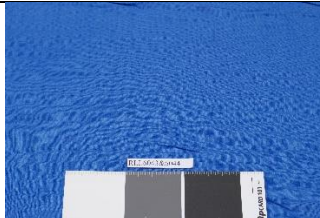





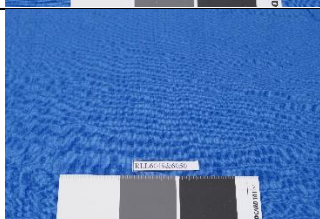






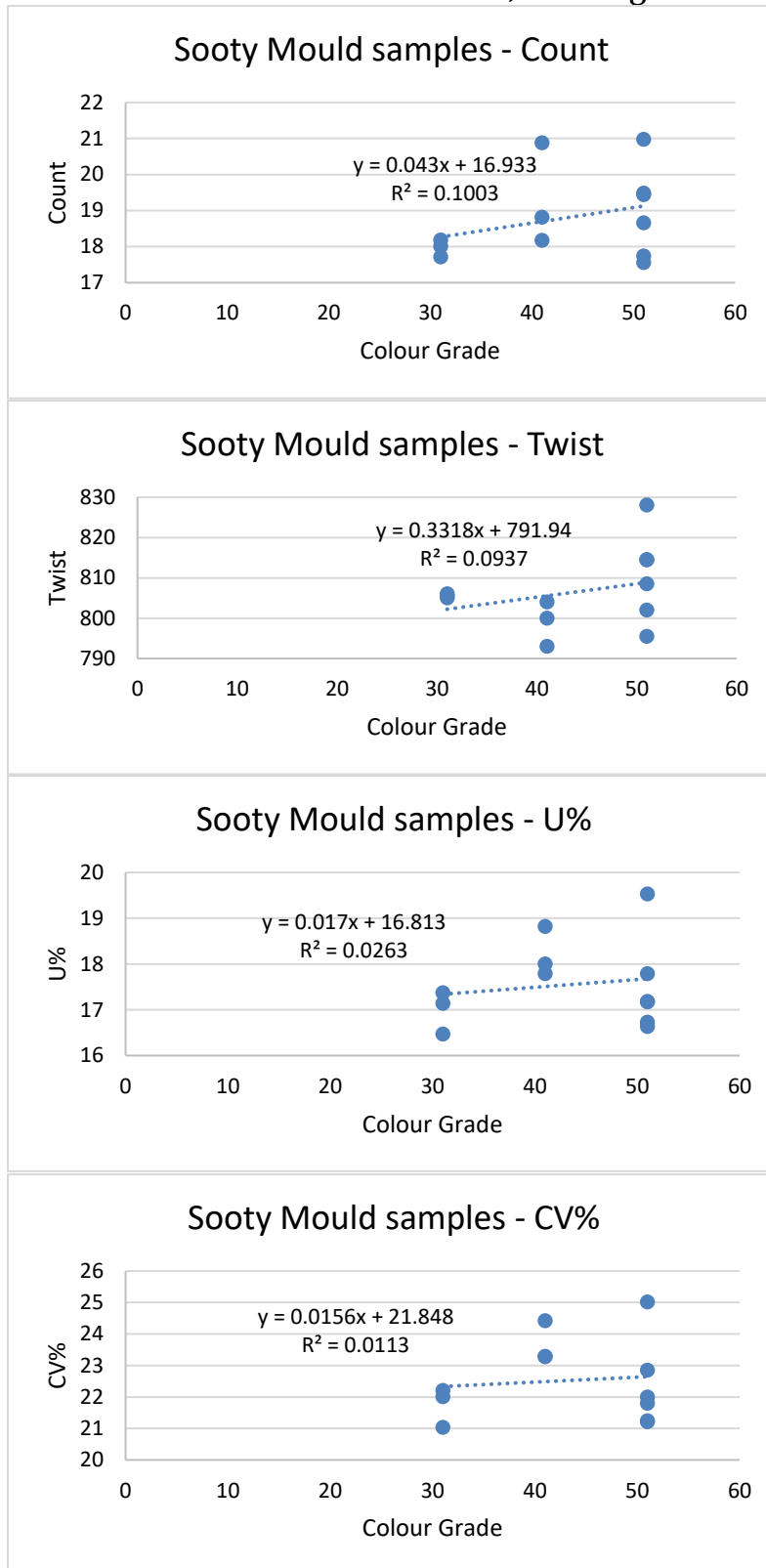
APPENDIX 7: Colour grades and Delta E values for pre- and post-dye rainfall affected (CPR) samples

Exposure (weeks after 1 st open boll)	RF (mm)	Grade	Delta E	Pre-Dye	Delta E	Post-Dye
Control	0 mm	11	0		0	
7.5	42.8 mm	11	1.07		0.29	
8.5	43.8 mm	11	1.01		0.28	
9.5	43.8 mm	21	0.47		1.47	
11	71.0 mm	31	1.84		1.11	
15	91.0 mm	31	2.17		2.16	
15	91.0 m	41	2.32		1.95	
16.5	142.0 mm	41	2.89		2.27	

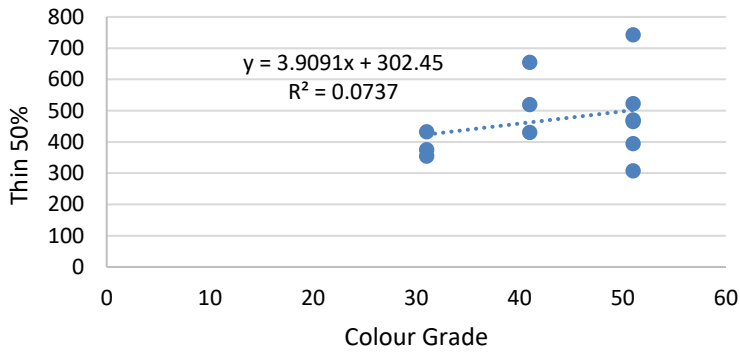
16.5	142.0 mm	51	3.20		1.81	
19.5	186.2 mm	51	3.42		2.18	
19.5	186.2 mm	61	3.46		2.93	
21.5	203.0 mm	61	3.73		2.61	

APPENDIX 8: Sooty Mould Yarn Test Results

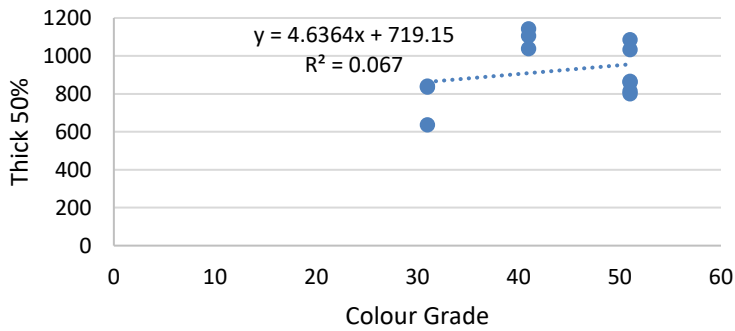
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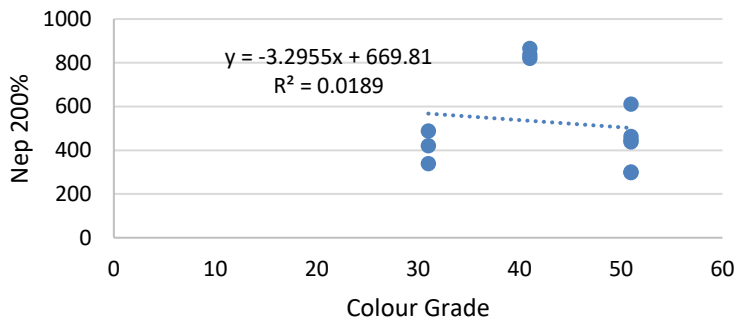
Sooty Mould samples - Thin 50%



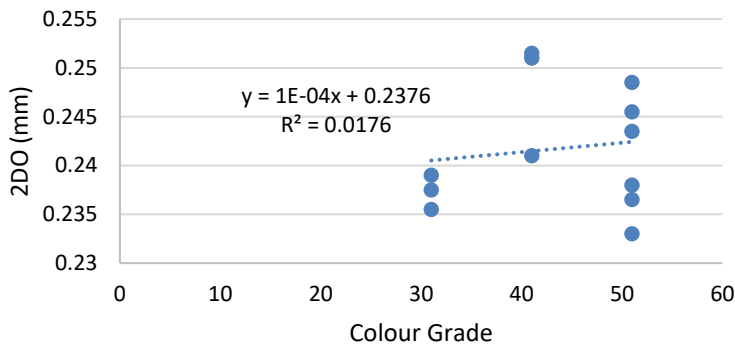
Sooty Mould samples - Thick 50%



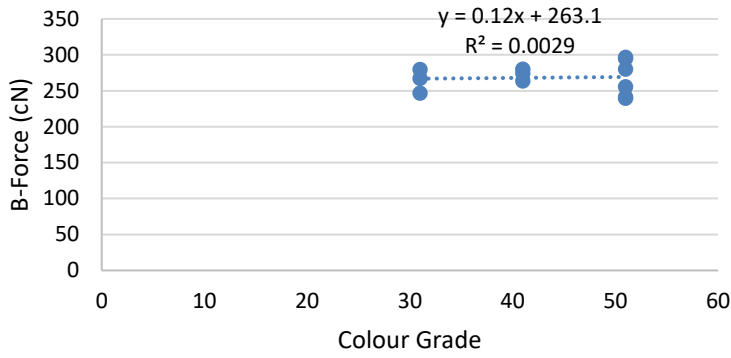
Sooty Mould samples - Nep 200%



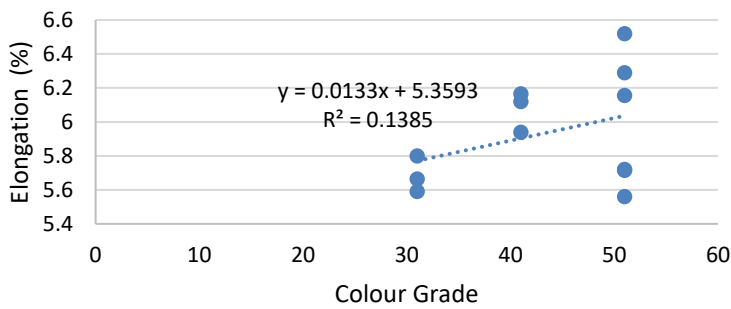
Sooty Mould samples - 2DO (mm)



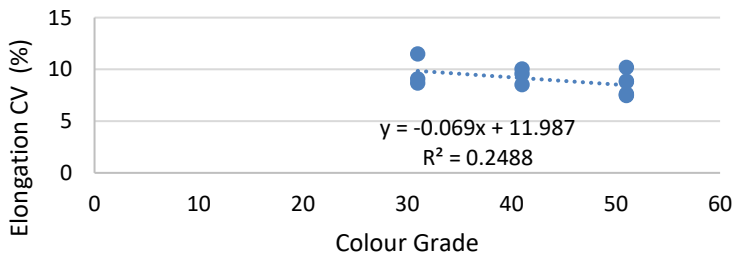
Sooty Mould samples - B Force (cN)



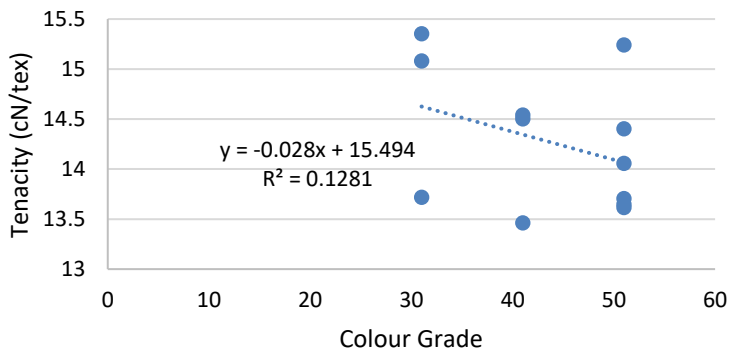
Sooty Mould samples - Elongation (%)



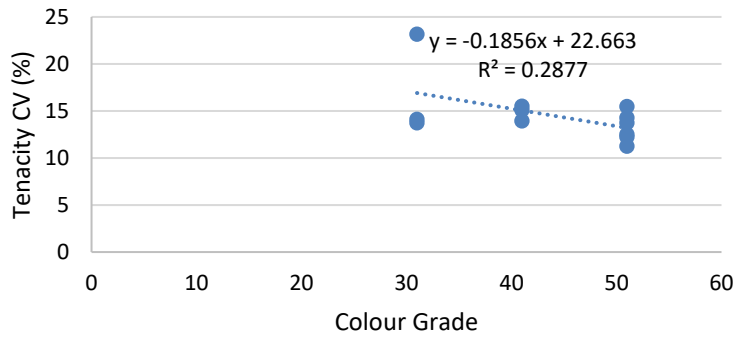
Sooty Mould samples - Elongation CV (%)



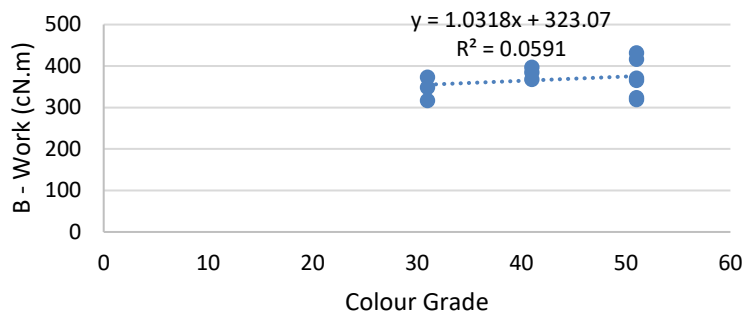
Sooty Mould samples - Tenacity (cN/tex)






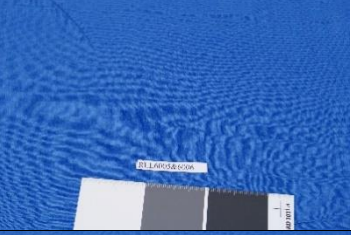









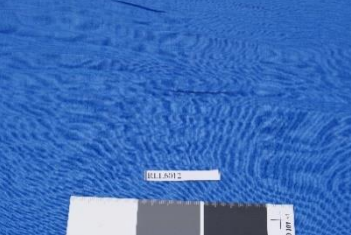
Sooty Mould samples - Tenacity CV (%)




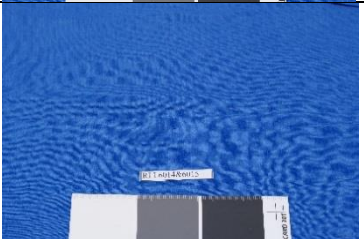







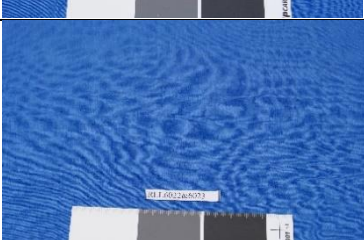



Sooty Mould samples - B - Work (cN.cm)



APPENDIX 9: Colour grades and Delta E values for pre- and post-dye sooty mould samples

Treatment	Grade	Delta E	Pre-Dye	Delta E	Post-Dye
Control	11	0		0	
Hand Pick	41	1.60		0.55	
Hand Pick	41	1.76		1.08	
Hand Pick	41	1.66		1.65	
Hand Pick	51	1.68		0.86	
Hand Pick	51	2.70		1.40	
Hand Pick	61	1.63		1.68	

Treatment	Grade	Delta E	Pre-Dye	Delta E	Post-Dye
Control	11	0		0	
Machine Pick	61	2.2		0.24	
Machine Pick	61	3.24		1.36	
Machine Pick	61	3.42		1.63	
Machine Pick	61	3.48		1.53	
Machine Pick	61	3.24		2.01	
Machine pick	61	3.12		1.29	