Study to investigate the feasibility of applying cuprous oxide to reduce sapstain in windthrown trees

REPORT INFORMATION SHEET

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Cover
Storm damage in *Pinus radiata* plantation north of Taupo, May 2011.

EXECUTIVE SUMMARY

Significant damage is sustained when storms occur in pine plantations in New Zealand, particularly as the crop reaches maturity. Much of the consequent economic impact is due to loss in value through sapstain and decay before the impaired timber can be recovered. A research programme is currently underway with the aim of providing information on threshold periods available for salvage operations in separate regions at different times of the year. However, a method of reducing the development of sapstain during this log recovery period would provide an additional management option.

A proposal was approved by the former Forest Biosecurity Research Council to investigate the possibility of applying a fungicide to protect fallen trees from sapstain fungi. This report present the results of a pilot study to spray freshly cut stems with cuprous oxide. If this treatment was successful, the way would then be open to test the effectiveness of an aerial application as a practical means of applying the chemical. An additional comparative treatment using orthene insecticide was included in the initial study because although most sapstain is caused by the wind- and rain-borne *Diplodia pinea*, bark beetles are known to introduce ophiostomatoid fungi which are also responsible for a proportion of the later developing sapstain.

The study was set up by felling 45 trees in a stand due for a late thinning early in February, 2011. One third of the stems were sprayed twice to runoff by hand with cuprous oxide (25g powder - 75% Cu_2O active ingredient - in 1L water plus 20% dothistroma spray oil), one third with orthene (0.8g orthene granules - 970g/kg acephate active ingredient - in 1L water), and the remaining third were left untreated as controls. Treatments were applied 1 day and 6 weeks after felling. In May, 15-16 weeks after felling, 6 discs were cut at 2 m intervals along the length of each stem, the first being taken several centimetres above the felling cut. In the workshop, each disc was photographed, after preparing the surface, and moisture content was determined by weighing a section of sapwood before and after drying. The percentage of the disc surface area affected by sapstain was measured using ESRI ArcGIS 9.3 software (Redlands, California) to outline and measure stain zone polygons on the disc images. Sapstain was defined to include brown radially arranged zones present on nearly all basal discs. Isolations were undertaken from an arbitrary selection of discs to investigate the fungi associated with the blue and brown staining. Data were analysed statistically to determine variation between treatments and between disc positions up the stem.

Although treatment with cuprous oxide initially appeared to be associated with a lower level of sapstain, when values were adjusted for moisture content there was no significant difference between treatments (mean sapstain percent: cuprous oxide, 21%; orthene, 25%; untreated controls, 26%). There were no significant differences between discs, except that the basal disc 1 (mean, 60%) had significantly more

sapstain than all others (means 12-20%). Disc 1 had also dried more rapidly (63% mean moisture content, dry weight basis) than all the others (means 122-161%). Most of the sapstain on disc 1 was due to the radial brown staining which was found to be associated with two unidentified basidiomycetous decay fungi. All the stain fungi isolated from the blue zones were *D. pinea*. There was no evidence that orthene had controlled bark beetle attack, which was low but significant on all treatments.

Unfortunately, the results of this study indicate that extending the work to include aerial spraying is unlikely to prove promising. However, the current region and season sapstain monitoring project is likely to provide much more promising data, judging by progress to date. It is also hoped that the other FBRC-approved study will prove helpful, namely to see if regularly updated climate information can be used to follow the rate of sapstain development during a recovery operation following a damaging storm (in a similar fashion to the manner in which fire risk is monitored).

Study to investigate the feasibility of applying cuprous oxide to reduce sapstain in windthrown trees

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Introduction

Sapstain and associated degrade of wood are the principal causes of economic loss resulting from storm damage in *Pinus radiata* plantations in New Zealand. A recent monitoring study following two storms in a pine forest in Nelson has provided useful information on the periods available for log recovery after such events (McCarthy *et al.*, 2010). This project is now being extended to obtain a broader picture that will include the effect of season and climate region on the rate of deterioration. With this current drive towards improving salvage efficiency, questions have arisen as to whether there may be additional ways of managing recovery operations to reduce losses after such events. One thought is that there might be merit in applying a fungicide at suitable intervals after a storm that will either protect damaged trees or else lower the incidence of spores of sapstain fungi such as *Diplodia pinea*. Is it possible to reduce the rate of wood degradation by periodic aerial spraying with cuprous oxide fungicide, for instance? Copper fungicides, together with a sticker/spreader, are recommended against *D. pinea* on *Pinus* species overseas (e.g. Peterson, 1981; Mulrooney, 2004), and phytotoxicity would not be an issue on fallen stems. It would be a simple matter to divert aircraft from routine aerial control operations against dothistroma needle blight (caused by *Dothistroma pini*) to storm damaged areas, when needed. A commitment was therefore made to the former Forest Biosecurity Research Council (FBRC) to test the feasibility of the idea by undertaking a small, preliminary, hand spray pilot study as follows:

"A sapstain model will predict when sapstain will occur, but it may not be possible to salvage all material before trees become susceptible. Fungicide is routinely applied to stands to control dothistroma needle blight and it may be possible to stop colonisation of logs by sapstain fungi using a similar method. There are a number of factors that make sapstain control difficult, i.e. amount of fungal inoculum present, surfaces of the log may be sheltered from spray, etc. A replicated trial will be undertaken where billets will be hand sprayed at intervals with copper fungicide. Moisture content and sapstain development will be monitored. If proof of concept is achieved an aerial spray trial will be planned for the following year".

It was decided to incorporate an additional treatment using orthene insecticide to prevent attack by bark beetles. Although most sapstain in New Zealand forests results from *D. pinea*, whose spores are air- and rain splash-borne, some is instigated by ophiostomatoid fungi (species of *Ophiostoma* and *Grosmannia*) whose sticky spores are carried by insects (Farrell *et al.*, 1997; Thwaites *et al.*, 2005; McCarthy *et al.*, 2010). Application of orthene would therefore provide a useful comparative treatment with that of the fungicide cuprous oxide.

This report outlines the results of this work and presents the conclusions.

Materials and Methods

Field site and study trees

The study was conducted in a rectangular area of dimensions 45 × 50 m (0.225 ha) on the north side of and adjacent to Burn Road, Cpt. 1049/3, Kaingaroa Forest, in a 10-year-old stand of *P. radiata* due to be thinned to waste. Stocking was 800 stems per ha, to be reduced to 500 stems per ha. The site was flat, level, and with only limited obstructing understorey weed growth. Trees were marked for thinning with the assistance of forest staff. Selected trees were then felled by chain saw on the following day, 1 February, 2011, taking care as far as possible, to leave stem lengths exposed for ease of access. The site was divided into three, roughly parallel, irregular shaped blocks, each enclosing 15 of the felled trees that were tagged for subsequent treatment (45 trees, total). Selected trees were left unpruned.

Treatment

One of three treatments was assigned to each set of 15 felled trees as follows: cuprous oxide (western block), orthene (eastern block) and untreated controls (central block). The formulation mix for each chemical was: cuprous oxide: 25g powder (75% Cu_2O active ingredient) in 1L water plus 20% dothistroma spray oil, well mixed during application; orthene: 0.8g orthene granules (970g/kg acephate active ingredient) in 1L water. Stems on each tree were sprayed twice to runoff on 2 February and 14 March, 2011 (1 day and 6 weeks after felling, respectively; Fig. 1). Coverage was complete (2-3 L applied per tree), apart from occasional portions on some trees that were inaccessible due to obstructing vegetation or debris, and extended for 12m or more from the cut end towards the top of the tree. Cut ends were also well sprayed to runoff. Control trees received no treatment. Weather remained dry for 24 hours or more after each application.

Harvest and evaluation

Trees were harvested on three separated days between 17 and 23 May, 2011 (15-16 weeks after felling; Fig. 2). Altogether, 6 discs (ca. 5-7 cm thick) were cut at 2 m intervals along the length of each stem, the first being taken several centimetres above the felling cut (only 5 discs were cut from 5 trees of stem length < 12m). Discs were stored in plastic bags, usually at 4°C overnight, and processed the next day in the work shop. Diameter over bark was measured using a diameter tape, after which bark was removed. An image of one face was recorded photographically after cutting each disc in half along the transverse plane using a band saw to create a smooth surface which was lightly misted with water to enhance features (Fig. 3). Sapwood moisture content (dry weight basis) was determined by weighing fresh and after oven drying. As much sapwood as possible was weighed (at the same time excluding any heartwood present) by making two sets of two parallel cuts at right angles, each inner cut placed ca. 2/3 along the radius from the cambium in to the pith, pooling the resultant four sapwood segments for a combined moisture content determination. Any signs of bark or tunnelling beetle attack were also documented.

Images were stored on an electronic database. Sapstain, as a percentage of total disc face area (delineated by the cambial circumference), was determined by manually outlining polygonal stain zones on each image using ESRI ArcGIS 9.3 software (Redlands, California). No allowance was made for heartwood which was assumed to be present only in small amounts, if at all. Because there is an element of subjectivity, all sapstain evaluations were made by one person. Sapstain was defined as any zone of blue (generally wedge shaped in outline) together with any immediately associated area of brown, if present (Fig. 4). Bleached regions were not counted as sapstain, even though they may be a precursor caused by fungal activity (they are not readily visible on the rough sawn disc face, and are therefore unlikely to be scored in industry assessments; Fig. 5). Circumferentially orientated brown zones of compression wood were not counted (Fig 6), but brown sectors radiating around basal disc 1 are potentially caused by colonising decay fungi and as such were treated as sapstain (Fig. 7). To check this assumption, isolation attempts were made from the brown discoloration zone, only (i.e. away from any blue sectors), of basal disc 1 taken from each of four arbitrarily selected orthene-treated trees. This was done using a culture medium selective for basidiomycete fungi consisting of 2% malt agar supplemented with 100 ppm streptomycin sulphate and 10 ppm benomyl to discourage bacteria and non-basidiomycete fungi, respectively. Isolations were also attempted from the blue zone of an arbitrarily selected disc taken from each of 4 copper-sprayed and 10 orthene-sprayed stems onto a non-selective medium (2% malt agar), to identify the causal fungi.

Statistical analyses were undertaken for percentage sapstain and moisture content by disc, tree and treatment, and conclusions drawn.

Figure 1 (a) and (b). Second spray application 6 weeks after felling (the first was applied after 1 day). Full protective gear was used for the orthene treatment.

Figure 2 (a) and (b). Harvesting discs 15 weeks after felling.

Figure 3 (a) and (b). Preparing and photographing disc surfaces.

Figure 4. Blue zones (control tree 16 disc 2; sapstain 88%)

Figure 5. Bleached zone (copper sprayed tree 8 disc 3; sapstain 0%)

Figure 6. Compression wood (copper sprayed tree 11 disc 5; sapstain 0%)

Figure 7. Radiating brown zones on basal disc close to felling cut (control tree 27 disc 1; sapstain 100%)

Results and Discussion

Initial inspection and analyses of results suggested that spraying with cuprous oxide had reduced the level of sapstain in felled stems by protecting them from the causal fungi (Fig. 8a; App. 1). However, unexpectedly it was found that mean moisture content was still sufficiently high in discs from treated stems (well over 100%) to have hindered the development of sapstain, when compared with values in untreated controls which had dropped to a level (mean less than 100%) allowing greater sapstain development (Fig. 8b; App. 2).

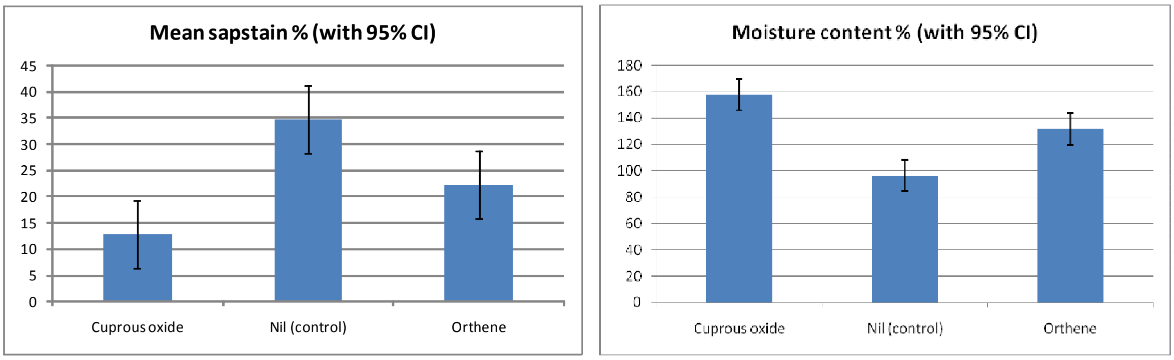

Figure 8 (a) and (b). Mean disc sapstain % (a) and moisture content % (b; dry weight basis) by treatment, with 95% confidence intervals.

When treatments were compared by analysis of covariance after adjusting for moisture content (fitted as a cubic equation to allow for the nonlinear relationship between sapstain and moisture content App. 3; cf. App.4), it was found that there was actually no difference between treatments in the level of sapstain present (Table 1). In other words, treatment with either cuprous oxide or orthene had not prevented the development of sapstain in stems 4 months after felling in early February.

Table 1. ANOCOVA comparing treatment and disc effects on sapstain after adjustment for moisture content.

Effect	df_1	df_2	F value	Significance
Treatment	2	42	0.63	0.54 NS
Disc	5	202	32.37	< 0.0001 ***
Treatment × disc	10	202	1.63	0.10 NS

Means adjusted to a common moisture content using the analysis of covariance are shown by treatment (Table 2; Fig. 9) and disc position (Table 2).

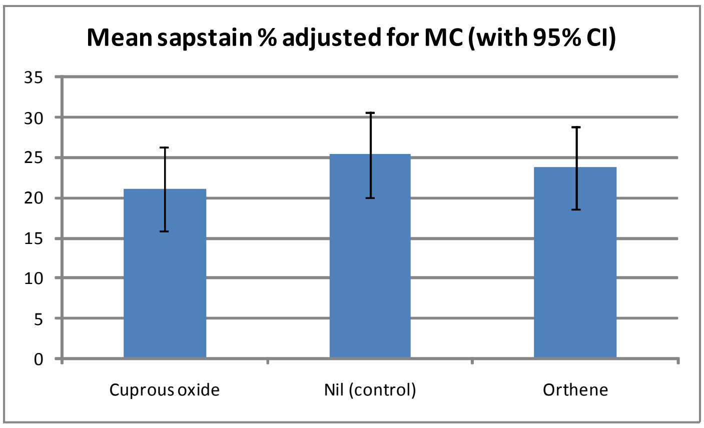

Figure 9. Mean sapstain percent adjusted to a common moisture content using analysis of covariance, with 95% confidence intervals.

Table 2. Means for sapstain percent by treatment and disc[1]

Treatment	Mean	Significance[2]
Cuprous oxide	21.1	a
Nil (control)	25.3	a
Orthene	23.7	a
Disc	**Mean**	**Significance[2]**
1	56.9	a
2	20.3	b
3	19.2	b
4	17.9	b
5	12.1	b
6	13.7	b

[1]Least squares means.
[2]Within each set, means with unlike letters are significantly different ($p<0.0001$).

The question arises as to why there was uneven drying between the different treatments. This effect was not explained by differences in stem diameter which were very similar between treatments (Fig. 10).

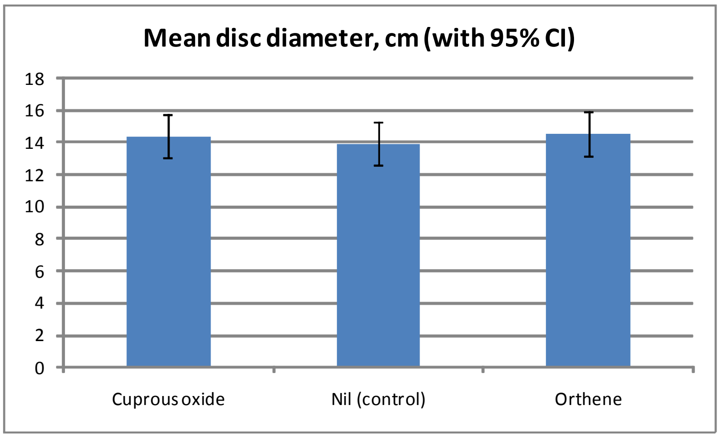

Figure 10. Mean disc diameter by treatment, with 95% confidence intervals.

It was also conjectured that spraying, especially with the cuprous oxide treatment which contained an oil sticker-spreader component, may have sealed the cut ends and slowed the rate of moisture loss. However, drying was in fact most rapid close to the cut end (disc 1), whether treated or not, making it very unlikely that this was the correct explanation (Fig. 11; App.2; drying was also a little more rapid in the uppermost disc 6 well up in the crown, green when felled, App. 2).

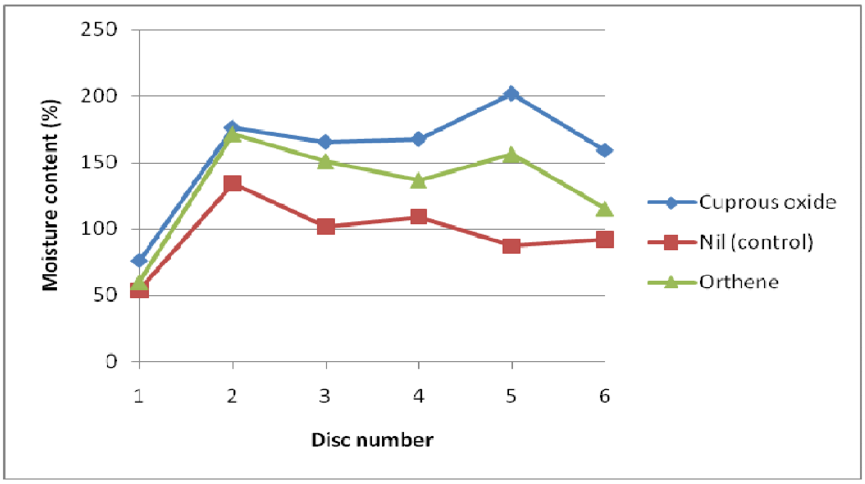

Figure 11. Mean moisture content of discs by treatment and disc position.

It therefore appears that the variation in rate of drying between treatments was due to differences in local environmental conditions between blocks. For instance, the occurrence of thinning gaps was possibly unevenly distributed across the trial area, potentially with greater clustering in a particular block or blocks, although such a trend was not apparent during a subsequent inspection of the site. Variation in drying was not anticipated over such a small trial area, and in future studies it will clearly be necessary to modify the design to include replication, either by complete randomisation or by randomised blocking. There could also be merit in incorporating oil or water alone treatments.

Would a treatment difference still have appeared if the trees had been harvested later, after there had been a greater development of sapstain (possibly entailing a third treatment application during the winter)? It is not possible to answer this question conclusively, but the present data give no hint of this. In the scatter graph (App. 4), a generous number of points representing discs treated with cuprous oxide have a significant amount of sapstain where they lie below the moisture content threshold of ca. 120% at which sapstain can occur, indicating that this treatment has failed to protect them. It should be noted that the majority (though not all) of these points represent discs with brown stain taken from basal position 1 close to the well-sprayed cut end.

Dark-stained sapstain fungi obtained from the blue zone on 12 of 14 arbitrarily selected discs were all *Diplodia pinea*, and no ophiostomatoid species were obtained (Table 3). Basidiomycete decay fungi were obtained using both the general and selective isolation media. With one exception (Basid 1 from disc 4 on copper treated tree 4), all decay fungi came from the brown zone at the disc 1 position close to the felling cut where drying was most rapid (Table 3). Two species (Basid 2 and Basid 3) were each isolated from several discs at this position. Single clamps were moderately common in cultures of all three basdiomycete fungi and they are still being identified. However, none are of *Phlebiopsis gigantea* or *Stereum sanguinolentum* which are common early colonisers of freshly cut or damaged *P. radiata* wood (McCarthy *et al*. 2010). No fruitbodies of basidiomycete decay fungi were observed on stems of study trees at time of harvest.

Bark beetle attack was recorded on 6 discs sprayed with cuprous oxide (7% of such discs), on 12 discs (14%) sprayed with orthene, and on 2 (2%) of untreated discs. Those identified (on 4 orthene-treated discs) were *Hylastes ater*. This comparatively low incidence of attack is consistent with the timing of the study which occurred mostly during Autumn. Nevertheless, this result does indicate that there was no evidence that the orthene insecticide treatment had prevented bark beetle colonisation. The general low incidence of beetle attack, the absence of any sign of beetle-vectored ophiostomatoid fungi and the lack of variation between treatments in controlling sapstain percent (after adjustment for moisture content) all conform to the presumption that most initial sapstain in New Zealand pine plantations is caused by *D. pinea* (McCarthy *et al*. 2010).

The apparent inability of cuprous oxide to reduce the level of sapstain when sprayed at high volume in this study was disappointing. Spraying by hand gave nearly complete coverage, even though it was not always possible to reach every part of the stem where there were occasionally obstructing branches and debris, or where access was sometimes limited beneath. Application by aerial spraying would face the additional challenges of a reduced spray volume and an obstructing canopy, if the latter remained moderately intact though patchy after a storm. These factors all imply that aerial spraying would not be an effective treatment in protecting damaged timber following storms.

Table 3. Fungi isolated from blue and brown sapstain zones from an arbitrary selection of discs.

Treatment and tree number	Disc position No.[1]	Culture No. isolation series	Isolation medium/ type of stain[2]	Species obtained (with No. isolated)[3]				
				Diplodia pinea	Basid 1	Basid 2	Basid 3	Colourless
Copper 3	1	L.362	Malt/ bluestain	5				
Copper 3	4	L.363	”	4	1			
Copper 4	1	L.364	”	5				
Copper 12	2	L.365	”	5				
Orthene 36	1	L.366	”	2		3		
Orthene 36	4	L.367	”	5				
Orthene 37	1	L.368	”					3
Orthene 38	1	L.369	”	2		2		
Orthene 39	3	L.370	”	5				
Orthene 40	3	L.371	”	5				
Orthene 41	1	L.372	”	5				
Orthene 42	1	L.373	”			5		
Orthene 42	4	L.374	”	5				
Orthene 43	1	L.375	”	4				1
Orthene 36	1	L.376	MEAB/ brown			6		
Orthene 38	1	L.377	”				6	
Orthene 41	1	L.378	”				6	
Orthene 42	1	L.379	”				6	

[1]Disc No. 1 is at the basal position close to the felling cut.

[2]Either ‘malt’ (2% malt agar, a general isolation medium) or ‘MEAB’ (a medium as defined in text selective for basidomycete decay fungi). ‘Malt’ isolations were made from the blue sapstained zone; ‘MEAB’ isolations came from the brown zone on basal disc 1 away from a blue zone.

[3]Minimum No. isolated out of 10 attempts per disc. ‘Basid’ isolates are basidiomycete decay fungi; ‘colourless’ is a non-sporulating, clampless, species with a hyaline mycelium lacking laccase enzyme indicating it is neither a stain fungus nor probably a basidiomycete.

Acknowledgements

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Appendices

Appendix 1: Statistical analyses for sapstain percent not adjusted for moisture content.

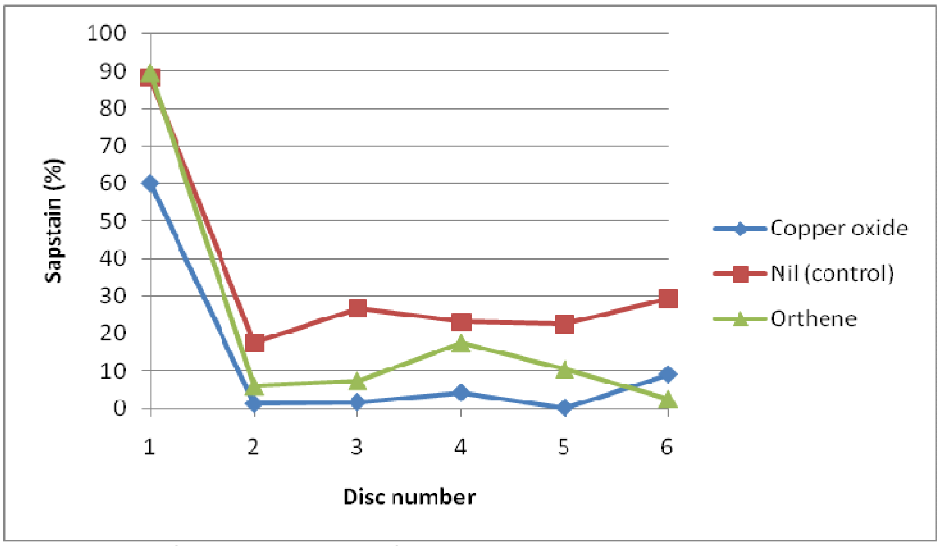
ANOVA comparing treatment and disc effects on sapstain (not adjusted for moisture content)

Effect	df_1	df_2	F value	Significance
Treatment	2	42	11.52	0.0001 ***
Disc	5	205	69.19	< 0.0001 ***
Treatment × disc	10	205	1.26	0.25 NS

Means for sapstain percent by treatment and disc (not adjusted for moisture content)[1]

Treatment	Mean	Significance[2]
Cuprous oxide	12.8	a
Nil (control)	34.8	b
Orthene	22.3	a
Disc	**Mean**	**Significance[2]**
1	79.2	a
2	8.3	b
3	12.6	b
4	14.9	b
5	11.1	b
6	13.8	b

[1]Least squares means.
[2]Within each set, means with like letters are not significantly different (p>0.05).



Mean sapstain of discs (not adjusted for moisture content) by treatment and disc position.

Appendix 2: Statistical analyses for moisture content percent (dry weight basis).

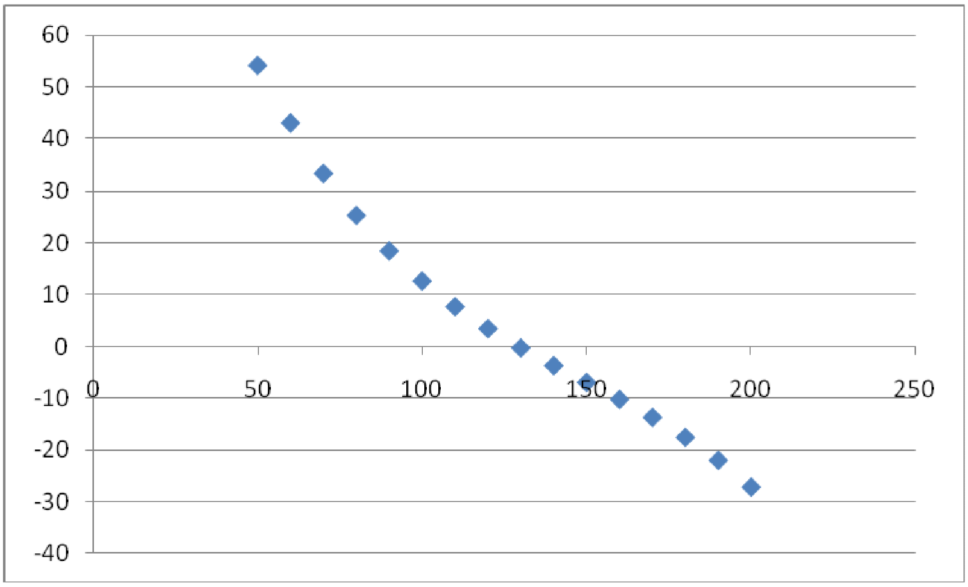
Effect	df_1	df_2	F value	Significance
Treatment	2	42	26.34	< 0.0001 ***
Disc	5	205	27.63	< 0.0001 ***
Treatment × disc	10	205	2.20	0.0190 *

Means for moisture content percent by treatment and disc[1]

Treatment	Mean	Significance[2]
Cuprous oxide	157.7	a
Nil (control)	96.3	c
Orthene	131.9	b
Disc	**Mean**	**Significance[2]**
1	63.3	a
2	160.6	c
3	139.4	bc
4	137.9	bc
5	148.4	bc
6	122.2	b

[1]Least squares means.
[2]Within each set, means with like letters are not significantly different (p>0.05).

Appendix 3: Plot of cubic transformation as fitted to moisture content percent during adjusted sapstain analysis.



Appendix 4: Scatter plot for all discs showing relationship between sapstain and moisture content by treatment.