Determining infection routes for seed-borne Fusarium

Data collection summary for 2005/2006

Output and Deliverables:

The projected target date for completion is March 31, 2006. Deliverables consist of:

1) *Compiled data in the form of Excel spreadsheets and/or figures outlining the incidence of Fusarium being isolated from surface sterilized seed from each of 15 seedlots, previously identified to have seedcoats contaminated with Fusarium spp.*
   i) *These data will provide valuable information as to whether seed-borne Fusarium previously thought to only exist as a seedcoat contaminant, might exist as internal seed infections.*

Seedlots comprising 5 tree species with *Fusarium* seed-contamination levels greater than 5% as identified during routine fungal assays are shown in Table 1. Twelve of these were available for withdrawal from the Tree Seed Centre to be tested for seed-borne *Fusarium.* White pine seedlot 60255 (highlighted) owned by Almforest Timber was not available as repeated attempts to contact Rainer Muenter at Almforest Timber for withdrawal permission were unsuccessful. White pine seedlot 61219 (highlighted) was also not available as it was all used in a 2005 sowing request. Permission was obtained from each of the other respective seedlot owners and samples of 1,000 seeds for each seedlot were withdrawn from storage at the Ministry of Forests and Range (MoFR) Tree Seed Centre and shipped to the AFS Lab. All seedlots with the exception of the Lw and Py samples, were A-class seed.

Table1. Percent *Fusarium* contamination by seedlot.

Seedlot	Species	Class	FUS%
61226	CW	A	5.8
60343	FD	A	6.2
6521	FDC	A	8
60274	FDC	A	5
61253	FDC	A	11.6
60254	PW	A	5
60255	Pw	A	8.8
60416	PW	A	5.8
61070	Pw	A	5.2
61156	PW	A	9.2
61219	Pw	A	29
61220	Pw	A	21.4
5243	LW	B	15.8
44219	PY	B	6

Based on the above, 500 seeds from each seedlot were surface sterilized and assayed as follows:

- 500 seeds were placed in a glass container and
- soaked in 30% H_20_2 at 3 X the volume of the seeds for 30 minutes
- seeds were then rinsed 3 times with sterile distilled water and
- then surface-dried and plated on *Fusarium* selective culture media (KM) and
- incubated at 24/18°C with 14/10 hour day/night photo period after which time they were
- examined for *Fusarium* at 5 days and 10 days
- fungal identifications were made to genus level only

Fungal assays for non-surface sterilized seed for each of the 12 identified seedlots were not proposed and not included in the workplan as these results are on record from previous tests. However, the results are reported as one single percent infestation for each seedlot. No replicate data were recorded. Thus, in order to perform statistical analyses to detect any differences that might exist between surface sterilized and non-surface sterilized seed within seedlots, fungal assays outside the scope of the work plan, on 500 non-surface sterilized seeds for each seedlot were conducted. Surface sterilized and non-surface sterilized seed were assayed with 16-23 replicates of 25 seeds for each seedlot. These data have been compiled in an Excel Worksheet, (*SS & Non-SS seed*) in the Excel Workbook, **"Kal and WFP Seed orchard Fusarium"**.

2) *Compiled data in the form of Excel spreadsheets and/or figures outlining the incidence of Fusarium being isolated from internal seed parts on seed identified to have Fusarium infections in step 1 above using a component plating procedure.*
   i) *These data will provide valuable information as to the extent of, and seed components infected by seed-borne Fusarium on seedlots identified in step 1.*

Fifty seeds from each seedlot for which *Fusarium* was isolated from surface sterilized seed, were examined to determine the extent if any, of internal mycelium using a procedure for component plating as described in Singh and Mathur (Singh and Mathur, 2004. Histopathology of seed-borne infections. CRC Press. Boca Raton, Florida). The interior of seedlots Lw 05243, Py 44219, Pw 61156, Pw 61220 and Cw 61226 for which *Fusarium* was isolated from surface sterilized seed were assayed using the following procedures:

- seeds were soaked in water at room temperature long enough to permit separation of the components, seed coat and pericarp, endosperm and embryo
- seeds were dissected aseptically to separate these components using a sterilized scapel, needles and forceps
- seed components were then sterilized by washing in a 1% chlorine solution
- seeds plated on *Fusarium* selective KM agar
- seeds were incubated for 7 days under near ultra-violet (NUV) light and examined for fungi after day 8

Internal seed parts for the above 5 seedlots were assayed with 6-8 replicates of 25 seeds for each seedlot. These data have been compiled in an Excel Worksheet, (*SS seed interiors*) in the Excel Workbook, **"Kal and WFP Seed orchard Fusarium"**.

3) *Compiled data in the form of Excel spreadsheets and/or figures outlining the incidence of Fusarium being isolated from the interior of conelets sampled from western larch and Douglas-fir seed orchard trees in Vernon and Victoria respectively. Samples will be made at weekly intervals from the time of reproductive bud elongation until the cones become closed and pendant.*
   i) *These data will provide valuable information as to whether seed-borne Fusarium infections might arise as a result of direct systemic infections via the parent tree.*

Laboratory tests to isolate any *Fusarium* growing systemically within developing western larch and Douglas-fir female strobili were made. From the time of reproductive bud elongation (23/03/05 Kalamalka (KAL) Forestry Centre; 22/03/05 Western Forest Product's (WFP) Saanich Forestry Centre) until receptive cones were closed and pendant (19/04/05 KAL; 27/04/05 WFP), 10 conelets from each of 5 western larch and 5 Douglas-fir trees from KAL and WFP respectively were sampled weekly.

It was unclear how surface sterilizing the conelets in 30% H_2O_2 for 30 minutes using the procedure for seeds would affect their soft tissues. Thus, prior to making fungal assays on the developing conelets, tests were made to determine a suitable soak time that would sterilize their surfaces without damaging the tissues. Ten conelets were soaked in 30% H_2O_2 for 0, 2.5, 5, 7.5, 10 and 12.5 minutes after which time they were plated on KM and assayed for the presence of any fungi on their surfaces. Fungi were observed to grow from the conelets soaked for less than 5 minutes while no fungi grew from the surface of those soaked longer. Therefore it was decided to use a 5 minute, 30% H_2O_2 soak to surface sterilize the conelets. The 50 conelets from each seed orchard were assayed as follows:

- conelets were surface sterilized in 30% H_20_2 for 5 minutes
- conelets were rinsed 3 times with sterile distilled water and surface dried
- each conelet was aseptically dissected longitudinally using sterilized scapel, needles and forceps after which each conelet half was,
- placed interior side down on KM and
- incubated at 24/18°C with 14/10 hours photo period (day/night)
- cultures were examined for *Fusarium* or any other fungi of note at 5 days and 10 days
- fungal identifications were to genus only

The interior surfaces of 50 conelets from each seed orchard were assayed for the presence of *Fusarium* and other fungi of note at weekly intervals from March 22 until April 27, 2005. These data have been compiled in an Excel Worksheet, (*Conelet fungi*) in the Excel Workbook, **"Kal and WFP Seed orchard Fusarium"**.