Tree Improvement Branch

Cone and Seed Improvement Program
BCMoF Tree Seed Centre

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BCMoF Fungal Assay Program

The BC Ministry of Forests has been co-ordinating a program for testing seedlots for pathogenic fungi since 1991. Results of this program have been presented in Edition 27 (1997) of this Newsbulletin; the Seed Handling Guidebook [SHG] (Kolotelo *et al.* 2001) and at various meetings. The intent of the program is to provide standardized seedlot test results on the incidence of the three significant seed-borne pathogens: *Caloscypha fulgens*; *Fusarium* spp. and *Sirococcus conigenus*. Michael Peterson's article on "Testing for seed-borne pathogens" in this Newsbulletin provides an excellent support document to this article and the Seed handling Guidebook provides the detailed test methodology for the fungal assays (Kolotelo *et al.* 2001).

The fungal assay results are an important tool for identifying high-risk seedlots and directing Integrated Pest Management (IPM) practices. The results of these tests are available on our seedlot information system (SPAR) and on the sowing request labels that are sent to nurseries with the seed. The fungal assays are performed on dry seed as the results are far more repeatable and more easily standardized. The topic of this Newsbulletin has encouraged me to review and share the data that we have gathered to date.

Fungal assay results are most useful when they are seedlot specific. It is also useful to have 'species' estimates for comparison and prioritization of IPM activities and this is the topic of this article. Initial species results, nursery feedback and the experience of pathologists has aided in the construction of a priority matrix for fungal assay testing (Table 1). These priorities guide sampling of seedlots with additional consideration given to seedlot balance; germination capacity, and operational use.

Species estimates of contamination (*Fusarium* spp.) or infection (*Caloscypha* and *Sirococcus*) can be presented in a variety of ways:

1) **% seedlots affected**[1] – indicates the proportion of tests for a pathogen that indicate a result > 0.0. This provides a 'reasonable' probability estimate for the occurrence of a specific fungi on the seed of a tree species.
2) **Affected Average** - provides the average level of contamination or infection for seedlots with a positive test result. Provides an indication of severity on a species basis. This could be used for prediction if one knew that the pathogen was present on the species of interest.
3) **Overall average** – provides the average contamination or infection level calculated based on all tests performed. The previous variable (Affected Average) only includes positive test results, but all test results are averaged in this statistic. Without any additional information this could be used as the estimate of the level of a pathogen on a tree species.

[1] Affected is used to cover incidences of both contamination and infection. Read either contamination or infection for the pathogen of interest.

4) **Maximum affected** – indicates the maximum test result obtained for a species:fungi combination. Provides an estimate of the worst-case scenario for seedlots of this species assayed to date.

5) **Sample size** – don't underestimate the importance of the sample size. An estimate performed on a large number of seedlots will be more accurate and precise than one performed on only a few seedlots.

Table 1. The priorities for fungal assay testing by pathogen and tree species.

	Fungi		
Species[2]	***Caloscypha***	***Fusarium***	***Sirococcus***
Ba	High	Medium	Low
Bg	Medium	Medium	Low
Bl	High	High	Low
Cw	Low	Medium	Low
Fdc	Low	High	Low
Fdi	Low	Medium	Low
Hw	Low	Medium	Low
Lw	Low	High	Low
Plc	Low	Low	Low
Pli	Low	Low	Low
Pw	Medium	High	Low
Py	Low	High	Low
SS	High	Medium	High
Sx	High	High	High
SxS	Medium	Medium	High
Yc	Low	Low	Low

In the past the % of seedlots tested was also presented. This was a difficult statistic to maintain as seedlots are continuously being used (expired) and others added to the inventory. This led to the question of what should be reported – should it be all seedlots ever tested or should it be seedlots that currently reside in the inventory? In the past all test results were presented and this makes maximum use of the data and can be considered the best 'Biological' estimates of the parameters. Other would argue that the results should be indicative of what is currently in the inventory and 'expired' seedlot data should not be included. This may offer a better predictive estimate of our current inventory, but it does not use all available data and would have lower precision that the previous estimate. Currently 80% of the seedlots tested still have a seed balance (are active), but and this varies somewhat by pathogen: *Caloscypha* [87%]; *Fusarium* [77%] and *Sirococcus* [86%]. The following detailed tables will use all of the available data, but an indication of the percentage of seedlots tested and still being used (active) is also included for reference.

RESULTS

The fungal assay program has had 5719 seed assays performed (*Caloscypha* – 1277 [22%]; Fusarium – 3553 [62%]; and Sirococcus [16%]. The assay results, by pathogen, are presented in Table 2 to provide a high-level comparison of the three pathogens. Assaying for *Fusarium* is

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[2] [2]Ba-Amabilis fir; Bg-grand fir; Bl-subalpine fir; Cw-western red-cedar; Fdc-coastal Douglas-fir; Fdi-interior Douglas-fir; Hm-mountain hemlock; Hw-western hemlock; Lw-western larch; Plc-coastal lodgepole pine; Pli-interior lodgepole pine; Pw - white pine; Py-yellow pine; SS-Sitka spruce; Sx-interior spruce; SxS-Sitka X interrior spruce hybrid; Yc-yellow cedar.

clearly a priority and this pathogen is especially problematic operationally with Douglas-fir and western larch seedlots.

In general, average pathogen levels are relatively low, but certain seedlots can have extremely high levels. Even a high pathogen level does not guarantee a disease problem. Keep in mind the disease triangle that indicates the pathogen, a susceptible host, and the proper environmental conditions must ALL be present for a disease to occur. The detailed results of each fungus will be discussed separately in the following sections.

Table 2. Summary of fungal assay results conducted by the BCMoF Tree Seed Centre between 1992 and 2005.

	Caloscypha	*Fusarium*	*Sirococcus*
Sample Size	1277	3553	889
% of Program	22%	62%	16%
% Seedlots affected	13.5	37.7	15.5
Affected Average	3.8	1.8	0.6
Overall Average	0.5	0.7	0.1
Maximum Value	37.6	75.4	7.8

Caloscypha fulgens (seed or cold fungus)

Caloscypha fulgens infects and kills the contents of the seeds. Seeds become hard and mummified and the fungus can spread through direct contact during stratification. This fungus has been shown to increase in the field with prolonged contact with the forest duff under cool and moist conditions, but proper cone storage can halt the spread of the fungus (Sutherland 1981). Looking at seeds in storage it was found that collections derived from squirrel caches had significantly higher levels of *Caloscypha* compared to collections from slash or from the ground (Sutherland 1979). In a comparison of cone collection methods, *Caloscypha* appeared more frequently on squirrel cache collections, but the authors indicate the differences were small and likely not practically important (Pigott and Peterson 1996). The debate on the quality of squirrel-cache collections continues. Is it *possible* to obtain good collections from squirrel caches. The answer is yes, but the risk of introducing *Caloscypha* is greater with this collection method. Older caches would have a higher probability of containing the fungus and wet, cool weather will increase the probability of a *Caloscypha* infection occurring in a cache. Serotinous lodgepole pine cones are probably the least at risk due to the sealed nature of the cones. There are obvious cost advantages to raiding squirrel caches, but these should be weighed carefully against the increased potential of the cold fungus infecting your seedlot.

The incidence of *Caloscypha* appears most prevalent on seedlots of subalpine fir (Bl) and populations consisting of white and Sitka spruce hybrids (SxS) (Table 3). I don't believe that it is coincidental that these species inhabit the cool, moist environments in which this fungi thrives. Although infection levels may not be as high in Amabilis fir, Noble fir or western white pine – the long stratification periods required for these species to break seed dormancy will provide a larger window for the fungi to bulk-up. These species are of concern as well as any seedlot in which extended stratification is employed to increase the germination rate or robustness of the seed to sub-optimal germination conditions. This fungus is able to out compete other fungi at cooler temperatures.

For Sitka spruce, approximately one in ten seedlots will be infected, but the average infection is quite high at 7.8% and this species also showed the highest observed level of *Caloscypha* (37.8%). Ponderosa pine also had a relatively high affected average of 5.2% indicating disease potential is present. Pathologists indicate that a seedlot with a *Caloscypha* infection of 5% or greater is significant and strategies should be employed to minimize the spread (Kolotelo *et al.* 2001; page 31).

Table 3. Results of testing for *Caloscypha fulgens* by the BC Ministry of Forests Tree Seed Centre 1991-2005.

Species	**Sample Size**	**% Seedlots Affected**	**Affected Average %**	**Overall Average %**	**Maximum Value**	**% tested Seedlots Active**
Ba	189	14.3	4.0	0.6	22.0	86.2
Bg	42	14.3	**5.1**	0.7	12.4	23.8
Bl	187	**31.0**	**5.0**	1.6	**32.8**	78.1
BN	21	4.8	2.0	0.1	2.0	81.0
Cw	1	0.0		0.0		100.0
Fdc	55	1.8	0.4	<0.1	0.4	76.4
Fdi	108	9.3	1.6	0.1	4.4	90.7
Hm	8	0.0		0.0		100.0
Hw	47	8.5	0.4	<0.1	0.4	89.4
Lw	14	0.0		0.0		92.9
Plc	5	0.0		0.0		100.0
Pli	41	0.0		0.0		73.1
Pw	74	6.8	1.8	0.1	4.8	83.8
Py	15	13.3	**5.2**	0.7	10.0	93.3
SS	74	10.8	**7.8**	0.8	**37.6**	97.3
Sx	356	11.5	2.2	0.3	16.0	89.6
SxS	34	**26.5**	3.4	0.9	16.0	97.1
Yc	6	0.0		0.0		100.0
TOTAL	**1277**			**0.5**		

Fusarium spp.

This pathogen group is reported only the genus level due to the taxonomic complexity of the genus, high cost of species identification and uncertainty of the impact of the various species on conifer tree seeds. The fungi is complex to identify, but also complex because it can be involved in a variety of disease problems including pre- and post- emergence damping –off, late season root rots and shoot blights. As opposed to the other two pathogens, Fusarium is 'primarily' contaminating the seed surface allowing for a wider range of sanitation methods to be effective. Compared to *Caloscypha* there is a large amount of published information regarding *Fusarium* and sanitation methods – see the Seed Handling Guidebook (Kolotelo et al 2001) for references.

Several species have greater than 50% of their seedlots contaminated with Fusarium: western larch, Douglas-fir; white and Ponderosa pine (Table 4). This means half of the seedlots will be contaminated and have the potential to cause disease. The overall average contamination levels are not high, but the maximum values obtained for some seedlots is quite alarming. The importance of this pathogen cannot be overstated. Pathologists indicate that a seedlot with a Fusarium contamination of 5% or greater is significant and strategies should be employed to minimize the spread.

It is also important to understand that although *Fusarium* can be seed-borne, but it can also enter the seed handling facilities through the air, organic debris and improperly cleaned equipment. Control of Fusarium-caused problems requires a thorough look at the entire seed handling system at your facility.

Table 4. Results of testing for *Fusarium* spp. by the BC Ministry of Forests Tree Seed Centre 1991-2005.

Species	**Sample Size**	**% Seedlots Affected**	**Affected Average %**	**Overall Average %**	**Maximum Value**	**% tested Seedlots Active**
Ba	260	31.9	1.2	0.4	14.0	66.5
Bg	59	40.7	**1.6**	0.7	7.0	74.6
Bl	222	31.5	**0.7**	0.2	2.8	67.1
BN	21	47.6	1.0	0.5	2.0	81.0
Cw	246	45.5	1.6	0.8	20.4	72.0
Fdc	374	59.6	2.7	1.6	75.4	55.9
Fdi	441	59.6	2.0	1.2	42.0	89.8
Hm	21	14.3	0.2	<0.1	0.2	100.0
Hw	139	32.4	0.8	0.3	4.8	90.6
Lw	199	64.3	2.4	1.5	43.2	75.4
Plc	12	8.3	0.1	<0.1	0.1	100.0
Pli	529	6.4	0.3	<0.1	1.2	62.8
Pw	99	57.6	2.7	1.6	29.0	82.8
Py	191	50.3	**1.7**	0.9	17.0	90.6
Sb	5	0.0		0.0		100.0
SS	82	22.0	**1.3**	0.3	6.4	95.1
Sx	592	26.7	1.7	0.4	39.8	88.3
SxS	34	23.5	1.6	0.4	4.6	100.0
Yc	22	27.3	0.4	0.1	0.8	81.8
TOTAL	**3553**					

Sirococcus conigenus (Sirococcus shoot blight)

The third pathogen is *Sirococcus* and it does not kill the seed, but causes a shoot blight that can spread rapidly through a crop or infect adjacent crops. Due to the potential build-up of this pathogen it is considered significant at a 1% level. To enable this greater precision a total of 1500 seeds are used in *Sirococcus* fungal assays (versus 500 for *Fusarium* and 250 for *Caloscypha*).

Sirococcus is primarily seed-borne on spruce species, but it has also been found on western larch, western hemlock and western white pine seeds (Table 5). Sirococcus has not been found on lodgepole pine seed, but it has been reported that spores could travel from infected spruce seedlots and infect lodgepole pine within the same nursery (Peterson 1996). Emphasis will continue to focus on spruce species followed by western larch and additional samples of western white pine will be assayed.

Table 5. Results of testing for *Sirococcus conigenus* by the BC Ministry of Forests Tree Seed Centre 1991-2005.

Species	**Sample Size**	**% Seedlots Affected**	**Affected Average %**	**Overall Average %**	**Maximum Value**	**% tested Seedlots Active**
Bg	1	0.0				100.0
Fdc	4	0.0				75.0
Fdi	6	0.0				66.7
Hw	81	7.4	0.3	<0.1	0.5	90.6
Lw	44	19.5	0.5	0.1	1.4	75.4
Plc	7	0.0				100.0
Pli	22	0.0				62.8
Pw	3	33.3	0.9	0.3	0.9	82.8
Sb	1	0.0				100.0
SS	85	20.0	**0.3**	0.1	1.5	95.1
Sx	600	15.7	0.7	0.1	7.8	88.3
SxS	33	36.4	0.8	0.3	2.4	100.0
TOTAL	**3553**					

I would appreciate feedback on our program. We will continue to perform fungal assays using our priority matrix, but feedback on seedlots with disease problems is always appreciated. This article provides detailed baseline information on a species level, but the specific seedlot test result, if available, should be the criteria to use in determining whether seed (or environmental) treatments are warranted.

References

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