If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October. If not, please provide a written report by 30 September.

Part 1 - Summary Details

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

CRDC Project Number: **CMSE1210**

Project Title: **Further Investigation of the effects of Quarantine Treatments on Cotton Properties focussing on Colour**

Project Commencement Date: 01/01/2012 **Project Completion Date:** 30/06/2012

CRDC Program: Value Chain

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Part 3 – Final Report Guide

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

Background

1. Outline the background to the project.

Due to Australia's age, extreme variable weather patterns and long-term geographic isolation, much of the country's fauna and flora is unique and diverse. This isolation as an island nation is rapidly changing as the barriers of time and distance become less relevant and international travel and trade increase. With this comes increased risk of exotic pests and diseases entering Australia to seriously affect its unique environment, native flora and fauna, tourism and lifestyle.

Quarantine plays a critical role to ensure that Australia remains free from serious pests, weeds and diseases present in other parts of the world. Australia places great importance on quarantine and has among the strongest quarantine measures of any country in the world. These protective measures are undertaken by the Australian Quarantine Inspection Service (AQIS).

In the case of cotton an import permit is not required [1]. However, cotton is treated to ensure that the consignment is free of live insects, soil and other debris (faeces, animal materials etc) and to verify that any quarantine risk material present will be dealt with during processing. The quarantine treatments used can be either by gamma irradiation or chemical (fumigation). Following the findings of project CTFT0904 (Preliminary Investigation into the Effects of Quarantine Treatments), AQIS have agreed to suspend the treatment of cotton samples with gamma irradiation due to the resultant damage to the physical fibre properties.

There are currently three chemical treatments prescribed by AQIS;

- Treatment T9020 which involves two fumigation treatments with ethylene oxide under an initial minimum vacuum of 50 kilopascals at 1200 g/m³ for 5 hours at 50^{o}C or at 1500 g/m³ for 24 hours at 21^{o}C .
- Treatment T9038 which involves the fumigation with methyl bromide at a rate of 32 g/m³ for 24 hours at 21^{o}C, above normal atmospheric pressure.

Although Australia is a net exporter of cotton, a large number of cotton samples are still imported into Australia on a yearly basis mainly to calibrate and evaluate instruments that objectively measure fibre properties. The Australian cotton industry through local classing facilities has participated in the International Cotton Advisory Committee (ICAC) Commercial Standardization of Instrument Testing of Cotton (CSITC) Round Trials since their inception in 2007. Australian High Volume Instruments (HVI) have performed particularly well in these Round Trials with a number of the instruments in the top 20 and with most instruments consistently below (i.e. better than) the worldwide average. The Cotton Classers Association of Australia (CCAA) have however noticed that the colour results (reflectance Rd and yellowness +b) of the Australian HVI instruments participating, have since 2010 been consistently above (i.e. worse than) the worldwide average.

The results from these trials have indicated that fumigation treatments by ethylene oxide may affect the colour of cottons. A desktop review of the literature concluded that there have been no studies conducted in the past to determine the effect of fumigation on the physical properties of cotton lint. In this report we present the results of further in-depth trials with various cottons to determine the effect of the fumigation treatments by ethylene oxide on the colour of cotton and whether this change in colour is permanent.

Objectives

2. List the project objectives and the extent to which these have been achieved.

The broad objectives of the project are to:

- Clarify the effect of fumigation on the colour of cottons from various origins.
- Determine if there is any residue on the cotton after treatment.
- Continue dialog with AQIS to change treatment of calibration cotton so that their physical properties are not affected.

Methods

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

As methyl bromide is considered an ozone depleter and its use is in the process of being withdrawn, the trials will concentrate on what effect the two ethylene oxide treatments have on the physical properties of cotton lint, focussing on colour.

In order for extensive trials to be conducted we sourced a range of calibration cotton from the USDA ranging from Upland White (11-71) to Spotted (23-63) and Tinged (34-54) as well as Pima cotton (1-6, with 1 being the best and 6 the worst grade) – Table 1. We received a total of 25 calibration cottons covering a wide spectrum of colour grades. Two 250 gram samples for each colour grade were used in this trial to allow for multiple HVI testing.

Table 1. Colour Grades and description of Upland cotton

Colour Grade	Description
11	Good Middling
21	Strict Middling
31	Middling
41	Strict Low Middling
51	Low Middling
61	Strict Good Ordinary
71	Good Ordinary
23	Strict Middling
33	Middling
43	Strict Low Middling
53	Low Middling
63	Strict Good Ordinary
34	Middling
44	Strict Low Middling
54	Low Middling

Prior to forwarding the samples to Australia for treatment and further analysis, the USDA tested the various samples on their master colorimeter. Eight measurements were taken per sample with each measurement made on a different piece/face. Upon arrival the untreated samples were also tested on a HVI 1000 by the Auscott Classing facility in Sydney, which is a BMP certified classing facility. The averages of the eight measurements are shown in Table 2. As one would expect there are no real differences in the results between the two facilities.

Table 2. Average colour values for the various cotton grades prior to treatment

Colour Grade	USDA Rd	USDA +b	AUS Rd	AUS +b
11	80.6	9.2	80.7	9.3
21	78.6	9.4	78.7	9.6
31	75.0	8.8	75.1	9.1
41	72.8	7.0	72.4	7.3
51	67.5	6.9	67.9	6.9
61	62.5	6.8	62.9	6.9
71	56.3	7.4	56.1	7.5
23	72.4	11.1	72.2	11.4
33	72.0	10.4	72.2	10.5
43	66.8	10.4	67.1	10.5
53	59.4	10.2	60.8	10.3
63	59.0	9.4	59.9	9.7
34	64.5	13.0	64.9	13.1
44	62.3	12.8	62.9	13.0
54	57.8	12.9	58.0	12.9
Pima 1A	70.8	12.3	71.0	12.4
Pima 1B	68.7	14.0	68.3	14.1
Pima 2A	68.7	12.2	68.3	12.3
Pima 2B	67.4	13.2	68.1	13.2
Pima 3A	65.4	11.3	65.8	11.4
Pima 3B	64.5	13.2	64.5	13.3
Pima 4	62.7	12.3	63.1	12.5
Pima 5A	62.8	11.4	62.9	11.3
Pima 5B	60.7	11.8	60.8	11.6
Pima 6	59.2	10.0	58.8	10.0

As the colour of Australian cotton is somewhat different to the USDA cotton we included three Australian cotton samples in the trial. The averages of the eight measurements, as tested by the Auscott classing facility, are shown in Table 3.

Table 3. Average colour values for Australian cotton samples

Cotton Description	USDA Rd	USDA +b	AUS Rd	AUS +b
Aussie 1	*	*	80.2	7.6
Aussie 2	*	*	73.9	7.1
Aussie 3	*	*	74.7	5.8

The samples were fumigated using ethylene oxide at R.A. Dibbs & Sons Pty/Ltd in Salisbury, Queensland who is accredited by AQIS.

After the fumigation treatment the samples were tested on the HVI instrument four times. The first test was conducted 5 days after treatment, the second test 40 days after treatment, the third test 60 days after treatment and the last test 93 days after treatment. In between these tests the samples were placed in trays and stored in a conditioned room.

Fibre Testing
Fibre samples were conditioned under standard conditions of 20°C +/-2°C and 65% +/-3% relative humidity for 24 hours as per ISO 139. The samples were then tested on an Uster Technologies (Knoxville, USA) 1000 High Volume Instrument (HVI), as per ASTM D5867, for Micronaire, staple length, length uniformity, staple strength, elongation and colour. Cotton colour is represented by the two measurements Rd (Reflectance) and +b (Yellowness). Prior to the commencement of the ginning season the instrument was qualified using the USDA HVI qualification standards and prior to testing each lot, five tests were conducted with the central tile to ensure that colour readings are within tolerance.

In order to determine the presence of ethylene oxide Aussie 2 was further analysed, as we had untreated and treated samples, by Infrared and Raman spectroscopy as well as wax extraction. All further analysis was carried out on all the samples. You might explain what each of these measurements will specifically show.

Infrared spectroscopy analysis
Infrared spectra were collected at a resolution of 4 cm^{-1} using a Perkin Elmer (UK) System 2000 Fourier-transform infrared (FTIR) spectrometer fitted with narrow band Mercury Cadmium Telluride detector. Attenuated Total Reflectance (ATR) spectra were obtained from the cotton fibres using a Specac (UK) 11900 variable angle accessory and a ZnSe internal reflectance element. Spectra were obtained from the extracts as cast films on KBr salt plates. The solvent was allowed to evaporate at room temperature. All samples were analysed in duplicate.

Raman spectroscopy analysis
Raman spectra were obtained at a resolution of 4 cm^{-1} using a Bruker RFS-100 FT-Raman spectrometer equipped with an Adlas Nd:YAG laser operating at 1.064 μm and a liquid nitrogen cooled Germanium diode detector. Loose fibre samples were held in a compression cell [2] and analysed using a 180^{o} backscatter geometry.

Cotton wax extraction and analysis
The cotton wax of selected cotton samples was extracted from the cottons in duplicate using the Conrad method [3]. Briefly, cleaned (no vegetable or leaf matter) and conditioned (22^{o}C, 65% relative humidity) cotton samples were Soxhlet extracted with hot ethanol solution (95%) for six hours. Waxes were isolated by extracting the ethanol solution with chloroform and the chloroform then back-extracted with water. Extractions were carried out in duplicate for each cotton sample. The chloroform soluble extracts were analysed by infrared spectroscopy.

The colour of the cotton was assessed both before and after the extraction using a Gretag Macbeth Colour-Eye 7000A spectrophotometer (Munich, Germany). Any residual vegetable and leaf matter trash was manually removed from the samples prior to analysis. The cotton samples were conditioned (22^{o}C, 65% relative humidity) prior to analysis. To ensure consistent measurement of the loose fibres, a mass of 0.512 g (0.005 g) cotton was evenly compressed to a volume of 3.2 cm^{3} in a polymethyl methacrylate disposable cuvette (BrandTech, CT, USA) and capped with a 10 mm Perspex cube with a 1 mm hole drilled through the centre. All samples were analysed in duplicate.

Gas Chromatography / Mass Spectroscopy (GC/MS)
A head space analysis method was developed for the detection and quantitation of ethylene oxide on cotton. This method is based on B.5.4 of AS ISO 10993.7-2003 [4]. Approximately 60 mg of cotton was accurately weighed into 6 mL headspace vials and sealed with Teflon

faced caps. Before sampling the vials were placed in an oven at 100°C for 1 hour and then in a block heater for 5 minutes at 80°C. The analysis was carried out on a Varian 3600 gas chromatograph and Saturn 2000 mass spectrometer fitted with a 30 m length x 0.32 mm ID x 0.25 µm film, SGE SOLGEL-WAX column. The headspace was sampled manually with a 100 µL sample taken and injected into the gas chromatograph. During analysis all temperatures were isothermal, injector at 200°C and oven at 35°C. The detection and analysis of ethylene oxide was by the combined response of ions 42 – 44 m/z.

Ethylene oxide reference material was purchased as a 50,000 ppm solution in dichloromethane from Supelco. The reference material was diluted in dichloromethane to prepare a standard series. The standards were analysed by injecting 1 µL of the ethylene oxide standards into headspace vials and analysis was performed in the same manner as the samples. The range of the method was 0.50 – 833 mg/kg of ethylene oxide on cotton with excellent linearity (R^2 = 0.9996) over this range. The limit of detection was calculated as 3x the signal height of the background. Cotton samples spiked with ethylene oxide solution had an average recovery (subtracting the spiked cotton results from the result of the untreated cotton) of 89%.

Results

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

As mentioned previously the samples were also tested for Micronaire, staple length, length uniformity, staple strength, elongation and colour, Rd (Reflectance) and +b (Yellowness), prior to and after fumigation. The results for prior to fumigation and 5 days after fumigation with ethylene oxide under an initial minimum vacuum of 50 kilopascals at 1200 g/m³ for 5 hours at 50°C and at 1500 g/m³ for 24 hours at 21°C are shown in Table 4.

As expected and noted in a previous study (CTFT0904) the two fumigation treatments did not have any effect on the physical properties of the fibre and hence all further HVI testing only measured colour.

The average colour results (Rd and +b), with their standard deviations, prior to and after fumigation with ethylene oxide for 24 hours at 21°C and 5 hours at 50°C are shown in Tables 5 and 6. The changes in the colour values after fumigation with ethylene oxide for 24 hours at 21°C and 5 hours at 50°C are shown in Tables 7 and 8.

The tolerances limits permitted by the CCAA for Rd is +/- 1.0 units and for +b is +/- 0.6 units. The changes in colour that are above this limit have been highlighted in yellow, while changes on the limit are highlighted in light blue. From the results it can be seen that it is mainly the reflectance of the cotton that has been affected irrespective of the fumigation method. In most cases the reflectance value has decreased which means that the cotton has become darker. It is also apparent that the cotton that seems to be affected is the Upland USDA cotton Grades 11, 21 and 31 as well as the ELS USDA cotton grades Pima 1A-3B. It is interesting to note that the Australian cotton which is generally whiter than the US cotton seemed unaffected by the fumigation treatments, with only a slight change to the +b value, with the cotton becoming slightly yellower.

Table 4: HVI fibre length, uniformity, SFI, strength and elongation for prior and after treatment

Comment [ban084 1]: There seems to be layout problem with table when printing

	Prior						5 days after treatment with 24 hours at 21oC						5 days after treatment with 5 hours at 30oC					
Bale ID	**Mic**	**UHML**	**UI**	**SF**	**Str**	**Elg**	**Mic**	**UHML**	**UI**	**SF**	**Str**	**Elg**	**Mic**	**UHML**	**UI**	**SF**	**Str**	**Elg**
11	3.9	1.04	81	14.9	28.3	6.6	4.1	1.04	80	15.6	27.3	6.9	4.03	1.03	80	14.5	26.8	6.6
21	4.5	1.03	81	14.2	28.3	6.7	4.4	1.04	80	14.5	26.5	6.4	4.45	1.05	81	13.8	27.4	6.4
23	4.2	0.97	80	17.5	27.2	6.9	4.2	0.98	80	17.0	25.9	6.4	4.20	0.98	80	16.5	26.1	6.3
31	4.3	1.00	79	16.8	26.4	6.8	4.2	0.99	79	17.5	25.1	6.7	4.29	1.00	79	16.8	26.2	6.7
33	5.1	1.11	82	10.3	30.5	8.8	5.1	1.12	83	10.2	31.2	8.4	5.08	1.11	82	11.0	30.9	8.5
34	5.0	1.08	82	10.7	29.5	7.8	5.0	1.07	82	11.0	29.4	8.0	5.01	1.09	82	11.1	27.9	8.1
41	4.5	1.07	80	12.8	29.1	7.0	4.5	1.07	79	14.2	27.6	7.1	4.28	1.07	79	14.4	28.4	7.3
43	4.8	1.09	82	11.8	29.5	8.0	4.8	1.09	80	12.3	28.9	8.2	4.79	1.10	81	12.1	28.9	8.2
44	4.9	1.08	81	12.6	27.6	7.2	4.8	1.11	81	11.8	27.3	7.0	4.78	1.10	81	12.8	28.1	7.5
51	4.6	1.07	81	12.8	27.8	6.2	4.5	1.07	81	13.2	27.3	6.1	4.53	1.09	81	12.3	27.1	6.3
53	4.4	1.05	81	13.5	27.2	7.1	4.3	1.03	81	14.3	25.9	6.9	4.40	1.07	81	13.0	27.0	6.7
54	4.6	1.07	80	14.3	26.4	6.9	4.6	1.08	80	15.9	25.9	6.9	4.50	1.05	79	15.2	24.6	6.2
61	4.7	1.07	81	13.0	27.4	6.4	4.8	1.08	81	12.8	26.5	6.8	4.69	1.10	81	11.8	28.1	6.4
63	4.4	1.05	81	13.1	26.9	6.5	4.4	1.04	80	14.1	26.5	7.0	4.48	1.04	81	14.6	26.9	7.0
71	5.0	1.03	79	17.8	24.3	6.0	4.9	1.04	78	17.8	25.2	6.3	4.97	1.05	78	17.2	24.8	6.2
Pima 1a	4.6	1.44	86	4.8	44.6	7.1	4.4	1.43	87	4.7	47.1	7.3	4.57	1.43	86	4.8	46.0	7.2
Pima 1b	3.1	1.52	87	4.7	39.5	6.5	3.1	1.47	87	4.7	41.1	6.2	3.18	1.49	87	4.7	40.3	6.2
Pima 2a	4.0	1.43	86	4.7	45.5	7.2	4.1	1.40	86	5.1	45.1	7.5	4.18	1.39	85	4.9	44.3	7.4
Pima 2b	3.7	1.45	85	4.8	43.2	6.9	3.6	1.38	85	5.4	42.8	7.2	3.66	1.40	85	4.9	42.8	6.8
Pima 3a	3.6	1.35	84	6.8	38.1	7.1	3.7	1.35	84	6.4	38.3	7.1	4.00	1.35	84	6.1	37.2	7.3
Pima 3b	4.0	1.48	87	4.7	44.7	6.8	3.9	1.40	86	4.8	42.8	6.5	4.08	1.44	86	4.8	42.1	6.6
Pima 4	2.9	1.38	85	5.4	41.0	6.8	3.1	1.31	83	7.9	37.0	6.7	3.03	1.30	83	8.5	39.8	7.6
Pima 5a	3.6	1.40	84	5.7	39.7	6.0	3.5	1.42	84	4.8	44.1	6.0	3.46	1.40	82	5.2	39.2	6.0
Pima 5b	3.4	1.43	85	4.9	41.4	6.3	3.3	1.37	85	5.2	41.1	6.6	3.58	1.38	85	5.3	40.8	6.3
Pima 6	3.3	1.36	83	8.0	39.2	6.3	3.1	1.36	84	6.1	42.0	5.8	3.13	1.34	84	6.8	42.8	6.3
Aussie 1	4.0	1.20	82	10.5	31.7	6.5	3.9	1.20	82	11.1	31.9	6.4	3.93	1.22	82	10.7	32.1	6.7
Aussie 2	4.6	1.23	83	9.6	32.5	5.9	4.5	1.23	83	10.0	32.5	6.1	4.51	1.22	82	10.5	32.4	6.0
Aussie 3	4.5	1.20	81	11.1	31.2	6.1	4.5	1.19	81	11.1	30.6	6.3	4.59	1.20	81	11.2	31.6	6.3

Table 5: Colour results for samples prior and after treatment of ethylene oxide at 24 hours at 21°C

USDA	Prior to treatment				5 Days after treatment				40 Days after treatment				60 Days after treatment				93 Days after treatment			
Grade	Rd	st	+b	st	Rd	st	+b	st	Rd	st	+b	st	Rd	st	+b	st	Rd	st	+b	st
11	80.7	0.31	9.3	0.2	79.3	0.30	9.8	0.27	79.1	0.5	9.7	0.16	79.3	0.41	9.7	0.25	79.4	0.40	9.6	0.30
21	78.7	0.33	9.6	0.13	77.5	0.23	10.1	0.22	77.2	0.21	9.8	0.25	77.5	0.52	9.8	0.40	77.4	0.20	9.8	0.20
31	75.1	0.40	9.1	0.11	74.2	0.26	9.6	0.18	74.0	0.25	9.5	0.20	74.1	0.32	9.3	0.34	74.6	1.30	9.5	0.20
41	72.4	0.41	7.3	0.11	72.2	0.44	7.6	0.17	72.2	0.35	7.7	0.15	72.8	0.28	7.6	0.23	72.4	2.70	7.4	0.20
51	67.9	0.58	6.9	0.14	67.6	0.46	7.2	0.18	67.1	0.47	7.3	0.28	67.3	0.67	7.1	0.43	67.4	0.40	7.3	0.20
61	62.9	0.81	6.9	0.19	63.4	0.85	7.0	0.22	63.4	0.57	7.2	0.18	63.5	0.96	7.2	0.26	63.4	0.50	7.3	0.20
71	56.1	0.62	7.5	0.21	56.2	0.64	7.7	0.12	56.1	0.49	7.6	0.18	56.3	0.54	7.6	0.21	56.5	0.60	7.6	0.20
23	72.2	0.46	11.4	0.18	71.6	0.36	11.9	0.15	71.4	0.36	11.9	0.22	71.7	0.28	12.0	0.13	72.2	0.50	11.8	0.10
33	72.2	0.72	10.5	0.11	71.8	0.38	10.9	0.13	71.6	0.41	10.8	0.14	72.0	0.41	10.9	0.27	71.2	0.60	10.9	0.20
43	67.1	0.42	10.5	0.15	67.2	0.41	10.7	0.15	66.8	0.24	10.6	0.15	67.1	0.39	10.6	0.21	67.2	0.70	10.7	0.20
53	60.8	0.41	10.3	0.32	60.1	0.47	10.4	0.20	60.3	0.5	10.3	0.09	60.8	0.46	10.4	0.16	60.3	0.40	10.5	0.10
63	59.9	0.46	9.7	0.18	59.7	0.37	9.9	0.25	59.7	0.32	9.8	0.25	59.7	0.50	9.7	0.15	59.3	0.70	10.0	0.30
34	64.9	0.52	13.1	0.19	64.4	0.60	13.3	0.13	64.2	0.45	13.1	0.11	65.1	0.35	13.1	0.16	64.6	0.90	13.1	0.30
44	62.9	0.44	13.0	0.15	62.2	0.37	13.0	0.19	62.3	0.5	12.8	0.24	62.5	0.56	12.8	0.37	62.4	0.50	12.8	0.40
54	58.0	0.53	12.9	0.25	57.4	0.66	12.9	0.19	57.7	0.39	12.8	0.27	58.0	0.42	12.8	0.23	57.9	0.70	12.9	0.40
Pima 1A	71.0	0.36	12.4	0.27	69.7	0.63	12.7	0.20	69.6	0.61	12.5	0.27	70.2	0.94	12.7	0.22	70.5	0.90	12.3	0.40
Pima 1B	68.3	0.64	14.1	0.18	67.5	0.50	14.2	0.33	67.2	0.5	14.1	0.25	67.5	0.28	14.2	0.33	69.1	1.10	13.7	0.70
Pima 2A	68.3	0.44	12.3	0.25	67.3	0.57	12.4	0.21	66.7	0.29	12.4	0.17	67.3	0.22	12.7	0.16	68.0	0.50	12.6	0.40
Pima 2B	68.1	0.58	13.2	0.27	67.1	0.69	13.4	0.27	67.1	0.69	13.1	0.30	67.2	0.90	13.4	0.24	67.8	0.70	12.8	0.30
Pima 3A	65.8	0.40	11.4	0.32	65.0	0.69	11.5	0.29	64.8	0.73	11.4	0.21	65.3	0.60	11.5	0.23	66.0	0.80	11.0	0.40
Pima 3B	64.5	0.31	13.3	0.33	63.3	0.89	13.9	0.18	63.0	1.08	13.8	0.25	63.7	0.84	14.1	0.18	63.6	0.60	13.2	0.30
Pima 4	63.1	1.00	12.5	0.26	62.6	0.62	12.8	0.42	62.1	0.85	12.7	0.22	62.3	1.28	12.5	0.24	62.4	0.80	12.6	0.20
Pima 5A	62.9	1.28	11.3	0.32	62.0	1.16	11.7	0.19	62.0	1.12	11.8	0.26	61.8	1.29	11.8	0.24	61.4	0.70	11.8	0.30
Pima 5B	60.8	1.62	11.6	0.35	60.5	0.91	12.2	0.13	59.9	0.51	12.0	0.30	60.4	0.94	12.0	0.33	61.5	1.00	12.0	0.30
Pima 6	58.8	0.72	10.0	0.35	58.5	1.09	10.5	0.35	57.3	1.7	10.2	0.28	58.2	1.32	10.2	0.40	59.7	0.90	10.1	0.40
Aussie 1	80.2	0.62	7.6	0.38	80.2	0.56	7.9	0.21	79.4	0.35	7.9	0.11	79.8	0.44	8.0	0.14	79.9	0.20	7.9	0.10
Aussie 2	73.9	0.47	7.1	0.27	73.8	0.50	7.6	0.26	73.4	0.58	7.5	0.15	74.0	0.81	7.6	0.25	73.8	0.30	7.7	0.20
Aussie 3	74.7	0.28	5.8	0.12	74.6	0.69	6.1	0.18	74.3	0.41	6.4	0.21	74.8	0.31	6.6	0.21	74.9	0.70	6.3	0.10

Table 6: Colour results for samples prior and after treatment of ethylene oxide at 5 hours at 50°C

USDA	Prior to treatment				5 Days after treatment				40 Days after treatment				60 Days after treatment				93 Days after treatment			
Grade	Rd	st	+b	st	Rd	st	+b	st	Rd	st	+b	st	Rd	st	+b	st	Rd	st	+b	st
11	80.7	0.31	9.3	0.2	79.6	0.26	9.5	0.21	79.3	0.26	9.4	0.12	79.6	0.37	9.8	0.17	79.2	0.40	9.5	0.40
21	78.7	0.33	9.6	0.13	77.6	0.42	9.9	0.22	77.1	0.32	9.7	0.11	77.5	0.32	9.8	0.21	77.4	0.40	9.7	0.30
31	75.1	0.40	9.1	0.11	74.4	0.31	9.4	0.18	73.9	0.26	9.2	0.07	74.3	0.29	9.4	0.13	73.8	0.30	9.4	0.30
41	72.4	0.41	7.3	0.11	73.8	0.50	7.3	0.18	73.4	0.44	7.4	0.14	73.6	0.65	7.5	0.16	72.7	0.40	7.5	0.30
51	67.9	0.58	6.9	0.14	67.5	0.57	7.2	0.16	66.8	0.49	7.0	0.16	67.2	0.39	7.2	0.17	67.3	0.70	7.2	0.20
61	62.9	0.81	6.9	0.19	63.5	0.63	7.1	0.11	62.9	0.57	7.0	0.13	63.1	0.56	7.2	0.26	63.5	0.50	7.1	0.30
71	56.1	0.62	7.5	0.21	56.3	0.66	7.5	0.17	56.4	0.58	7.4	0.15	56.7	0.41	7.5	0.22	56.5	0.70	7.6	0.10
23	72.2	0.46	11.4	0.18	71.9	0.63	11.8	0.17	72.0	0.39	11.5	0.19	72.1	0.43	11.7	0.15	71.7	0.50	11.9	0.20
33	72.2	0.72	10.5	0.11	71.2	0.51	10.8	0.12	70.9	0.47	10.7	0.16	71.6	0.41	10.8	0.16	72.0	0.40	10.8	0.20
43	67.1	0.42	10.5	0.15	67.0	0.33	10.5	0.22	66.8	0.42	10.3	0.15	67.2	0.23	10.7	0.18	67.1	0.20	10.6	0.10
53	60.8	0.41	10.3	0.32	60.4	0.65	10.3	0.26	59.9	0.58	10.2	0.25	60.3	0.66	10.4	0.12	60.3	0.60	10.2	0.30
63	59.9	0.46	9.7	0.18	59.8	0.57	9.8	0.19	59.3	0.45	9.7	0.13	59.8	0.29	9.9	0.14	59.5	1.10	9.8	0.30
34	64.9	0.52	13.1	0.19	63.9	0.97	13.2	0.2	63.6	1.01	13.0	0.20	64.4	0.63	13.0	0.26	65.1	0.40	13.1	0.10
44	62.9	0.44	13.0	0.15	62.1	0.55	12.9	0.24	62.3	0.60	12.8	0.24	62.6	0.57	12.8	0.33	62.5	0.30	12.9	0.20
54	58.0	0.53	12.9	0.25	57.4	0.30	12.9	0.26	57.4	0.48	12.7	0.32	57.9	0.54	12.8	0.39	57.7	0.40	12.7	0.30
Pima 1A	71.0	0.36	12.4	0.27	70.4	0.64	12.3	0.32	70.1	0.83	12.5	0.28	70.5	0.78	12.6	0.42	69.9	0.70	12.6	0.20
Pima 1B	68.3	0.64	14.1	0.18	68.8	0.70	13.8	0.22	68.2	0.82	13.8	0.34	68.6	0.65	13.6	0.41	67.4	0.20	14.1	0.40
Pima 2A	68.3	0.44	12.3	0.25	68.0	0.56	12.6	0.25	67.7	0.69	12.3	0.32	68.0	0.66	12.2	0.37	67.4	0.50	12.4	0.30
Pima 2B	68.1	0.58	13.2	0.27	67.2	0.72	13.2	0.31	67.1	0.81	13.0	0.20	67.6	0.62	13.2	0.30	66.8	0.30	13.3	0.20
Pima 3A	65.8	0.40	11.4	0.32	65.6	0.77	11.2	0.19	65.1	0.76	11.4	0.33	65.7	0.45	11.2	0.44	65.8	0.70	11.5	0.30
Pima 3B	64.5	0.31	13.3	0.33	63.4	0.42	13.5	0.35	62.9	0.65	13.5	0.20	64.0	0.31	13.5	0.49	63.3	0.90	13.8	0.20
Pima 4	63.1	1.00	12.5	0.26	62.5	0.80	12.5	0.23	62.1	0.75	12.4	0.21	62.6	0.66	12.5	0.22	62.4	1.20	12.7	0.30
Pima 5A	62.9	1.28	11.3	0.32	61.9	0.55	11.5	0.35	61.2	0.72	11.8	0.11	61.5	0.78	11.7	0.27	62.1	0.40	12.0	0.30
Pima 5B	60.8	1.62	11.6	0.35	61.4	0.95	11.8	0.21	60.9	1.63	11.7	0.42	61.1	1.23	12.0	0.45	60.1	0.90	11.8	0.40
Pima 6	58.8	0.72	10.0	0.35	59.8	0.90	10.0	0.27	59.5	0.90	10.0	0.32	60.0	0.86	10.1	0.35	59.3	1.10	10.4	0.50
Aussie 1	80.2	0.62	7.6	0.38	80.3	0.43	7.7	0.25	79.5	0.36	7.8	0.21	79.9	0.43	7.9	0.18	79.7	0.50	8.1	0.20
Aussie 2	73.9	0.47	7.1	0.27	73.8	0.82	7.3	0.09	73.4	0.54	7.4	0.12	73.8	0.55	7.5	0.18	73.6	0.60	7.7	0.20
Aussie 3	74.7	0.28	5.8	0.12	74.8	0.51	6.2	0.21	74.3	0.50	6.1	0.16	74.9	0.62	6.2	0.11	74.9	0.30	6.5	0.30

Table 7: Colour changes after treatment of ethylene oxide after 24 hours at 21°C

	Prior	Prior	5 days	5 days	40 days	40 days	60 days	60 days	93 days	93 days
USDA	AUS	AUS	Change	Change	Change	Change	Change	Change	Change	Change
Grade	Rd	+b	Rd	b+	Rd	b+	Rd	b+	Rd	b+
11	80.7	9.3	-1.4	0.5	-1.6	0.4	-1.4	0.4	-1.3	0.3
21	78.7	9.6	-1.2	0.5	-1.5	0.2	-1.2	0.2	-1.3	0.2
31	75.1	9.1	-0.9	0.5	-1.1	0.4	-1.0	0.2	-0.5	0.4
41	72.4	7.3	-0.2	0.3	-0.2	0.4	0.4	0.3	0.0	0.1
51	67.9	6.9	-0.3	0.3	-0.8	0.4	-0.6	0.2	-0.5	0.4
61	62.9	6.9	0.5	0.1	0.5	0.3	0.6	0.3	0.5	0.4
71	56.1	7.5	0.1	0.2	0.0	0.1	0.2	0.1	0.4	0.1
23	72.2	11.4	-0.6	0.5	-0.8	0.5	-0.5	0.6	0.0	0.4
33	72.2	10.5	-0.4	0.4	-0.6	0.3	-0.2	0.4	-1.0	0.4
43	67.1	10.5	0.1	0.2	-0.3	0.1	0.0	0.1	0.1	0.2
53	60.8	10.3	-0.7	0.1	-0.5	0.0	0.0	0.1	-0.5	0.2
63	59.9	9.7	-0.2	0.2	-0.2	0.1	-0.2	0.0	-0.6	0.3
34	64.9	13.1	-0.5	0.2	-0.7	0.0	0.2	0.0	-0.3	0.0
44	62.9	13.0	-0.7	0.0	-0.6	-0.2	-0.4	-0.2	-0.5	-0.2
54	58.0	12.9	-0.6	0.0	-0.3	-0.1	0.0	-0.1	-0.1	0.0
Pima 1A	71.0	12.4	-1.3	0.3	-1.4	0.1	-0.8	0.3	-0.5	-0.1
Pima 1B	68.3	14.1	-0.8	0.1	-1.1	0.0	-0.8	0.1	0.8	-0.4
Pima 2A	68.3	12.3	-1.0	0.1	-1.6	0.1	-1.0	0.4	-0.3	0.3
Pima 2B	68.1	13.2	-1.0	0.2	-1.0	-0.1	-0.9	0.2	-0.3	-0.4
Pima 3A	65.8	11.4	-0.8	0.1	-1.0	0.0	-0.5	0.1	0.2	-0.4
Pima 3B	64.5	13.3	-1.2	0.6	-1.5	0.5	-0.8	0.8	-0.9	-0.1
Pima 4	63.1	12.5	-0.5	0.3	-1.0	0.2	-0.8	0.0	-0.7	0.1
Pima 5A	62.9	11.3	-0.9	0.4	-0.9	0.5	-1.1	0.5	-1.5	0.5
Pima 5B	60.8	11.6	-0.3	0.6	-0.9	0.4	-0.4	0.4	0.7	0.4
Pima 6	58.8	10.0	-0.3	0.5	-1.5	0.2	-0.6	0.2	0.9	0.1
Aussie 1	80.2	7.6	0.0	0.3	-0.8	0.3	-0.4	0.4	-0.3	0.3
Aussie 2	73.9	7.1	-0.1	0.5	-0.5	0.4	0.1	0.5	-0.1	0.6
Aussie 3	74.7	5.8	-0.1	0.3	-0.4	0.6	0.1	0.8	0.2	0.5

Table 8: Colour results for samples after treatment of ethylene oxide at 5 hours at 50°C

	Prior	Prior	5 days	5 days	40 days	40 days	60 days	60 days	93 days	93 days
USDA	AUS	AUS	Change	Change	Change	Change	Change	Change	Change	Change
Grade	Rd	+b	Rd	b+	Rd	b+	Rd	b+	Rd	b+
11	80.7	9.3	-1.1	0.2	-1.4	0.1	-1.1	0.5	-1.5	0.2
21	78.7	9.6	-1.1	0.3	-1.6	0.1	-1.2	0.2	-1.3	0.1
31	75.1	9.1	-0.7	0.3	-1.2	0.1	-0.8	0.3	-1.3	0.3
41	72.4	7.3	1.4	0.0	1.0	0.1	1.2	0.2	0.3	0.2
51	67.9	6.9	-0.4	0.3	-1.1	0.1	-0.7	0.3	-0.6	0.3
61	62.9	6.9	0.6	0.2	0.0	0.1	0.2	0.3	0.6	0.2
71	56.1	7.5	0.2	0.0	0.3	-0.1	0.6	0.0	0.4	0.1
23	72.2	11.4	-0.3	0.4	-0.2	0.1	-0.1	0.3	-0.5	0.5
33	72.2	10.5	-1.0	0.3	-1.3	0.2	-0.6	0.3	-0.2	0.3
43	67.1	10.5	-0.1	0.0	-0.3	-0.2	0.1	0.2	0.0	0.1
53	60.8	10.3	-0.4	0.0	-0.9	-0.1	-0.5	0.1	-0.5	-0.1
63	59.9	9.7	-0.1	0.1	-0.6	0.0	-0.1	0.2	-0.4	0.1
34	64.9	13.1	-1.0	0.1	-1.3	-0.1	-0.5	-0.1	0.2	0.0
44	62.9	13.0	-0.8	-0.1	-0.6	-0.2	-0.3	-0.2	-0.4	-0.1
54	58.0	12.9	-0.6	0.0	-0.6	-0.2	-0.1	-0.1	-0.3	-0.2
Pima 1A	71.0	12.4	-0.6	-0.1	-0.9	0.1	-0.5	0.2	-1.1	0.2
Pima 1B	68.3	14.1	0.5	-0.3	-0.1	-0.3	0.3	-0.5	-0.9	0.0
Pima 2A	68.3	12.3	-0.3	0.3	-0.6	0.0	-0.3	-0.1	-0.9	0.1
Pima 2B	68.1	13.2	-0.9	0.0	-1.0	-0.2	-0.5	0.0	-1.3	0.1
Pima 3A	65.8	11.4	-0.2	-0.2	-0.7	0.0	-0.1	-0.2	0.0	0.1
Pima 3B	64.5	13.3	-1.1	0.2	-1.6	0.2	-0.5	0.2	-1.2	0.5
Pima 4	63.1	12.5	-0.6	0.0	-1.0	-0.1	-0.5	0.0	-0.7	0.2
Pima 5A	62.9	11.3	-1.0	0.2	-1.7	0.5	-1.4	0.4	-0.8	0.7
Pima 5B	60.8	11.6	0.6	0.2	0.1	0.1	0.3	0.4	-0.7	0.2
Pima 6	58.8	10.0	1.0	0.0	0.7	0.0	1.2	0.1	0.5	0.4
Aussie 1	80.2	7.6	0.1	0.1	-0.7	0.2	-0.3	0.3	-0.5	0.5
Aussie 2	73.9	7.1	-0.1	0.2	-0.5	0.3	-0.1	0.4	-0.3	0.6
Aussie 3	74.7	5.8	0.1	0.4	-0.4	0.3	0.2	0.4	0.2	0.7

As the change in reflectance has remained for more than three months (93 days), it appears that this change is permanent. When comparing these changes to the USDA Upland and ELS colour charts (Figure 1 & 2) which are uploaded in all HVI instruments we note that these small changes can affect the colour grade results. These changes in the reflectance values will result in the HVI instrument wrongly classifying the cotton (as in CSITC Round Trials) one grade higher (i.e. worse than), for example the Grade 11 cotton will be graded 21 and the 21 will be graded 31.This will lead to the instrument failing to calibrate and also unable to qualify the instrument.

Figure 1. HVI Colour chart for Upland cotton

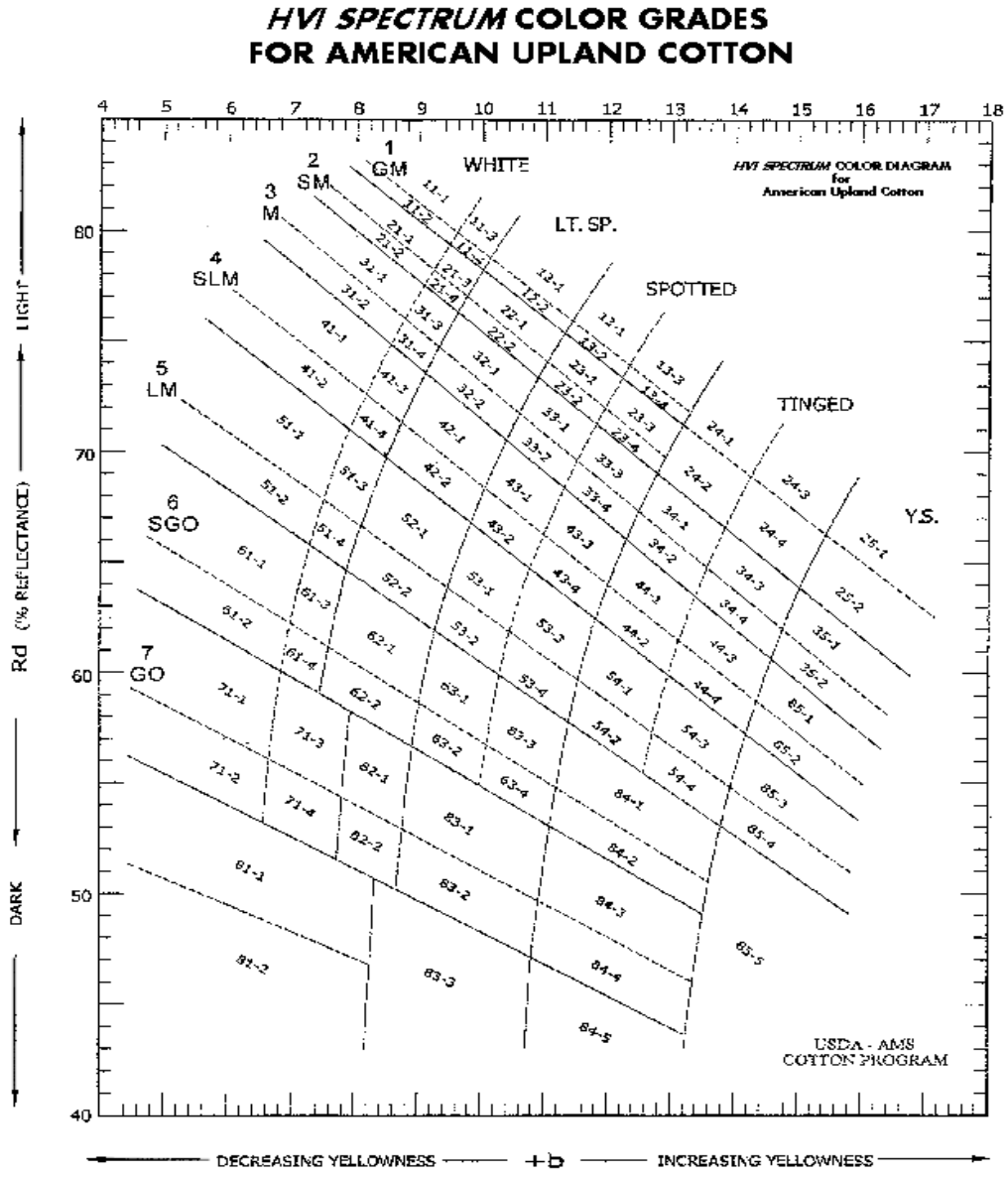
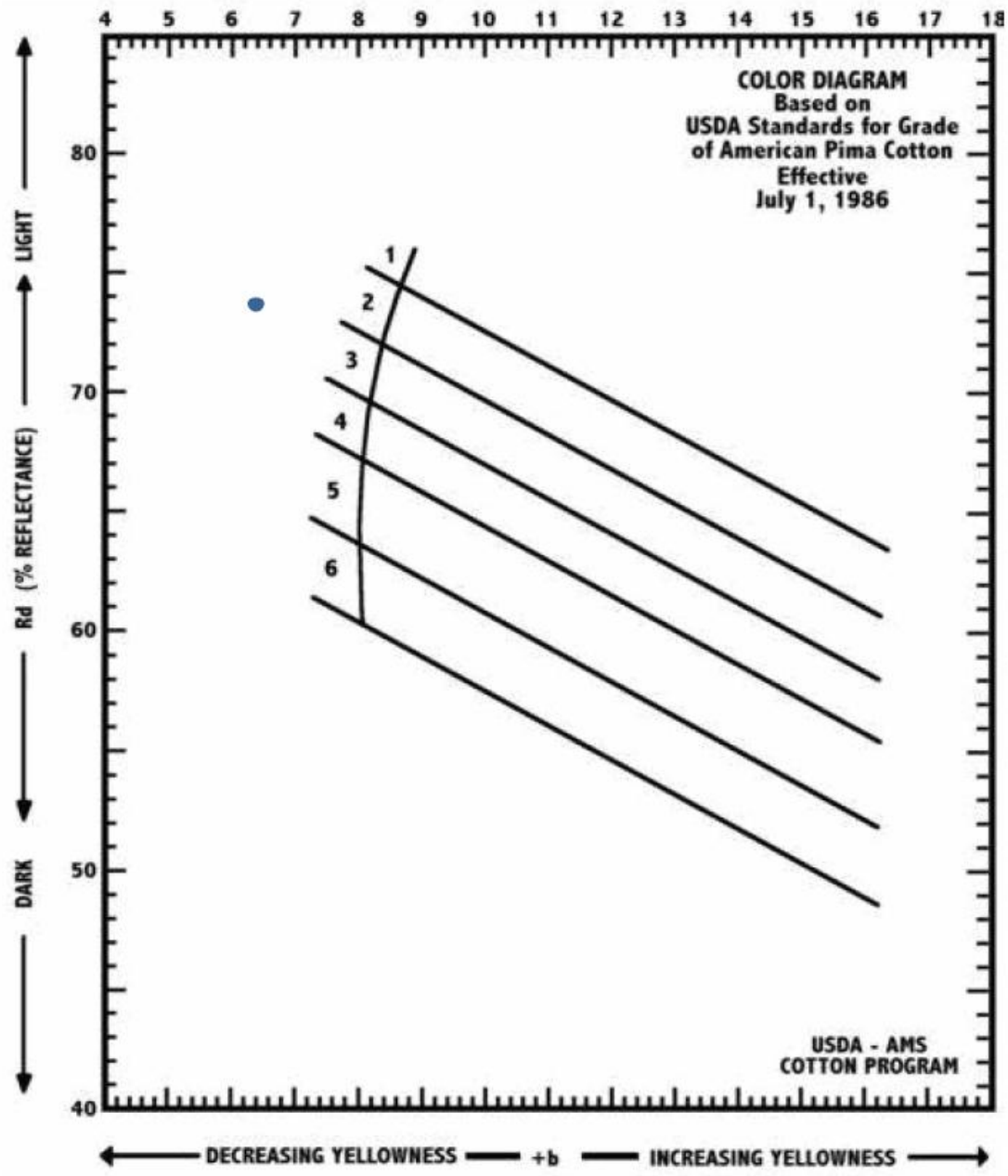


Figure 2. HVI Colour chart for ELS cotton



Infrared and Raman analysis of cottons

There are three ways in which ethylene oxide can interact with cotton after a fumigation treatment.

1. Ethylene oxide can be retained unchanged within the cotton. This residue may persist for some time before final disappearance as a result of volatilization.
2. The formation of small molecules including ethylene chlorohydrins, bromohydrin and ethylene glycol. These species form by the reaction of ethylene oxide with inorganic halides or water present within the cotton.
3. Ethylene oxide can react with the hydroxyl groups of the cotton itself forming alkylated or hydroxyethylated derivatives. The reaction of ethylene oxide with foodstuffs has been known to affect colour, taste and texture [5].

Among the possible reactions presented above, a few can take place under the mild conditions of fumigation. Hydrolysis of ethylene oxide by water present in the cotton and surrounding air can be catalysed by acid which is likely present in raw cotton from the microbial degradation of plant matter. The presence of acid would also likely catalyse the reaction of ethylene oxide with the hydroxyl groups of the cellulose molecules comprising the cotton. Ethylene oxide can undergo a polymerization reaction forming low molecular weight glycols. This reaction is accelerated by the presence of water [6].

Infrared spectroscopy was used to determine if any chemical or structural differences could be detected between the ethylene oxide and untreated cottons that exhibited colour differences. Infrared spectroscopy is sensitive to chemical changes such as the dervativation described above as well as fibre oxidation and the degree of perfection in the lattice structure of the crystalline regions [7].

An investigation was carried out using the Attenuated Total Reflectance (ATR) technique which provides information from the top few microns of the fibre surface. Typical ATR spectra are shown below.

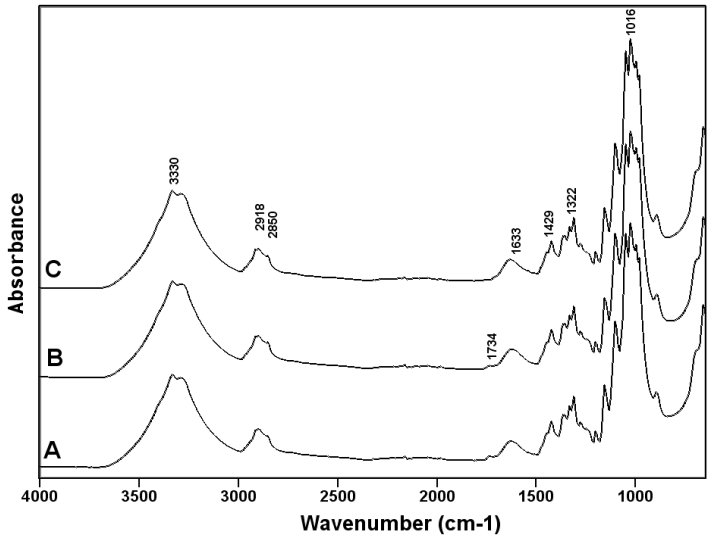


Figure 3. Infrared ATR spectra obtained from cotton wax extracts: A) Aussie 2 untreated, B) Aussie 2 treated at 21°C after 5 days and C) Aussie 2 treated at 50°C after 5 days.

The spectral features can be assigned as follows: 3330 cm^{-1} intermolecular hydrogen bonded O-H stretching, 2918, 2860, 1439 and 1322 cm^{-1} vibrations of the CH_2 groups, the strong multi-component feature centered near 1016 cm^{-1} C-O-C β-glycosidic linkages, alcoholic C-O stretch and C-C and C-O-C ring modes [8].

The only differences found between the spectra shown in Figure 1 can be attributed to differences in adsorbed water (1633 cm^{-1}) and in ester content (1734 cm^{-1}), the latter likely being a fatty acid ester contaminant. These features were found to vary randomly, even between sub-samples of the same cotton and thus cannot be related to the effects of the treatments.

Raman spectroscopy is complementary to the infrared technique in that while infrared is sensitive to changes in functional groups, Raman is more sensitive to the conformation of the molecular backbone and bond with high electron polarizability such as conjugated C=C bonds. This type of bonding is typical of that often found in dye chromophores. Raman spectra obtained from a series of cotton samples collected 5 days after treatment are shown below.

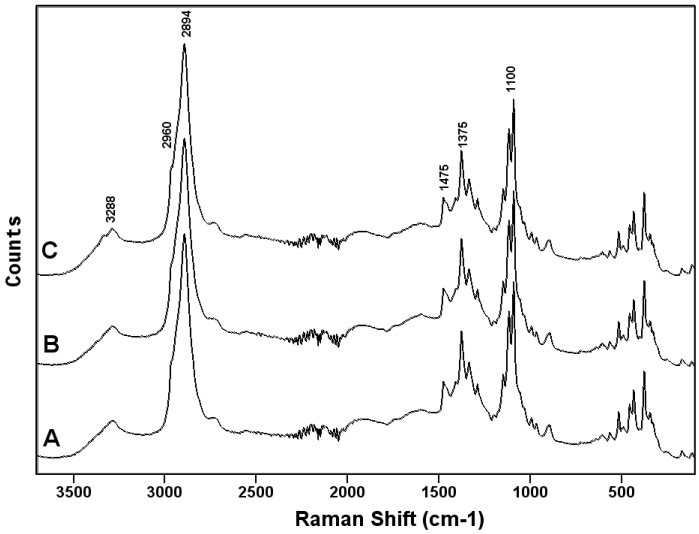


Figure 4. Raman spectra obtained from cotton wax extracts: A) Aussie 2 untreated, B) Aussie 2 treated at 21°C after 5 days and C) Aussie 2 treated at 50°C after 5 days.

The strong features at 2960 and 2894 cm^{-1} as well as the weak feature at 1475 cm^{-1} and the moderate feature at 1375 cm^{-1} can be associated with CH_2 groups [9]. The moderate feature at 1100 cm^{-1} is assigned to the β-glycosidic linkages [6] which are part of the cellulose backbone. The broad weak feature at 3288 cm^{-1} reveals the presence of hydrogen bonded C-O-H groups on the α-D-glucose rings which are also part of the backbone [6]. No evidence for the presence of dye chromophores could be detected and no differences were found between the treated and untreated cottons.

Extraction and analysis of cotton wax

Cotton wax extractions were undertaken in an attempt to determine if the colour or reflectance changes detected in the cotton by the HVI measurements could be associated with the wax degradation or reaction of the cotton wax with the ethylene oxide.

Colour measurements were made for cotton fibres both before and after extraction. Yellowness Index (YI-E313), Δb* and ΔE (CMC 2:1) were determined. Reflectance (Rd) was not determined for these samples, however L* values have been shown to strongly correlate with Rd values [10]. The results are shown in Table 9.

Table 9. Typical mean colour differences obtained after wax extraction by the Conrad method

Sample	Delta (Δ)			
	YI-E313	b*	E (CMC 2:1)	L*
Aussie 2 Untreated 5 days	-1.02	-0.33	0.68	1.62
Aussie 2 Untreated 60 days	0.45	0.21	0.20	-0.03
21°C Aussie 2 5 days	0.28	0.26	0.53	1.33
21°C Aussie 2 60 days	0.28	0.20	0.27	0.54
50°C Aussie 2 5 days	-0.52	-0.15	0.4	0.94
50°C Aussie 2 60 days	-0.88	-0.34	0.36	0.32

There are no significant differences between the yellowness of the extracted and un-extracted cottons at the 95% confidence limit. The ΔL* results suggest that the extraction of the cotton wax has increased the brightness of the cotton irrespective of whether the cotton was fumigated or not. The effect appears to be reduced for the aged cotton but none of the ΔL* values obtained for the treated – untreated pairs are statistically significant at the 95% confidence limit. The extracted wax was analysed by infrared spectroscopy as due to its low concentration on the cotton fibres surface very little information about its chemical state would be gleaned from the infrared spectra obtained from the cotton itself. Typical spectra of the extracted waxes are shown in Figure 5.

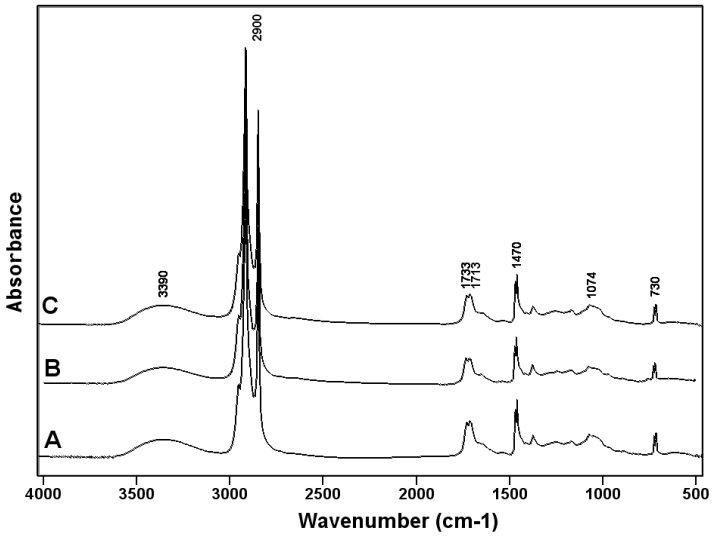


Figure 5. Infrared spectra obtained from cotton wax extracts: A) Aussie 2 untreated, B) Aussie 2 treated at 21°C after 5 days and C) Aussie 2 treated at 50°C after 5 days.

These spectra are typical of cotton wax [11] and exhibit dominant CH_2 stretching vibrations near 2900 cm^{-1} with the corresponding deformation and rocking vibrations observed at 1470 and 730 cm^{-1}, respectively. Other significant features include the broad –O-H stretching mode at 3390 cm^{-1}, ester and free acid C=O stretching modes at 1733 and 1713 cm^{-1} and C-O-H stretching modes at 1074 cm^{-1}. These features are indicative of the presence of components including fatty acids and esters, hydrocarbon waxes and fatty alcohols.

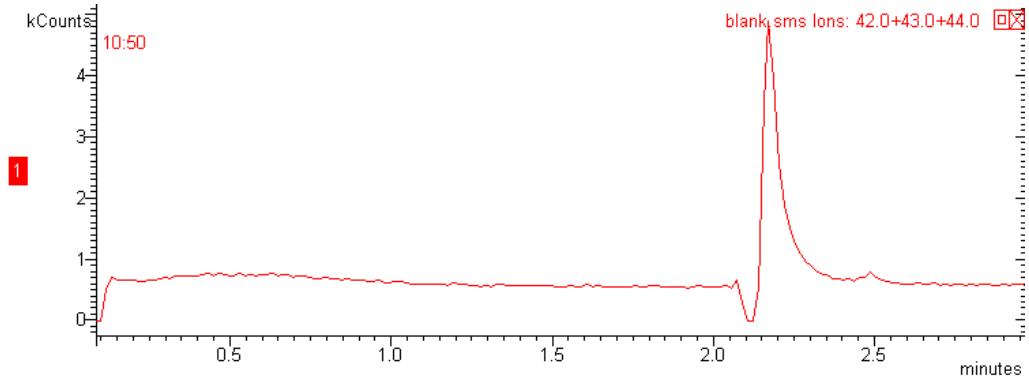
Possible signs of oxidation could include intensity changes in C-O-H, C=O and C-OH stretching vibrations. Ethylene oxide has the potential, in the presence of heat or alkaline catalyst, to react with free fatty acids present in the wax to form mono- and di-esters [12]. This reaction is unlikely under the conditions used during the fumigation process. No change in the relative intensities of free acids to esters can be detected.

In summary, no significant differences can be detected between the cotton fibres or the cotton wax extracts obtained from the treated and untreated samples.

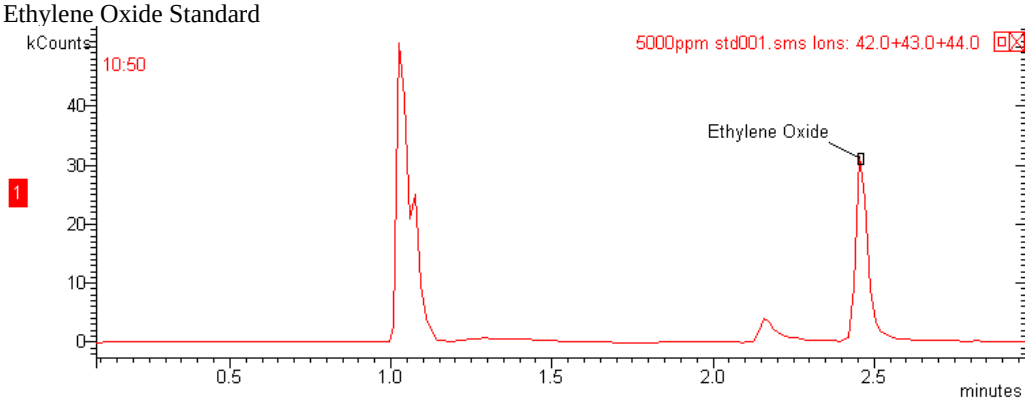
GC/ MS analysis of cotton

GC/MS analysis of the vapours given off by the cotton samples after incubation at 100^{o}C for 1 hour was carried out in order to determine if there was any residual ethylene oxide present from the fumigation. Under the chromatographic conditions used, ethylene oxide had a retention time of 2.46 minutes and the total run time was 3 minutes. Typical chromatograms are shown as Figure 6. The chromatogram shown as the top trace is typical of a blank run where no cotton was present. Only a weak peak is evident at a retention time of about 2.16 minutes. The middle chromatogram trace shows the ethylene oxide standard eluting as expected. The lower chromatogram trace is from the analysis of a cotton sample 3 months after fumigation at 21^{o}C. From this result it is clear that even after storage in under standard conditions there is a residual of ethylene oxide in the cotton.

Blank – No Cotton in Vial



Ethylene Oxide Standard



Sample – 21^{o}C, Aussie 2 at 3 months

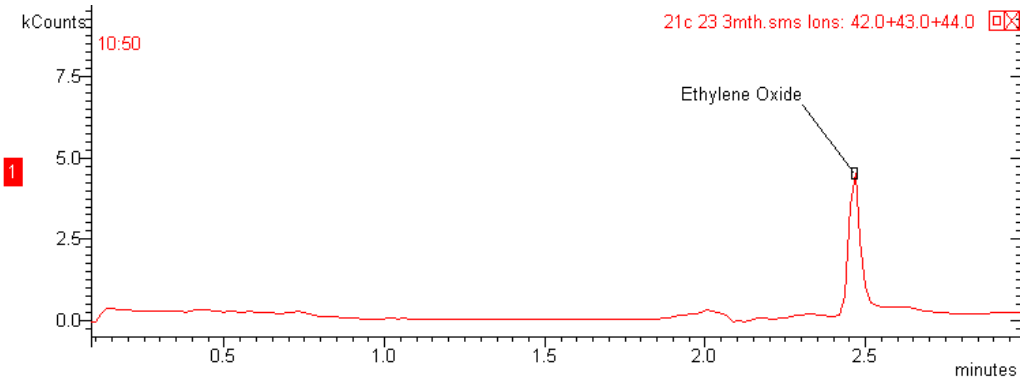


Figure 6. Typical chromatograms: blank chromatogram (top), ethylene oxide standard (middle) and Aussie 2 cotton sample treated at 21°C and sampled after 3 months (bottom).

The residual ethylene oxide concentration in milligrams per kilogram (mg/kg) detected on the fumigated cotton are given in Table 10.

Table 10: Residual ethylene oxide detected after treatment

Sample	Ethylene Oxide concentration (mg/kg)					
USDA Grade	21°C 5 days	21°C 60 days	% change	50°C 5 days	50°C 60 days	% change
11	6.9	7.3	5%	4.1	3.8	-8%
21	4.0	2.8	-29%	4.3	3.9	-10%
31	4.2	11	151%	4.8	3.0	-36%
41	6.0	6.6	10%	3.8	3.5	-8%
51	7.8	7.6	-2%	3.8	2.4	-38%
61	6.3	4.9	-23%	4.3	1.8	-59%
71	6.7	3.7	-45%	2.8	2.1	-25%
23	7.1	4.1	-42%	2.9	1.8	-36%
33	2.6	5.4	110%	3.9	2.7	-32%
43	7.9	2.2	-72%	3.2	4.9	52%
53	11	6.6	-42%	4.3	4.0	-7%
63	8.1	8.4	5%	7.3	3.3	-55%
34	7.0	3.7	-46%	2.7	1.9	-27%
44	6.7	7.2	8%	4.7	4.4	-6%
54	2.4	4.5	87%	1.8	1.5	-19%
Pima 1A	4.1	2.8	-31%	2.4	1.2	-47%
Pima 1B	2.4	1.5	-37%	2.3	4.4	94%
Pima 2A	3.1	2.1	-33%	2.1	1.4	-31%
Pima 2B	6.3	2.4	-62%	3.6	2.0	-45%
Pima 3A	17	3.3	-81%	5.1	2.8	-46%
Pima 3B	8.0	4.1	-49%	2.0	3.5	76%
Pima 4	6.3	2.5	-60%	2.0	3.4	66%
Pima 5A	4.9	3.0	-39%	3.2	2.8	-14%
Pima 5B	2.5	1.2	-50%	3.9	1.8	-53%
Pima 6	2.1	2.4	11%	2.5	2.1	-15%
Aussie 1	2.3	1.4	-38%	4.0	3.2	-18%
Aussie 2	4.6	1.6	-65%	6.4	2.7	-58%
Aussie 3	3.6	2.0	-46%	3.9	2.4	-37%

Residual ethylene oxide was detected on all cotton samples, although there were no apparent trends, the residual levels of all treated cottons changed with time,

For the US Upland White cottons treated at 21^{o}C, fifty seven percent showed decreased ethylene oxide levels while in the case of the Spotted colour cottons 60% showed decreases with time. Thirty three percent of the Tinged colour cottons showed decreased ethylene oxide levels. The majority of the Pima cottons, with the exception of Pima 6, showed decreased ethylene oxide levels. All the Australian cottons showed decreases.

For the US Upland White cottons treated at 50^{o}C, all showed decreased ethylene oxide levels with time and in the case of the Spotted colour cottons, 80% showed decreased ethylene oxide levels with time. The Tinged colour cottons all showed decreased ethylene oxide levels with time. The majority of the Pima cottons, with the exception of Pima1B, 3B and 4, also showed decreased ethylene oxide levels. All the Australian cottons showed decreasing ethylene oxide levels with time.

As presented earlier in this report, the US cottons whose colour was most affected (reduction in Rd) by the fumigation process were the Upland USDA Grades 11, 21 and 31 as well as the ELS USDA cotton grades Pima 1A-3B. The ethylene oxide content of a majority of these cottons decreased with time after treatment. The Grade 11 cotton treated at 21^{o}C showed a 5% increase in residual ethylene oxide over the 50 days while over the same time period the cotton treated at 50^{o}C decreased by 8%. For the Grade 21 cotton treated at 21^{o}C, the ethylene oxide residual decreased by 29%, while the same cotton treated at 50^{o}C decreased by only 10%. The Grade 31 cotton treated at 21^{o}C exhibited the largest increase (151%) while the 50^{o}C treated cotton decreased by 36%. The ethylene oxide residuals in the Pima cottons treated at 21^{o}C all decreased by a minimum of 31%, whereas all the Pima cottons treated at 50^{o}C also decreased by a minimum of 31% with the exception of 2B and 3B which increased by a minimum of 76%.

It is interesting to compare the ethylene oxide residuals detected on the cotton samples to those detected on wheat flour and other comedies including sultanas, cocoa beans, ground nuts and lentils after fumigation treatments. In general it was found that when these materials are freely aired at 25^{o}C, the ethylene oxide levels dropped below a residual of 1 parts per million (ppm) within 14 days (for solids, 1 ppm = 1 mg/kg) [5]. These levels of ethylene oxide are considerably lower than those detected for even the 50 day aged cotton samples. When fumigated wheat was sealed in air–tight conditions a residual of 50-100 ppm was detected after 14 days and traces were found after 90 days. At lower temperatures the ethylene oxide was found to dissipate more slowly.

The change in the reflectance values of the Upland USDA Grades 11, 21 &31 appears to be permanent as the lower Rd values have lasted for longer than 3 months. We surmise that the cause of this could be associated with the ethylene oxide damaging the surface wax layer causing it to become pitted and less smooth resulting in the surface of the fibre reflecting light more diffusely. As the Australian cotton is whiter, with lower +b values it seems that the damage by the ethylene oxide is not as apparent as noted with the US cottons. This hypothesis will be tested in future work.

From the variability of the results reported above and the lower level of ethylene oxide found in wheat and other commodities after fumigation, it is clear that further work needs to be carried out on cotton to further refine our understanding of the effects of the fumigation process. One of the major drawbacks of the current study is that the fumigation and testing was carried out at multiple locations. This required the cotton to be shipped back and forth to different labs several times during the experimentation. Based on the current results it is clear that only colour measurements are actually required to further this study and these can be carried out using a spectrophotometer instead of a HVI instrument. It is probable that with more stringent controls of the fumigation process and sample analysis a correlation between ethylene oxide content and cotton colour may be found.

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.

This study has shown that the fumigation of cotton lint samples with ethylene oxide for 24 hours at 21^{o}C and 5 hours at 50^{o}C, as per AQIS requirement had no effect on the physical properties (such as length, strength and Micronaire) of the fibre. The study however found that fumigation with ethylene oxide did result in changes in the colour value and subsequently the colour grade of the cotton. In most cases the reflectance value (Rd) decreased while the yellowness (+b) was unaffected, which in essence means that the fibre has become darker. This was most apparent for the Upland USDA cotton Grades 11, 21 and 31 as well as the ELS USDA cotton grades Pima 1A-3B. The change in the Rd values appears to be permanent and it is interesting to note that the Australian cotton which is generally whiter than the US cotton seemed unaffected by the fumigation treatments, with only a slight change to the +b value, with the cotton becoming slightly yellower.

Work in this area has provided the CCAA with information to successfully lobby AQIS to change import requirements for calibration cotton from the USA.

6. Please describe any:-
   a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
   b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
   c) required changes to the Intellectual Property register.

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?

Work in this area has provided the CCAA with information to successfully lobby AQIS to change import requirements for calibration cotton from the USA.As part of their treatments for cotton, AQIS has added a condition for raw cotton fibre imported as a sample to calibrate HVI machines (condition PC6465) which allows classing facilities to import calibration cotton from the USA which will not be treated if the requirements as stipulated on their import permit are met. This has resulted in an improvement in the performance of the Australian HVI instruments participating in the CSITC Round Trials, as well as providing confidence in the calibration and qualification of the HVI instruments.

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

8. 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)

   B. Have you developed any online resources and what is the website address?

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Any cotton imported into Australia is treated to ensure that the consignment is free of live insects, soil and other debris (faeces, animal materials etc) and to verify that any quarantine risk material present will be dealt with during processing. The quarantine treatments used can be either gamma irradiation or chemical (fumigation). Following previous studies AQIS have agreed to suspend the treatment of samples with gamma irradiation due to the resultant damage to the physical fibre properties. There are two quarantine treatments currently prescribed by AQIS which involves chemical (fumigation) with ethylene oxide. The two treatments involve fumigation under an initial minimum vacuum of 50 kilopascals at 1200 g/m³ for 5 hours at 50°C or at 1500 g/m³ for 24 hours at 21°C.

This study has shown that the fumigation of cotton lint samples with ethylene oxide for 24 hours at 21°C and 5 hours at 50°C, as per AQIS requirement had no effect on the physical properties (such as length, strength and Micronaire) of the fibre. The study however found that fumigation with ethylene oxide did result in a permanent change in the colour value and subsequently the colour grade of the cotton. In most cases the reflectance value (Rd) decreased while the yellowness (+b) was unaffected, which in essence means that the fibre has become darker. This was most apparent for the Upland USDA cotton Grades 11, 21 and 31 as well as the ELS USDA cotton grades Pima 1A-3B. These changes in the reflectance values will result in the HVI instrument wrongly classifying the cotton one grade higher (i.e. worse than), for example the Grade 11 cotton will be graded 21 and the 21 will be graded 31.This will lead to the instrument failing to calibrate and it will also impossible to qualify the instrument. It is however interesting to note that the Australian cotton which is generally whiter than the US cotton seemed unaffected by the fumigation treatments, with only a slight change to the +b value, with the cotton becoming slightly yellower.

Although the results vary considerably and there are no definite trends, in general this change in the Rd value is related to a decrease in residual ethylene oxide. We surmise that the cause of this could be associated with the ethylene oxide damaging the surface wax layer causing it to become pitted and less smooth resulting in the surface of the fibre reflecting light more diffusely. As the Australian cotton is whiter, with lower +b values it seems that the damage by the ethylene oxide is not as apparent as noted with the US cottons. This hypothesis will be tested in future work.

We recommend that further work needs to be carried out on cotton to further refine our understanding of the effects of the fumigation process.

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