Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

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Seed Sanitation Methodology for Amabilis fir

Objectives:

To recommend an operational procedure for reducing seed-borne pathogens on *Abies* spp. to reduce the risk of disease incidence and increase seed use efficiency.

Materials and Methods:

This trial investigated seed sanitation treatments on six seedlots of Amabilis fir (Ba), two seedlots of Grand fir (Bg) and two seedlots of subalpine fir (Bl)[1]. The seedlots were selected for the presence of contamination with either *Calsoscypha fulgens* or *Fusarium* spp. (Table 1). A 50-gram sample of each seedlot was treated with one of the six seed treatments (including a Control) presented in Table 2. The treatments with H_2O_2 were conducted after soaking (except T6) and the seed thoroughly rinsed following treatment. Data was collected on the moisture content during stratification; resin vesicle damage rating after treatment and germination capacity (GC); incidence of *Caloscypha* (CAL%) and *Fusarium* (FUS%) following stratification. Germination and fungal assay testing were performed in accordance with procedures presented in Kolotelo *et al*. (2001). The criteria for the assessment of resin vesicle damage are presented in Table 3.

The treatment means will be presented for each variable followed by statistical analyses. For GC, CAL% and FUS% the normality of the data will be compared to the arcsin (squareroot(x)) transformation that is suggested for binomial distributions (i.e. percentages) (Zar 1974). The ANOVA was used to test the significance of the variables using the PROC GLM procedure in SAS with seedlot being a random factor and treatment being a fixed factor. For all analyses an $\propto$ level of 0.05 was used. The term 'significant' will be used in the text to indicate statistical significance. An indication that a treatment resulted in the lowest or highest levels for a trait translates to it not being significantly different from the lowest or highest absolute value. The mean separation test used to determine significant differences between treatments was the Bonferroni t-test of differences.

Replicate information is available For GC [4 X 100], resin vesicle damage [5 X 20] and CAL% [10 X 25] and the variation will be partitioned to the following sources: Seedlot **:** Treatment **:** Seedlot* Treatment interaction **:** and Error. For FUS% no replicate data is available and the interaction term could not be tested. All seedlots were not contaminated with both fungi. To address the question of how to reduce pathogen levels only seedlots infected with *Caloscypha* or contaminated with *Fusarium* will used in the analyses. This strategy also improves the normality of the data by removing entire seedlots showing no occurrence of one of the pathogens.

A second analysis will look at all factors as random to compare the proportion of variance accounted for by each term using PROC VARCOMP in SAS. The main difference between these analyses is that the first (GLM Model) assumes we are only interested in the investigated treatments and the second (VARCOMP Model) that these treatments represent a random sample of all possible treatments available. The results from both analyses help to elucidate the important elements of seed sanitation in *Abies* spp.

[1] Funding for the fungal assays of the non-eligible Bl were supplied by BCMOF Extension Services and seed treatments, chemicals and germination testing are an in-kind contribution from the BCMOF Tree Seed Centre.

Table 1. The seedlots used and their respective test results (from SPAR).

Species	Seedlot	GC%	CAL%	FUS%
Ba	41428	80	22.0	0.0
Ba	41436	86	3.6	2.8
Ba	41156	50	0.4	0.6
Ba	43761	74	9.6	0.0
Ba	46179	64	0.0	12.1
Ba	61069	66	0.0	7.2
Bg	39215	58	10.0	2.0
Bg	7379	60	0.0	6.2
Bl	2504	59	0.0	2.8
Bl	44918	70	32.8	0.0

Table 2. The five treatments used to evaluate the optimal seed sanitation methodology in *Abies* spp.

Treatment	[H2O2]	Duration
T1 – Control	None	0.0
T2	3%	0.5 hours
T3	3%	2 hours
T4	3%	4 hours
T5	Mycostop	Seed dressing
T6[2]	3% (pre-soak)	2 hours

Table 3. Criteria for evaluating resin vesicle damage.

Category	Description
0	No resin vesicle damage apparent;
1	Light resin vesicle damage; less than 25% of the resin vesicles have been ruptured and/or less than 25% of the surface area is covered by resin
2	Moderate resin vesicle damage; between 26 to 50% of resin vesicles have been damaged and/or between 26 to 50% of the surface area is covered
3	Moderately-High resin vesicle damage; between 51 to 75% of resin vesicles have been damaged and/or between 51 to 75% of the surface
4	High resin vesicle damage; ; between 76 to 100% of resin vesicles have been damaged and/or between 76 to 100% of the surface area is covered

Results:

The trial results will be presented separately by the variable investigated followed by a discussion of their significance.

Moisture Content

For overcoming dormancy in Ba and Bl a split stratification regime is used where a high moisture content (mc) is maintained for 28 days at 2° C and then the seed is dried to a surface dry state and cold stratification continues for an additional 56 days. Moisture content was monitored non-destructively using target moisture

[2] T6 did not have fungal assays performed. The main question was whether pre-soak sanitation treatments would reduce GC and/or cause resin vesicle damage.

content calculations (Kolotelo *et al.* 2001: pg. 57) to ensure appropriate moisture content was maintained in stratification. In Ba the mc varied from 41% to 46.3% before dryback and from 33.4% to 36.2% following dryback. For Bl the range was smaller (only 2 seedlots), but the Mycostop (T5) treatments were kept moist before dryback as it was thought that the drying might be detrimental to the *Streptomyces* sp. No dryback occurs in Bg and the moisture ranged from 34.5 to 38.3% except the Mycostop treatment that was higher at 47.0% mc.

Germination Capacity

The average GC results ranged from 64 to 68% for the six treatments and from 55% to 84% for the 10 seedlots examined. There was no consistent GC response across seedlots when the H_2O_2 treatment duration was lengthened from 0.5 to 4.0 hours. For Bg, one Bl and one Ba seedlots, the use of H_2O_2 treatments prior soaking produced the lowest GC. Although differences were not large it appears that post-soak H_2O_2 treatments [T3 = 68%] are preferred to pre-soak treatments [T6 = 65%]. The Mycostop treatment also resulted in a 3% decrease in GC.

For ANOVA the raw data was used as the arcsine(squareroot(x)) transformation did not improve the normality of the data. The analysis indicated that the Seedlot and Treatment effects were statistically significant, but the interaction term was not. An investigation of which treatments were significantly different through approximate least squares means separation tests did not indicate any significantly different comparisons. Looking at the variance components it is clear that Seedlot is the most important factor accounting for 83% of the variation while treatment only accounted for 1%!

Resin Vesicle Damage

The resin vesicle damage ranged from 1.1 to 1.7 for the treatments indicating less than 25% of the resin vesicles were damaged across the six treatments. For seedlots the range was much greater at 0.6 to 2.8 indicating that some seedlots had an average of between 25 and 50% of their resin vesicles damaged (Bl 2504; Ba 41156). A comparison of GC and resin vesicle damage across all seedlots is presented in Figure 1. The relationship between the resin vesicle damage rating and GC was relatively poor with an r-squared value of 0.13.

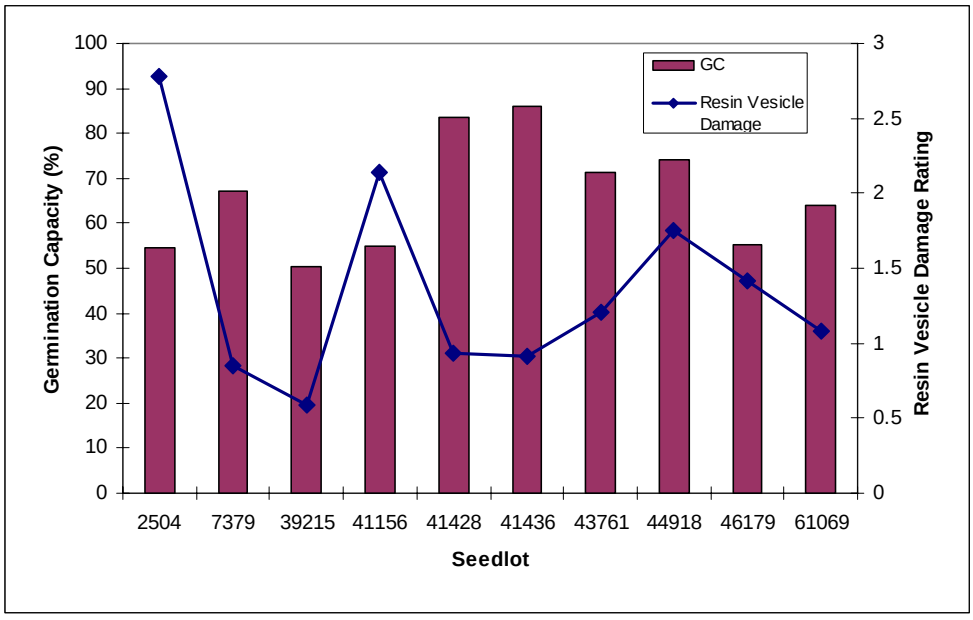

Figure 1. The average germination capacity and resin vesicle damage rating of *Abies* spp. seedlots averaged over all treatments.

The ANOVA indicated that all terms were statistically significant and to determine if significant treatment effects were present the analysis was performed separately by species. The VARCOMP model estimated that 78% of the variation in resin vesicle damage was due to Seedlot and 8.4% due to treatment.

In the ANOVA by species, for Bg and Bl the seedlot effect was the only statistically significant source of variation. For Ba, Seedlot and Treatment were statistically significant and least squares mean separation tests indicated that Treatments 2 and 4 were significantly different. Extending the H_2O_2 treatment from 0.5 to 4.0 hours significantly increased the incidence of resin vesicle damage.

Caloscypha fulgens

Only five of the ten seedlots showed any incidence of *Caloscypha fulgens*. One seedlot [Bg 39215] showed an average level of 32.3%, but all other infected seedlots were under 6%. The Mycostop treatment (T5) produced the lowest levels of *Caloscypha* in all seedlots. In Bg 39215 the four-hour H_2O_2 treatment resulted in a large increase in *Caloscypha*. For all other seedlots the four-hour treatment results were not significantly different from the Mycostop treatment.

The raw data was used on the five seedlots showing *Caloscypha* as the transformation did not improve the normality of the data. The ANOVA indicated that all sources of variation were statistically significant. Analyses were performed for each seedlot and in all cases the treatment effect was statistically significant. The treatment responses differed by seedlot (the significant Seedlot*Treatment interaction) and increasing H_2O_2 durations did not have a consistent effect on *Caloscypha* levels. An examination of the variance components associated with the five infected seedlots indicated that 85% of the variance was due to seedlot and only 0.1% to treatment.

Fusarium spp.

Fusarium was found in six of the seedlots examined and ranged from 0.7% to 84%. The treatment means (of seedlots with *Fusarium*) ranged from 19.9% [T2] to 32.5% [T5]. A comparison of the GC with the incidences of *Caloscypha* and *Fusarium* (averaged over all treatments) is presented in Figure 2. The relationship between these variables was quite weak with the relationship between GC and *Fusarium* being the least weak [$r^2 = 0.14$].

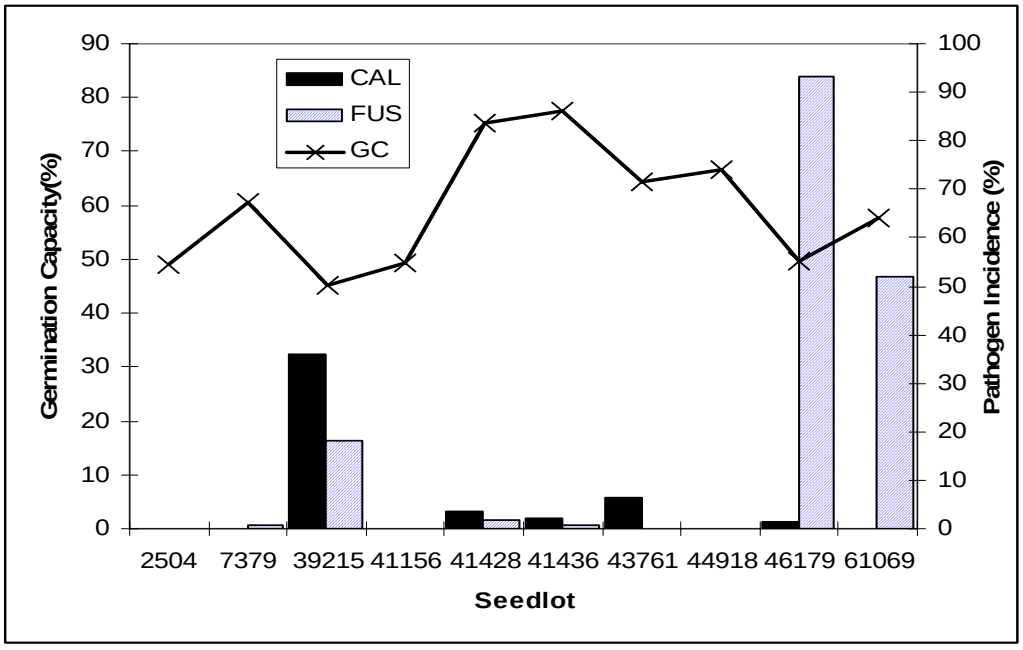

Figure 2. The results of GC, Caloscypha infection and Fusarium contamination averaged over all treatments.

The distribution of the data was not improved by transformation and the raw data was used for the analysis. Replicate data was not available and therefore the ANOVA can only test the significance of Seedlot and Treatment. For *Fusarium* the effect of Seedlot was significant, but Treatment was not significant. It is uncertain whether an interaction is present (although highly suspect based on the other variables) and due to the lack of replicate data the seedlots cannot be analyzed individually. The VARCOMP analysis indicated that 86% of the variance was due to seedlot and an estimate of 0.0% for treatment.

Discussion:

The overwhelming result of this seed treatment trial was the significance of the individual seedlots and their unique response to sanitation treatments. Significant Seedlot*Treatment interactions were found for resin vesicle damage and *Caloscypha*. For GC a significant interaction was not found ($p = 0.11$), but no significant differences between treatments were found. Using the random effects model the importance of Seedlot was obvious as it explained a majority of the variation in all cases: GC = 83%; Resin Vesicle Damage = 78%; *Caloscypha* = 85% and *Fusarium* = 86%. Previous work with ten Bl seedlots also illustrated that the 'best' treatment varied by seedlot although 3% H_2O_2 for one hour appeared to be the best treatment for controlling *Fusarium* (Neumann 1997).

Although treatment effect were small compared to seedlots some conclusion can be drawn from this trial. The general trend was that seedlots with more resin vesicle damage tended to have greater increases in damage following prolonged treatment. For Ba, there was a significant increase in resin vesicle damage by extending H_2O_2 treatment duration from 0.5 to 4 hours. This extension also resulted in a reduction in GC in Ba from 72 to 66%. Although the results were not statistically significant for Bg and Bl, one seedlot of each species showed increased damaged with increased durations of treatment. To be cautious it is recommended that *Abies* spp. should not be treated for more than two hours with 3% hydrogen peroxide.

The lack of a strong treatment effects on GC does not negate the importance of seed treatments it just illustrates that the selected seed sanitation treatments appear to have a small impact on the GC (i.e. no deleterious effects, but no benefit of the attempted seed sanitation treatments on GC). This agrees with previous results of Edwards and Sutherland (1979) who found that no H_2O_2 treatments were beneficial to germination in one Ba and one Bg seedlot. For unstratified seeds they found that with progressively longer H_2O_2 treatments the GC was reduced.

The moisture content of the samples was monitored as *Abies* spp. (particularly Ba and Bl) respond positively to a change in moisture content during stratification (Edwards 1996). The moisture contents corresponded to the general recommendations of 45% before and 35% after dryback for Ba and Bl.

For *Caloscypha* the contamination level varied considerably by seedlot ranging from 0.0% to an average of 26.2% for Bg 39215. For all seedlots the Mycostop treatment resulted in the lowest *Caloscypha* levels. This was unexpected as the Mycostop was expected to have a greater impact on the fungi contaminating the surface of the seeds (i.e. *Fusarium*) compared to the fungi infecting the seed (*Caloscypha*). Compared to the control Mycostop treatment resulted in a 3% reduction in GC. With the exception of Bg 39215 the four hour H_2O_2 treatment was as effective as Mycostop in reducing *Caloscypha*. The result of Bg 39215 is puzzling as the increase in *Caloscypha* with 4 hours of H_2O_2 was not accompanied by an increase in resin vesicle damage, but it did result in a decrease in GC. Mycostop therefore shows some promise as a pre-stratification treatment for seedlots infected with *Caloscypha,* but there is a small cost in germination.

The results of Fusarium do indicate a Seedlot*Treatment interaction although this could not be tested due to a lack of replicate data. The use of H_2O_2 reduced *Fusarium* in some seedlots, but in other cases the four-hour treatment actually increased the Fusarium contamination level. The Mycostop treatment did not control *Fusarium* very well, especially with seedlots exhibiting a high Control level of this pathogen.

Fungal assay data indicates that a higher proportion of Ba and Bg seedlots are contaminated with *Fusarium*, but that actual pathogen levels are higher for *Caloscypha*. In Bl, *Caloscypha* and *Fusarium* have

approximately equal chance of occurrence, but as with the other species *Caloscypha* occurs at higher levels. With the long stratification regime required for Ba and Bl germination the *Caloscypha* level is critical and can cause significant seed mortality.

Conclusions:

Although the results are not consistent across seedlots and one cannot recommend a universal seed sanitation treatment for *Abies* seedlots the following are general guidelines for the use of seed sanitation in *Abies* spp.

1) The results of hydrogen peroxide treatment vary by seedlot and implementation should be used with caution and preferably when one knows that a disease problem is present during stratification or in the nursery based on historical records.
2) The use of 3% hydrogen peroxide seed treatments generally has no impact on seed germination.
3) Hydrogen peroxide treatments (3%) for *Abies* spp. should not exceed 2 hours in duration as this may result in increased resin vesicle damage.
4) Hydrogen peroxide treatments (3%) should be performed after the initial 48-hour soak that is used for *Abies* spp.
5) The Mycostop biological treatment appeared promising as a control for *Caloscypha*, but results were not promising with *Fusarium*. A 3% decrease in GC was estimated by the use of Mycostop.

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