INTERNATIONAL WOOL TEXTILE ORGANISATION

TECHNOLOGY & STANDARDS COMMITTEE **CAIRO MEETING**

Commercial Technology Forum **May 2006**

Chairman: A.G.DE BOOS (Australia) **Report No: CTF 2**

Relationship between greasy and processed dark fibre contamination from Damara crossbred lambs in Merino wool

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SUMMARY

Merino fleeces from 10 groups of sheep exposed to various breeding scenarios with Damara rams and Damara crossbred lambs were tested by SARDI for dark fibre as individual animals using staple wool (based on DTM-13-01) and core sampled as groups then worsted processed by CSIRO-TFT. The cores and tops were tested by AWTA Ltd. with the benzol method and the tops also tested by Centexbel using the Optalyser method (IWTO-55-99).

The combined fleece measurements for each batch were closely related to the dark fibre content of top measured by the benzol method and by the Optalyser. Single tests on core sample wool (20g) explained 97% of the variation in the benzol top test and 95% of the Optalyser test. Around 40% to 50% of the dark fibre in the core sample was detected in the top. This case study adds to evidence that presale testing of wool lots for dark fibre is feasible and also that dark fibre testing of tops could be undertaken with the benzol method.

It is confirmed that contacts with Damara crossbred lambs can produce Merino wool contamination that persists in worsted processing to downgrade the quality of the top produced. This result substantiates a risk penalty applied for this breed type on farm within the Dark and Medullated Fibre Risk scheme.

The benzol and Optalyser dark fibre tests on top show close relationships with the combined dark fibre counts from testing individual fleeces using the CSIRO-DFD balanced lighting method (DTM-13-01) and with the benzol core test. Such clear relationships highlight the potential of the benzol method as a means of determining dark fibre levels in greasy wool and tops.

INTRODUCTION

Damara fat tail sheep were introduced to Australia in 1996 and adopted by some Merino producers wanting to diversify due to low wool prices. Michell Australia Pty. Ltd. and SARDI initiated research in 1999, as greasy wool lots from such flocks were starting to appear in the market, to assess the level of contamination involved and provide a basis for wool industry concerns (Fleet *et al.* 2001, 2002b). Following this initial research, revisions to the AWEX Code of Practice for Clip Preparation (AWEX 2004) for bale branding, specification and vendor declaration requirements were introduced, with woolclasser education and producer awareness projects that continue for adoption of the Dark and Medullated Fibre Risk Scheme (Hansford *et al.* 2003). AWTA Ltd. started wool sale lot assessments in 2001 (Fleet *et al.*

2002c) and has since developed a lower cost test for dark and medullated fibres and applied it to larger samples of Australian wool lots (AWTA *et al.* 2004; Balasingam and Mahar 2005).

While the practice of using Damara for crossbreeding with Merino ewes continues, the use of fat-tail and fleece-shedding sire breeds represents a small minority of matings to Merino ewes (Curtis and Croker 2005). However, a substantial proportion of the Australian Merino ewe flock is mated to sire breeds other than traditional Merino; some of which may be causing wool contamination (Fleet 1998). Hooper and Connell (2001) surveyed broad acre farms with more than 200 sheep in 1999/2000 and found 65% of Merino ewes were mated to Merino rams, Curtis and Croker (2005) in a sample of 1700 producers with 500 or more sheep in 2005 found that 73% of Merino ewes were joined to Merino rams and ABARE (2005) reports that nearly 25% of Merino ewes were mated to short and long wool rams to produce first cross lambs in 2004/05.

Apart from a finding that effects of transferred fibre from Awassi sheep are minimised by the worsted process (Hatcher *et al.* 1999), there is a need to validate the benzol test so it can be interpreted in terms of processing expectations. SARDI retained since 1999, for future processing evaluation, the Merino fleeces used for assessing contamination arising from Damara crossbreeding. In a collaborative initiative with AWI and AWTA Ltd these wools have now been mini-scale processed to top by CSIRO-TFT and are being measured for dark and medullated fibre contamination.

The hypothesis examined in this report is that Damara crossbred dark fibre persists in worsted processing to affect wool tops as measured by the benzol and Optalyser dark fibre tests.

MATERIALS AND METHODS

Fleet *et al.* (2001, 2002ab) describe the sheep and management on farm. Briefly, 5 management groups are involved as follows:

- Batches 1 and 2: Merino ewes single-sire mated to Merino rams (2) and shorn with 8-months wool growth at the end of mating.
- Batches 3 and 4: Merino ewes single-sire mated to Damara rams (2) and shorn with 8-months wool growth at the end of mating.
- Batch 5 and 6: Merino ewes that reared a Damara crossbred lamb(s) and shorn with 8-months wool growth 3.5 months after weaning of the lambs.
- Batches 7 and 8: Merino ewes that reared a Damara crossbred lamb(s) and shorn with 7.5 months wool growth the day after weaning of the lambs.
- Batches 9 and 10: Merino lambs that had run continuously (including 8 weeks in a feedlot) with Damara crossbred lambs for 7-months.

The Damara crossbred lambs were mostly coloured and shed mainly pigmented fibre. The unskirted fleece (excluding belly wool) from each ewe and wool shorn from each lambskin (after slaughter) were individually bagged.

Care was taken to ensure urine stain was removed pre-shearing or that the crutch-shear interval was short. This precaution is clearly important for minimising dark fibre risk (Hansford *et al*. 2003) and also critical for the relationships with dark fibre in top when these tests cannot discriminate between different types of colouration (i.e. fibres coloured by melanin pigment from fibres coloured by urine stain).

Fleece measurement

Fleet *et al.* (2001 and 2002b) and Fleet and Bennie (2002) describe the fleece sampling and measurement procedure. Briefly, from each fleece within management groups, 104 staples were grid sampled and aqueous scoured in fine mesh bags. The number of scoured staples measured was based on anticipated content of dark fibre. For batch 1 to 4, all of the scoured staples (104) for each fleece

sample were inspected and the average weight was 41g; batch 5 and 6 had a sub-sample of 20g and batch 7 to 10 a sub-sample of 10g of the scoured staples inspected for each fleece.

The inspection method was based on DRAFT TM-13-01 using the CSIRO Dark Fibre Detector with staple webs inspected between glass plates in air.

Fibres with a darkened section ≥ 3mm were scored for darkened length (A = 3mm to 10mm; B >10mm) and CSIRO Darkness Level, and extracted and mounted on clear adhesive tape with clear transparency overlay. The extracted fibres were examined with a microscope for confirmation of melanin pigmentation. Fleet *et al.* (2001) and Fleet and Bennie (2002) provide details on the characteristics of the pigmented fibres. For example, 87% of the pigmented fibres had CSIRO Darkness Fibre Scale, levels 7 and 8 (dark tan and black) that correspond to Very Dark Fibre (VDF) as assessed by Optalyser (Longree *et al.* 1996).

The individual measurements for the fleeces comprising each processing batch were combined in a manner to reflect their expected weighted contribution of pigmented fibre. For the purpose of this report the combined fleece measurements for each batch will be referred to as the staple test and for this test the dark fibre referred to consists of only melanin-pigmented fibres.

Processing batches

Preparation and sampling of the groups of fleeces was in the order 1 to 10 with a thorough initial cleaning of facilities and cleaning between management groups. Each of 12 or 13 fleeces from each batch was grid sampled to provide another reference sample of 104 staples. Fleeces with weights exceeding that needed for processing (22 to 25kg of greasy wool) were reduced to the required amount by grid sampling further staple wool. The fleeces for each batch were then progressively combined in 2 stages and the wool from each stage pneumatically cored in a mini-scale press, and the cores from both stages progressively sampled and combined to produce 2 samples of 250g.

The ten batches were processed to top by CSIRO-TFT, with other batches consisting of display samples from single sale lots compiled by AWTA, in order of increasing contaminant fibre level. However, after scouring some brands were noted and removed from batches 7 and 8. As a precaution, batch 8 was placed after batch 10 in the processing order. Although samples of noil and card wastes were collected separately for each batch, only the results of the tops were available for this report.

Hoschke and Smith (1982) and Hansford (1997) provide details of the mini-scale processing which involved a single combing with a ratch setting 34mm on an NSC PB29 comb. Thorough cleaning of all equipment used (individual components of each machine) and the surrounding environment was performed throughout all stages of the processing. This was done to ensure that no extraneous contamination and no cross contamination would occur between batches. The processed samples were delivered by CSIRO to AWTA Ltd for sampling and the balance was delivered to SARDI.

AWTA measurements

Core test

Core sample wool was tested as described in AWTA *et al.* (2004). Two x 10g samples (oven dry weight) of cleaned core wool was tested from each batch. The threshold for detection of darkness was established using the CSIRO Dark Fibre Scale, level 6 (Foulds *et al.* 1982 and 1984); the threshold for fibre length was 3mm (i.e. only fibres with darkened length >3mm were counted). Using this technique, which was initially developed by CSIRO with funding from AWI, benzyl alcohol is added to a sample of wool in a specially designed plastic bag. The bag is sealed and then mounted on a purpose-built stage for manual inspection. This AWI:AWTA technique is referred to as the benzol method in this report.

Top test

Wool tops were tested using the benzol method (i.e. substantially the same technique as used in the core test procedure). Three changes to the core test procedure were that:

1. the samples were not Shirley Analysed prior to testing. A 5g (oven dry weight) sample of top was spread evenly over an area of approximately 300mm x 100mm prior to insertion into the bag;
2. the minimum darkened length to be included as dark fibre was increased from 3mm to 10mm in line with specifications set out in current IWTO Methods for detection of Dark Fibres in tops (viz IWTO-55-99 and Draft TM-13-01 both of which specify a minimum dark fibre length of 10mm); and
3. a total of six (6) 20g (oven dry weight) samples, rather than (1) 20g sample was tested per batch.

Optalyser measurements

At SARDI a single ball from each batch was covered in a fine mesh bag and allowed to condition (20^{o}C and 65% RH) before collecting samples for Opatalyser (IWTO-55-99) measurement. A 20m length of sliver (measured on a bench) was drawn off the ball and weighed. On the basis of the determined weight per unit length (tex) a further length of sliver was drawn off to provide around 300g for the Optalyser measurement. Centexbel measured dark fibre in top using the Optalyser instrument (using the supplied tex data) with each measurement involving the mean of 5 subsamples each of about 25g; except one subsample reduced to 13g due to sliver preparation problems**.** Note that previous trials have demonstrated that Optalyser classification for VDF relate directly to CSIRO Dark Fibre Levels 7 – 8 and DF relates to CSIRO Dark Fibre Level 5 – 6 (Delfosse *et al.* 1992; Longree *et al.* 1996).

Statistical analysis

In view of the AWTA samples being based on an oven dry weight these measurements were adjusted for 16% regain to provide a similar basis. The SARDI, AWTA and Optalyser data for dark fibre were correlated and various simple linear regression analyses undertaken. Since information was available for length score on the extracted fibres from SARDI fleece sample tests, these scores were each analysed separately and in combination.

RESULTS AND DISCUSSION

Relationship between different tests

The two raw wool tests (staple and core tests) were highly correlated with each other and with the top tests (Table 1). The mean levels of dark fibre contamination for the benzol core and for both top tests (benzol and Optalyser) suggest a trend for reduction in dark fibre levels associated with the wool processing; perhaps through preferential loss of contaminant fibre that has different characteristics to the wool bulk as found in previous work (Hatcher *et al.* 1999).

Clearly, dark fibre contamination from Merino contacts with Damara crossbred lambs is persisting in a predictable manner to wool top in this case study. The high level of persistence is in contrast to the poor persistence (over 89% loss of dark fibre in processing fleece to top) found in Merino wool contaminated from pen contacts with adult purebred Awassi sheep (Hatcher *et al.* 1999). Possible reasons for the different levels of persistence of dark fibre in the two trails include:

- the breed of “exotic” sheep involved (i.e. the often coloured, hair fleece-shedding Damara contrasting with purebred Awassi that have tan coloured legs and face but predominantly white fleece of carpet wool type that requires shearing); and,

- the age of the "exotic" sheep; with the Damara trial involving lambs which generally have a propensity to shed hairy fibre from the fleece. The adult Awassi sheep would have mature carpet wool fleeces and likely only isolated pigmented fibre being shed; and,
- variations in the processing (e.g. comb ratch setting, re-combing) might lead to further reduction of contaminant fibre levels than found in this report. However, even if absolute dark fibre numbers were reduced further by processing manipulations, such precautions would lead to extra costs (increased waste and processing steps) and would be unlikely to completely remove the risk of very objectionable contaminant dark fibres appearing in the end-products.

Table 1: Correlations between the raw wool and top results

Test	Mean (s.d.)	Optalyser (>10mm)	Fleece (>10mm)	Fleece (≥3mm)	Core (≥3mm)
Benzol Top (df≥10mm)	5.8 (6.3)	0.99 ****	0.95 ****	0.91 ***	0.99 ***
Optalyser Top (df>10mm)	5.1 (5.1)		0.98 ****	0.95 ****	0.98 ***
Staples (df>10mm)	11.3 (12.4)			0.99 ****	0.92 ***
Staples (df≥3mm)	20.5 (22.6)				0.88 ***
Benzol Core (df>3mm)	12.9 (12.8)				

Significance: n.s. not significant; *P*≥0.05; * *P*<0.05; ** P<0.01; *** *P*<0.001; **** *P*<0.0001

Table 2 shows the correlations between the length categories for the staple-based test, benzol core and top tests and the Optalyser test. Each length category was highly correlated with the benzol core and both top results. The B-length dark fibres from the SARDI measurements have a higher correlation than the A-length (3 – 10mm) fibres with the top tests (that exclude short dark fibre (≤10mm)). Note the mean dark fibre count for the core test (12.9df/10g) is much higher than the count for either the benzol top test (5.8df/10g) or the Optalyser test (5.1df/10g) but lower than the equivalent length threshold count for the staple tests (20.5df/10g).

Table 2: Correlations between raw dark fibre length, benzol tests and Optalyser

		Staple		Optalyser	
Test	Mean (s.d.)	(3 –10mm)	(>10mm)	DF	VDF
Benzol Top (df≥10mm)	5.8 (6.3)	0.84**	0.95****	0.92***	0.99****
Benzol Core (df>3mm)	12.9 (12.8)	0.81**	0.92***	0.93***	0.97****
Staples (df 3mm – 10mm)	9.2 (10.5)		0.94****	0.73*	0.90***
Staples (df >10mm)	11.3 (12.4)			0.91***	0.98****
Optalyser Top (DF)	0.51 (0.50)				0.92***
Optalyser Top (VDF)	4.6 (4.6)				

Significance: n.s. not significant; *P*≥0.05; * *P*<0.05; ** *P*<0.01; *** *P*<0.001; **** *P*<0.0001

Table 3 provides the regression statistics for the relationships between the staple test (≥3mm or >10mm) lengths and the core test or top tests. Of all fibres counted in the staple test around 26% were detected in the top test. It would be expected that none of the A length fibres (3mm – 10mm) counted in the staple test would be counted in the top test, which only detects fibres ≥10mm, but not only the short fibres are being excluded as only 48% of B-length fibres persisted into top. Optalyser showed similar relationships to the benzol test with 21% for all fibres in the staple test and 40% for B-length fibres being counted. Figures 1 and 2 show the relationship between the combined staple tests for B-length fibres (>10mm)

and the benzol and Optalyser tests on tops. The number of B-length fibres was similar to the benzol core test count and explained more variation in the core test than the staple test with the same length basis (i.e.≥3mm). Furthermore, the core test detected only 50% of that predicted by the staple tests (≥3mm) suggesting fibre loss arising from sampling and preparation. The Shirley Analyser process in the benzol core test could be interfering with the counts by separating short dark fibre. The approximately equal relationship indicated by the slope of 0.95 between the benzol core test and the B-length (>10mm) staple test suggests that the benzol core test is detecting the appropriate levels to match the occurrence of dark fibres which are specified for tops (i.e. those fibres >10mm).

Table 3: Degree of persistence to top depends on the pigmented fibre length

df/10g scoured staples	Model R-square	Slope (s.e.)	Intercept
Benzol Top (≥10mm) v. Staple Test			
All lengths (≥3mm)	0.83 ***	0.26 (0.04) ***	0.52 n.s.
B-length (>10mm)	0.89 ****	0.48 (0.06) ****	0.32 n.s.
Optalyser Top (>10mm) v. Staple Test			
All lengths (≥3mm)	0.90 ****	0.21 (0.02) ****	0.69 n.s.
B-length (>10mm)	0.95 ****	0.40 (0.03) ****	0.57 n.s.
Benzol Core (>3mm) v. Staple Test			
All lengths (≥3mm)	0.78 ***	0.50 (0.10) ***	2.64 n.s.
B-length (>10mm)	0.85 ***	0.95 (0.14) ***	2.16 n.s.

Significance: n.s. not significant; *P*≥0.05; * *P*<0.05; ** P<0.01; **** *P*<0.001; *P*<0.0001***

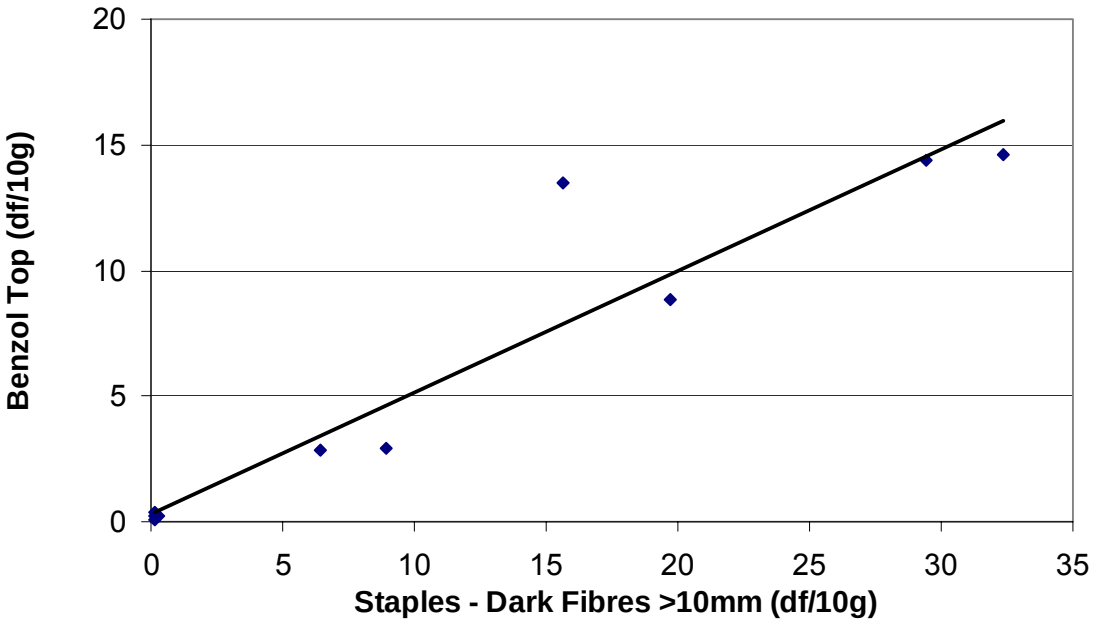

Figure 1. Association of the benzol test on top with the staple test for dark fibre >10mm length

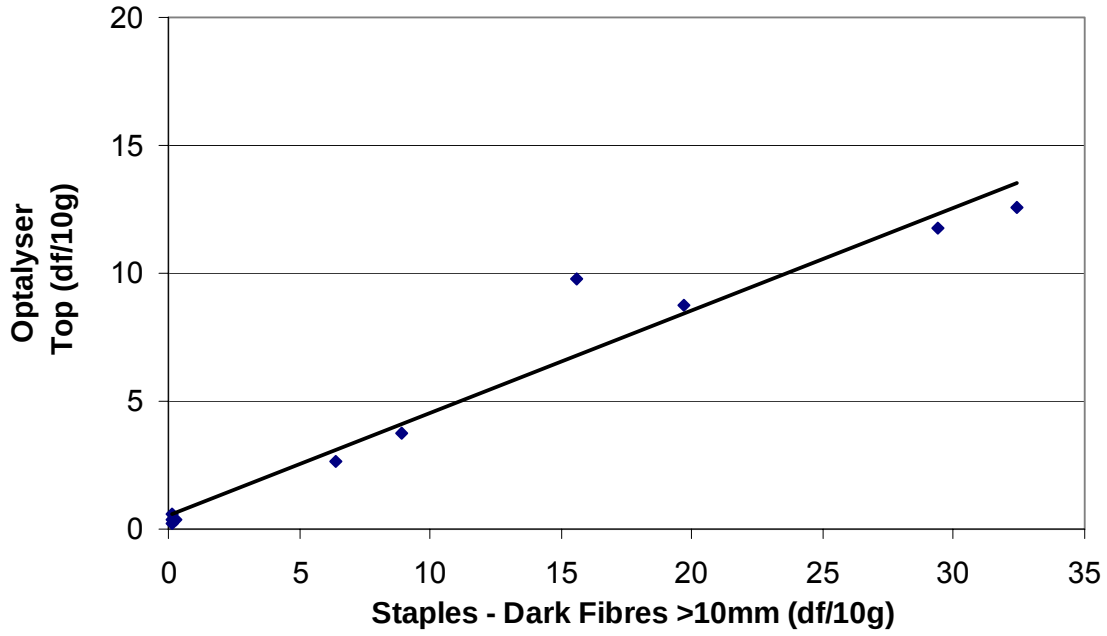

Figure 2. Association of the Optalyser test on top with the staple test for dark fibre >10mm length

Relationship between Benzol and Optalyser tests

Table 4 provides the regression statistics for comparisons made between the benzol core and top tests and the Optalyser tests. The benzol core test explained 95% of the variation in the Optalyser test on top 97% of the benzol test on top. The benzol top test result suggests that around 49% of the dark fibre count from the core test will persist to top (as fibres with darkened lengths ≥10mm) while the Optalyser regression indicates 39%. This difference may be the result of sampling. There are relatively few points on which to base the regression and the Optalyser test is based on a single length of sliver while the benzol tests were derived from several segments of sliver from all 3-balls of top produced for each batch.

Table 4: Comparison of Benzol and Optalyser dark fibre measurements

Benzol Top (≥10mm) v. Benzol Core Test (≥3mm)			
Benzol Core (>3mm)	0.97 ****	0.49 (0.03) ****	-0.51 n.s.
Optalyser Top (>10mm) v. Benzol Core (≥3mm)			
Benzol Core (>3mm)	0.95 ****	0.39 (0.03) ****	0.09 n.s.
Benzol Top (≥10mm) v. Optalyser Top (>10mm)			
Dark fibre (>10mm)	0.84 ***	11.53 (1.76) ***	-0.06 n.s.
Very dark fibre (>10mm)	0.98 ****	1.36 (0.07) ****	-0.45 n.s.
All dark fibre (>10mm)	0.98 ****	1.23 (0.07) ****	-0.49 n.s.

Significance: n.s. not significant; $P \geq 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

Figures 3 and 4 show the benzol and Optalyser tests on top, respectively, in relation to the benzol test on core samples. For batches 1 to 4 at the core test there were <3df/10g and this was associated with very low (<0.6df/10g) contamination in the top. For the other 6 batches, there were between 5 and 30 df/10g in the core test and 30% to 53% of these were detected in top based on the slopes of the relationships shown in Figures 3 & 4.

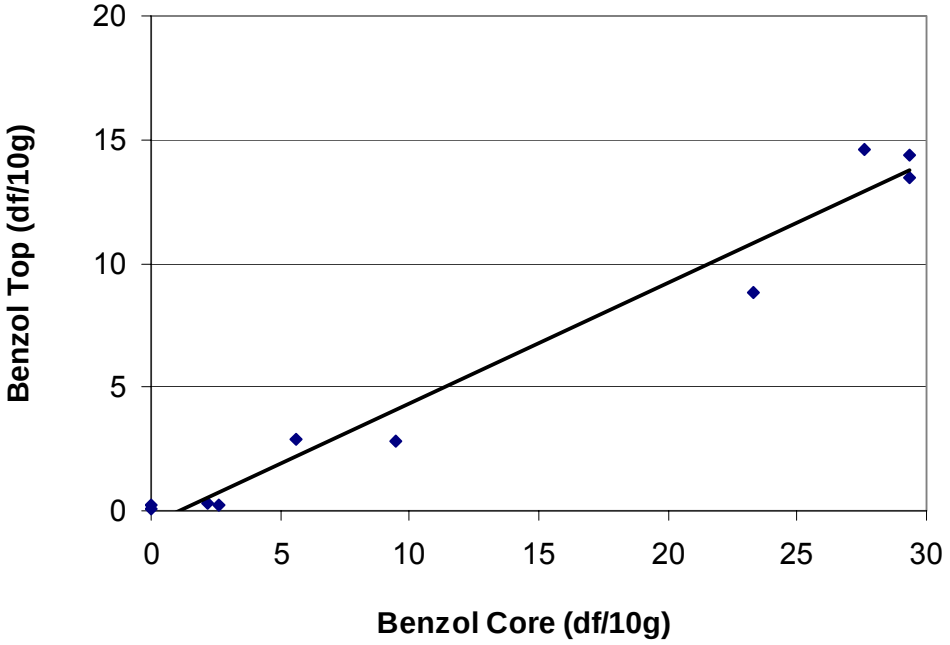

Figure 3. Association between the benzol tests on top and cores

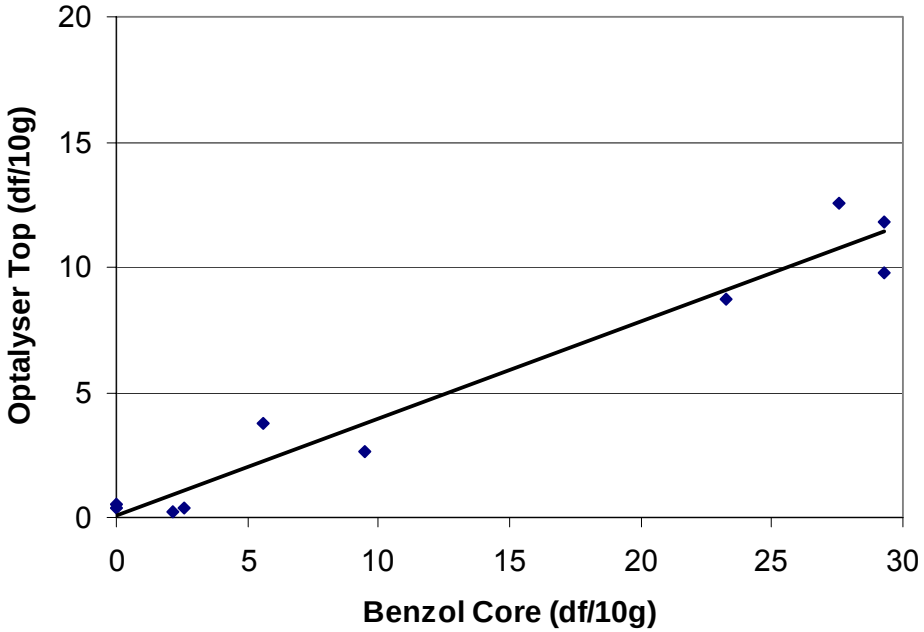

Figure 4. Association between the Optalyser test on top and benzol test on cores

The benzol top test explained 98% of the variation for all dark fibre from Optalyser. Since the Optalyser test was included in this trial as a benchmark with which to compare the benzol top test, this result, and the relationship shown in Figure 5 confirm the excellent performance of the benzol test in this trial.

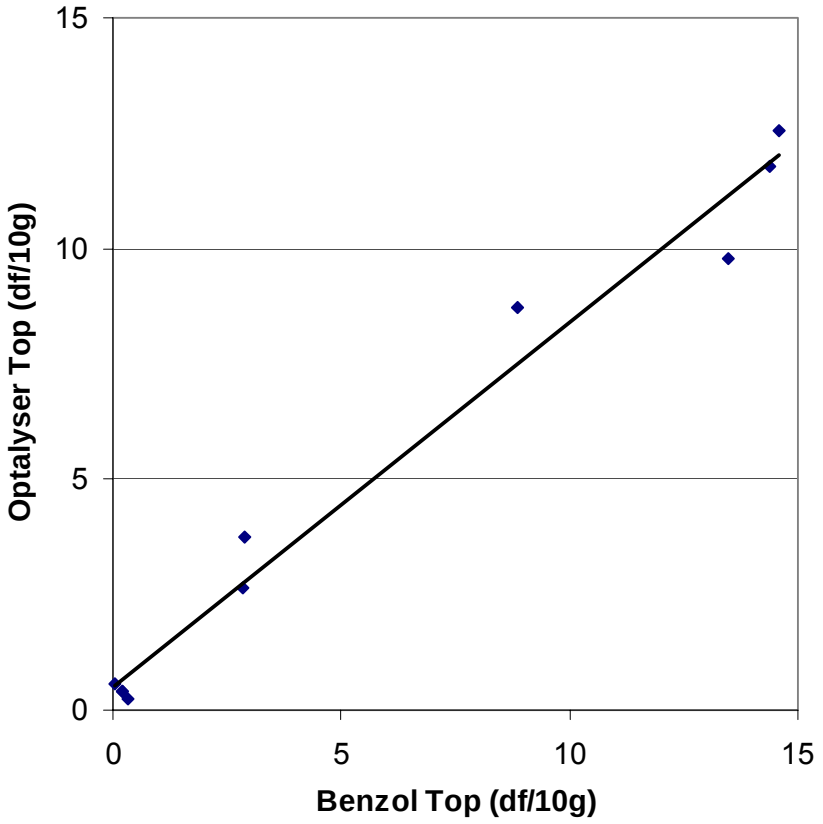

Figure 5. Association between the Optalyser and benzol tests on top

Levels of contamination in different tests

Table 5 provides the dark fibre counts as determined on the different samples (pooled fleece test, batch core and batch top). Based on previous work (Fleet *et al.* 2001; 2002b and Fleet and Bennie 2002) the expected levels of dark fibre contamination in these 10 batches could be grouped into 3 different categories:

- Low levels of contamination for batches 1, 2, 3 and 4;
- Medium levels of contamination for batches 5 and 6; and
- High levels of contamination for batches 7, 8, 9 and 10.

In terms of farm management, mating a single Damara ram in a large mob of ewes (65) for 5.5 weeks (Batches 1 and 2 v. Batches 3 and 4) did not have any detectable effect in the small quantities of top measured. The lack of effect in this case study may arise because there was no prior or subsequent exposure to Damara crossbred lambs in the period of wool growth – just a brief contact with a single Damara ram. Wool from ewes that had reared a Damara crossbred lamb but for which shearing was delayed for 3.5 months (Batches 5 and 6) had some pigmented fibre detected in top (3 to 4 per 10g) that would be considered excessive for critical end use (white or pastel shades) (Foulds *et al.* 1984). In contrast, wool from Merino ewes or lambs shorn immediately after separation from Damara crossbred lambs (Batches 7 to 10) had extremely high levels of pigmented fibre in top (i.e. 9 to 15 per 10 g top).

Table 5: Levels of dark fibres per 10g of scoured wool or top in each batch

Expected Dark Fibre		Staples			Benzol Method				Optalyser Top (>10mm)	
Contamin-ation	Batch	≥3mm	>10mm	Rank	Core (≥3mm)	Rank	Top (≥10mm)	Rank	All DF	Rank
Low	1	0.20	0.12	1	0.0	1	0.04	1	0.56	4
Low	2	0.22	0.16	2	2.16	3	0.34	4	0.24	1
Low	3	0.34	0.26	4	2.59	4	0.22	2	0.40	2
Low	4	0.36	0.16	2	0.0	1	0.22	2	0.40	2
Medium	5	17.65	8.91	6	5.60	5	2.89	6	3.74	6
Medium	6	15.42	6.43	5	9.48	6	2.84	5	2.64	5
High	7	47.15	29.42	9	29.31	9	14.40	9	11.80	9
High	8	65.31	32.39	10	27.59	8	14.57	10	12.56	10
High	9	25.34	15.61	7	29.31	9	13.49	8	9.78	8
High	10	33.40	19.71	8	23.28	7	8.84	7	8.74	7

Table 5 shows how robust the rankings of the four different test methods are. Not once does a ranking cross over the boundary between the Low, Medium and High groups. Each of the two greasy wool tests and two top tests ranks the groups of fleeces in the same order, indicating the contamination in top due to contact with Damara crossbred lambs can be detected in greasy wool. In particular, the benzol method has demonstrated a similar performance to more established detection methods for both greasy wool and top testing.

CONCLUSION

Contamination of Merino wool caused by rearing contacts with Damara crossbred lambs does persist in worsted processing to affect contamination levels in the tops. However, there is a reduction in contaminant fibre levels between raw wool and top, the proportion of which appears to depend on the length classes used for the contaminant fibre.

Farm management procedures can influence levels of contamination, but caution is required in assessing the commercial impact of particular procedures. In this trial, the effect of rearing Damara crossbred lambs was to introduce commercially important levels of contamination in the tops, even though the levels of contamination were reduced by delaying ewe shearing for 3.5 months after weaning.

The benzol and Optalyser dark fibre tests on top show close relationships with the combined dark fibre counts from testing individual fleeces using the CSIRO-DFD balanced lighting method and with the benzol core test. Such clear relationships highlight the potential value of the benzol method as a means of determining dark fibre levels in wool of various forms.

ACKNOWLEDGMENTS

We thank staff at Minnipa Research Centre (SARDI) for the management of the sheep and Tom Ashby who provided the Damara rams. Wendy Fulwood, Tony Fotheringham and Meridith Treloar (SARDI) inspected the fleece samples and Kylie Chenoweth (SARDI) assisted in the preparation and coring of the

processing batches. Judy Turk (formerly of Michell Australia Pty. Ltd.) provided oversight and advice regarding the processing protocol. DMF operators at AWTA Ltd, Dr Martin Prins, Andrew and Dennis Jones and their team at CSIRO-TFT mini-scale processed the batches of fleeces and Dr Michel Longree (Centexbel) measured the wool tops by Optalyser. Dr Paul Swan (AWI) and Ian Ashman (AWTA) provided comments on the original manuscript. Funding for this project is provided by Australian wool producers and the Australian Government through Australian Wool Innovation Limited

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