

Fungal Assay Program History



Dave Kolotelo
Connections Through Seed
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Special Thank you to Michael Peterson (*Applied Forest Sciences Ltd.*) for providing pathology services to the TSC and BC for the past 25+ years

Michael also recently received the Green Timbers Award for his contributions to reforestation in BC



CONGRATULATIONS
and enjoy your retirement



Fungal Assay Program History

- **1985 to 2000** – **Forest Pest Clinic** at Pacific Forestry Centre (PFC) was the foundation for the current program
- **1992** – large program (877 seedlots) to perform tree seed bioassays for Fusarium by PFC – **John Dennis**
- **1993 to 2000** – Fungal Assays performed on contract by AFS (**1997 to 1999** MOF assisted in program funding)
- **2000** – Forestry Nursery Health Clinic closed
- **2001** – AFS operating pest clinic on a fee-for service basis (**2003** – FGC (PMTAC) funded the fungal assays)
- **2017** –MOU with Ministry of Agriculture Plant Health Clinic to perform services – **Vippen Joshi / David Trotter**
- Funding from TSC base budget (~ \$6000 per year currently and up to \$25 000 when dealing with backlog)

A Few Highlights

- **1994** – Sample sizes determined (95% confidence level)
 - *Caloscypha fulgens* - 250 seed to detect 5% ± 2.5%
 - *Fusarium* spp. - 500 seeds to detect 5% ± 2.0%
 - *Sirococcus conigenus* - 1500 seeds to detect 1% ± 1.0%
- **1996** – Seed Pathology Meeting
 - Paige Axelrood
 - John Dennis
 - Dave Kolotelo
 - Melody Neumann
 - Michael Peterson
 - David Trotter
 - Established Fungal Assay priorities



Species	Caloscypha	Fusarium	Sirococcus
BA	Medium	Medium	Low
BL	High	High	Low
CW	Low	Low	Low
FDC	Low	High	Low
FDI	Low	Low	Low
HW	Low	Medium	Low
LW	Low	Medium	Low
PLI	Low	Low	Low
PW	Low	High	Low
PY	Low	High	Low
SS	High	High	High
SX	High	High	High
YC	Low	Low	Low

Those initial priorities formed the foundation for the program there have been some 'minor' adjustments over time (in pink)

– Fusarium media question?

Komada	Nash & Snyder
2067 tests already using this media	New media type, would be a different test
Specifically for <i>F. oxysporum</i> , but others also show up	More general to all Fusarium
Trichoderma may mask Fusarium	
Experienced with this media	Need to develop expertise

- We voted and **Komada won** – primarily # tests to date

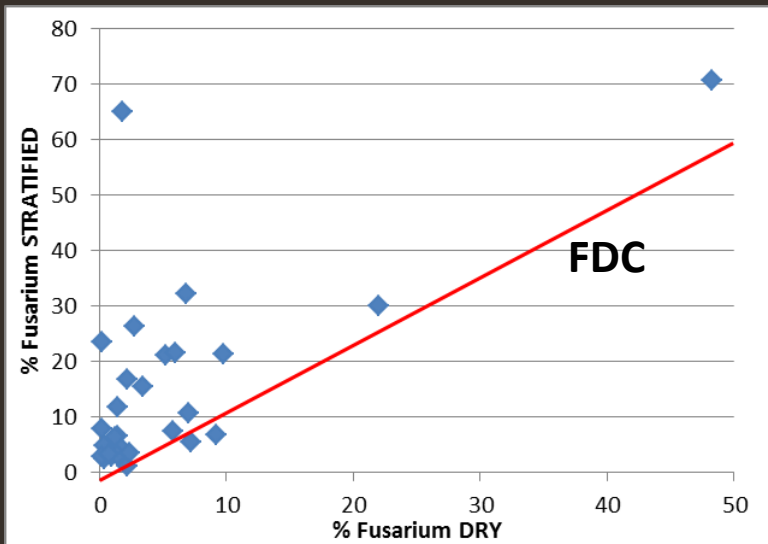
– Dry vs. stratified test results?

Dry	Stratified
2067 tests already performed dry	Would be new
More reproducible results No Timing constraints	Slight MC differences may impact test Stratification duplication complicates test
Consistent material	High variability in bulking up rates
Better baseline	Possibly better correlation with disease incidence in nursery

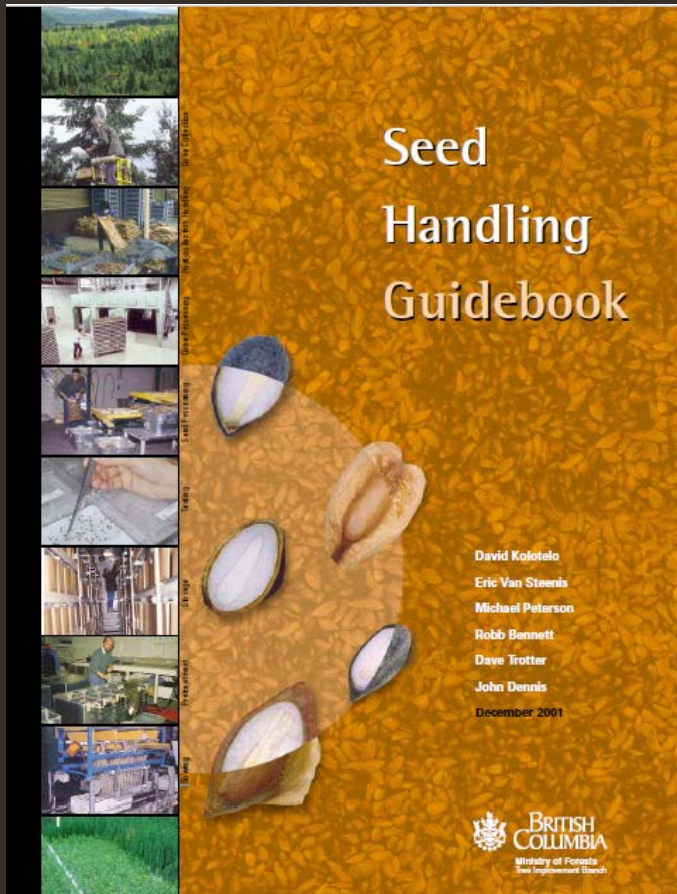
- **Dry testing won** - # tests to date & reproducibility

– Critical Levels for each Pathogen (significant problem)

- 5% for *Caloscypha* – *pathogen can bulk-up in stratification*
- 1% for *Sirococcus* – *found on SX can then infect Pli, Py*
- Fusarium not as simple – 5% agreed to ... eventually
- Assay doesn't differentiate % of different Fusarium species (species testing was cost prohibitive 20 years ago)
- Bulking-up (dry to stratified) % Fusarium is highly variable



Extension Efforts



Fungal Assays Reduce the Impact of Seed-borne Pathogens

The identification and quantification of potential seed pathogens is a critical and cost-effective step to direct integrated pest management (IPM) practices, limit seedling losses and improve seed use efficiency.

Program Priorities

- 1984 - Applied Forest Science (A) determines appropriate sample sizes for 95% confidence in each fungal assay. Results in brackets indicate the standard deviation.
 - Colletotrichum Fulvum** 200 seeds required to detect a 5% infection (±2.5%) level
 - Fusarium spp.** 500 seeds required to detect a 5% contamination (±2.5%) level
 - Sirospora conopsea** 1500 seeds required to detect a 1% infection (±0.5%) level
- 1990 - A seed pathology group (J. Karkool, J. Dennis, D. Kocabel, M. Neumann, M. Peterson and G. Thibet) determines species priorities and develops standards for fungal assay testing.

Testing is performed on dry seed allowing for greater test repeatability by reducing variability in seed condition that could occur with isolated or sterilized seed. This decision retained the value of approximately 2000 industrial tests.

Species priorities for fungal assays (Table 1) established specific, repeatable and flexible test species. Fungal species priorities. Actual tests performed estimated by the seedlot size, germination capacity, genetic class, use, feedback from clients and budget resources.

Interpretation of the Results

The results are based on all tests performed, although some seedlots have expired since testing. The variables are defined below: Table 1, but all results are probably typical, so we look at the results for Quebec (Quebec for QP) for Fusarium testing. There is a 56% probability of a seedlot being contaminated and the average contamination level is 2.8%. The worst case scenario was 94% providing an indication of how bad the situation can be.

A common question is at what level does a pathogen become significant? This is not an easy question to answer as other factors such as the germination environment, seed treatment and moisture content can have a significant impact on actual disease occurrence. For Fusarium spp. and Colletotrichum Fulvum a level of 5% or more is considered significant, but for Sirospora conopsea a level of 1% is considered significant as this pathogen can spread to adjacent seedlings quite rapidly.

Pathologists were uncomfortable assigning a significance level to Fusarium as disease incidence could be significantly influenced by actual Fusarium species (assays to species level are currently cost prohibitive, variability in degree of subunit ratios for sterilized seed and differences between the species). A more conservative approach is therefore warranted in predicting potential disease incidence from Fusarium fungal assay results.

Table 1. The results of the fungal assay testing program.

Species	Colletotrichum Fulvum (CF)			Fusarium spp. (FUS)			Sirospora conopsea (SIRO)		
	Probability	Average Infection	Standard Deviation	Probability	Average Contamination	Standard Deviation	Probability	Average Infection	Standard Deviation
BC	14.1	1.8	0.9	33.2	1.2	14.0	0.0	0.0	0.0
AB	19.0	1.0	0.4	11.8	1.9	11.0	0.0	0.0	0.0
SK	4.9	2.0	2.0	86.0	1.0	2.0	0.0	0.0	0.0
MB	2.0	2.0	2.0	47.4	1.6	20.4	0.0	0.0	0.0
ND	7.1	1.4	0.7	88.0	2.8	84.0	0.0	0.0	0.0
NE	8.2	1.6	1.6	87.6	1.6	42.0	0.0	0.0	0.0
SD	7.4	0.4	0.4	10.3	0.2	0.2	7.4	0.3	0.8
IA	2.0	2.0	2.0	82.0	2.7	1.8	19.2	0.6	1.4
IL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IN	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MI	0.4	1.8	1.8	84.0	2.4	20.0	0.0	0.0	0.0
WI	8.1	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0
MO	8.8	1.6	0.8	11.4	1.4	11.0	0.0	0.0	0.0
KS	20.4	0.1	0.0	20.0	1.8	18.0	0.0	0.0	0.0
OK	0.0	0.0	0.0	38.0	0.0	1.8	0.0	0.0	0.0
CO	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

More Information

More details on the probability pathogen-fungus and seedlot variability include: [see the spread column in the final 'Seedling Outcomes' table](#) [http://www2.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/tree-seed/tree-seed-centre/cone-seed-improvement-program](#)

More details regarding Fusarium on the forest site as "Fusarium species a Risk Category" perspective in forest seedling production: [http://www2.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/tree-seed/tree-seed-centre/cone-seed-improvement-program](#)

PGCC 2010

- <https://www2.gov.bc.ca/gov/content/industry/forestry/managing-our-forest-resources/tree-seed/tree-seed-centre/cone-seed-improvement-program>
- https://www2.gov.bc.ca/assets/gov/farming-natural-resources-and-industry/forestry/tree-seed/tree-seed-centre/tsc_fungal_assay_poster_2010.pdf
- Upcoming Tree Seed Working Group News Bulletin article

Disease Incidence Feedback Loop

- FDC root rot issues at some nurseries
 - *Fusarium* spp.
 - Seed-borne
 - Air, water, media- borne
 - *Pythium* spp.

Proposed Program Changes

- Eliminate *Sirococcus conigenus* testing
- Remove requirement to identify companion fungi
 - not on SPAR, many fungi not pathogens
 - Possible exceptions include :
 - *Pythium*
 - *Phomopsis* (LW)
 - *Cylindrocarpon*
- Investigate development of media-less direct PCR assay to identify *Fusarium* to the species level