

**The Susceptibility of *Populus trichocarpa* Provenances
in the Pacific Northwest to *Septoria musiva* and *Septoria populicola***

by
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Abstract

A *Populus trichocarpa* provenance trial in Harrison Mills had tested positive in 2009 and 2010 for *Septoria musiva*, a pathogen that reduces the photosynthetic activity of its host through necrotic spots on the leaf surface and has the potential to girdle and kill the host through cankers that develop on the branches and main stem. A closely related pathogen, *Septoria populicola*, has also been reported in the provenance trial; this particular pathogen is native to British Columbia and causes only non-lethal minor leaf lesions, but is impossible to distinguish from *Septoria musiva* without conducting DNA-based analyses. This study is aimed to assess the frequency of *S. musiva* and *S. populicola* infections on provenances of *P. trichocarpa* to determine if there is a pattern of differential susceptibility. The provenances of the *P. trichocarpa* extend from Alaska, through British Columbia and down into Oregon; these were grouped into two categories: a North and South population. The proportions of *Septoria musiva* and *Septoria populicola* infections were compared between the north and south populations to determine if there is a difference in infection proportions between the regions. The north and south proportions were also compared between 2010 and 2011. Although the number of infections were always higher in northern than in southern provenances, no significant differences were found between northern and southern infection proportions for *Septoria musiva* or *Septoria populicola* in 2010 or 2011. A comparison between regional infections in 2010 and 2011 was also not significantly different from one another for *Septoria musiva*. Although no significant differences was observed in the infection proportions of *Septoria populicola* between the southern populations over 2010 and 2011, a significant difference was observed in the infection proportions between northern regions in 2010 and 2011.

Keywords: Septoria musiva, Septoria populicola, Populus trichocarpa, black cottonwood, Mycosphaerella populorum, Mycosphaerella populicola

Introduction

Poplars are integral in Canada due to their vast abundance and potential in the country's forest industry; in addition, poplars have several desirable biological characteristics such as rapid, indeterminate growth, hybridization characteristics that make them ideal for culturing (Feau, Mottet, Perinet, Hamelin, & Bernier, 2010). As the demand for energy increases, and the limits of the fossil fuel economy projected to peak within the next several decades, the demand for renewable energy is rapidly increasing (McPhail, Cigolotti, & Moreno, 2012).

Black cottonwood (*Populus trichocarpa* Torr. & A. Gray) is native to British Columbia and is also distributed along the northwestern coast of North America from California to Alaska; the poplar embodies all the characteristics that make them ideal for cultivation. In BC, black cottonwood has already been utilized for lumber harvest, pulping and riparian restoration; its role in the future biofuel endeavors is also met with high expectations. As such, several plantations across the province have been cultivating the tree for the economical applications listed.

As with all other poplars, *P. trichocarpa* is host to many diseases and other biotic stress factors, especially in areas of intensive culturing, as the selection of cottonwood by breeders is often placed on wood quality and growth characteristics (Ostry & McNabb Jr., Susceptibility of *Populus* species and hybrids to disease in the North central United States, 1985). Two such diseases include *Septoria musiva* Peck. (telomorph: *Mycosphaerella populorum* Thomps.) and *Septoria populicola* (telomorph: *Mycosphaerella populicola*). *Septoria populicola* is native to British Columbia, while *Septoria musiva* has historically been localized in eastern North America

(Ostry, 1987; Krupinsky, 1989). The injury symptoms of the two pathogens are identical in which they cause circular, necrotic spots on the foliage, which leads to reduced photosynthetic leaf area, as well as premature senescence of leaves (Bier, 1939). However, a key difference in the injury symptoms is that *S. musiva* has been associated with canker development in the main stem and branches, which can potentially girdle and kill the tree (Bier, 1939; Ostry, 1987; Newcombe & Ostry, 2001; Callan, Leal, Foord, Dennis, & van Oosten, 2007; Feau, Mottet, Perinet, Hamelin, & Bernier, 2010). The canker causing ability of *S. musiva* has is detrimental and can lead to plantation failure (Bier, 1939; Ostry, 1987). *S. musiva*, despite being native to eastern Canada, has recently been reported and isolated from several poplar plantations within Fraser Valley, British Columbia (Callan, Leal, Foord, Dennis, & van Oosten, 2007); as *P. trichocarpa* has never been exposed to *S. musiva* in western Canada, the pathogen's establishment in the province could be detrimental to local *P. trichocarpa* populations.

A black cottonwood plantation comprised of *P. trichocarpa* from 35 different drainages was established in Harrison Mills, BC in 2007. Several trees in that plantation have tested positive for *S. musiva* and *S. populicola*. Currently, the *S. musiva* disease outbreak is localized within the plantation and black cottonwood adjacent to the plantation has not shown any symptoms of infection. As the two pathogens are morphologically identical with overlapping characteristics, and have injury symptoms that are virtually indistinguishable, the two pathogens must be identified and distinguished at the genetic level through the use of *ITS* (Internal Transcribed Spacer) primers developed by Feau et al. (2005).

The threat of *S. musiva* has now reached the Pacific Northwest; it is important to determine if there are patterns in the *P. trichocarpa* provenances in their susceptibility to the

two pathogens and whether or not some provenances display less susceptibility. A field study was conducted at the plantation in Harrison Mills with the objective to determine if any *P. trichocarpa* within the plantation exhibit any resistance to *S. musiva* and *S. populicola*; the plantation had been sampled by Beauseigle et al. (2010) many elements of this study have been drawn on their work with various changes to sampling methodology, laboratory protocol, and statistical analyses. This study divides the 7 geographical regions delineated by Beauseigle et al. 2010 into a northern and southern population and uses a subsample of 306 trees out of over 3000 trees within the plantation to gain a coarse scale analyses.

Methods and Materials

Description of the Plantation

Established in 2007, provenance trial EP1123.02 is a *Populus trichocarpa* plantation located in the Fraser Valley at Harrison Mills, B.C. (49° 13'42.00 N, 121°54'51.30). It is comprised of 516 clones from 35 different drainage regions and distributed within the plantation in a randomized complete block design; each individual tree plot had a spacing of approximately 2.5m x 2.5m. Eight repetitions for each block was planted with each clone is represented 4-8 times within the block, depending on the availability of clonal material. Fertilizers were not applied to the plantation.

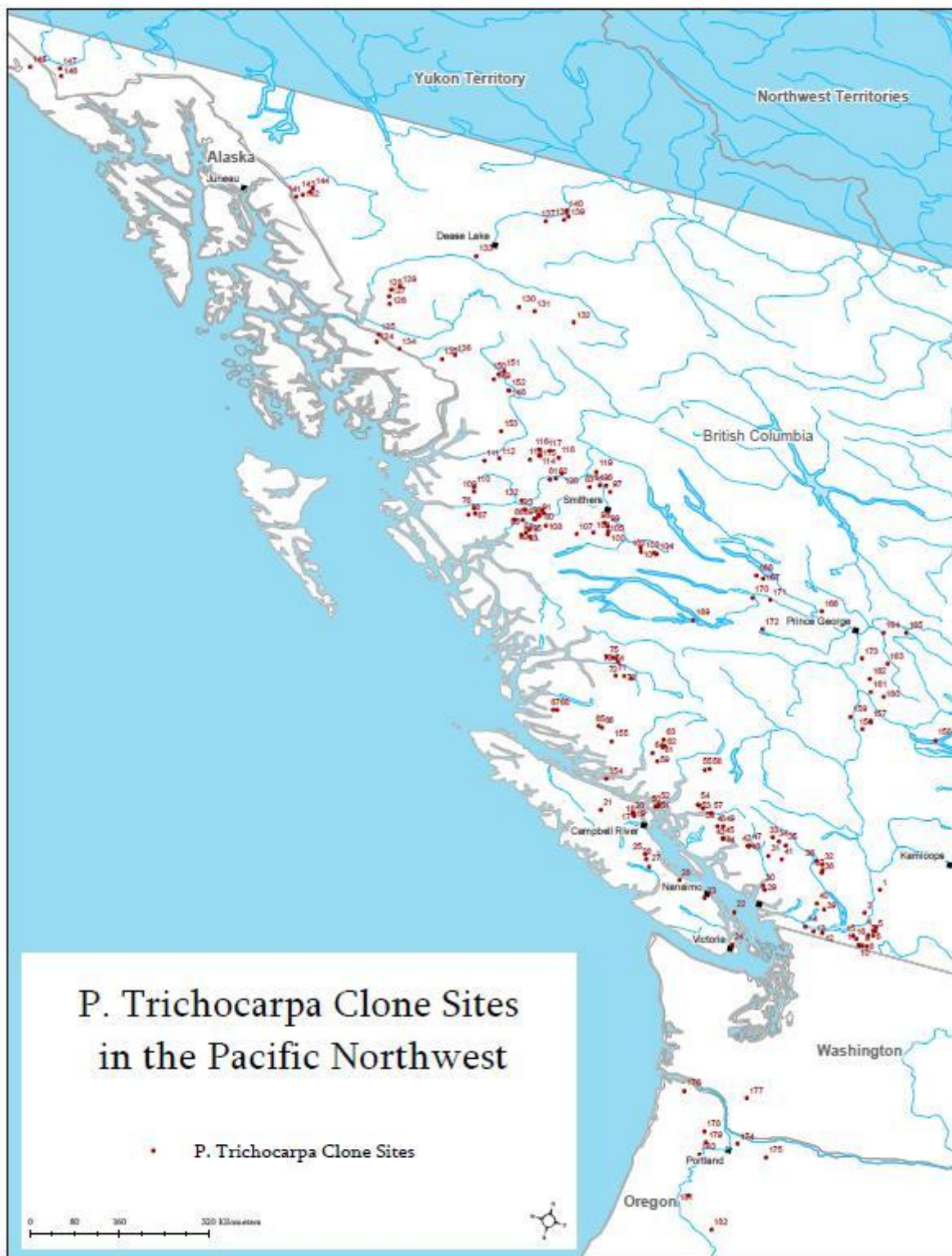


Figure 1 – A map of the origins of each *P. trichocarpa* within the plantation at Harrison Mills. Obtained from Dr. Harry H. Kope, Forest Practices and Investment Branch, British Columbia, Ministry of Forests, Lands, and Natural Resource Operations, Victoria, British Columbia)

Septoria spp. Identification

Identification of leaf spots that were potential *Septoria* spp. lesions was based on characterization in literature (Bier, 1939; Ostry & McNabb, 1985; Sinclair and Lyon, 2005). Lesions on the leaves are generally localized in the lower branches and are characterized as circular necrotic spots that are black in colour. Pycnidia can sometimes be seen within the center of the lesions. Cankers are also characterized as dark, necrotic protrusions from the stem or branches. The figures below illustrate features that are generally associated with *Septoria musiva* and *Septoria populicola*; cankers in the figures belong exclusively to *Septoria musiva*:



Figure 2 – (On left) Photograph of *P. trichocarpa* that has been infected with multiple *Septoria musiva* cankers around the stem (Photograph by Fung, 2012). (On top right) Example of *Septoria musiva* leaf spots on hybrid poplar (Photograph from Sinclair & Lyon, 2005). (On bottom right) Juvenile tree showing symptoms of *Septoria* spp. leaf spot injury. (Photograph from Ostry et al., 1989)

Leaf Sample Collection

Each tree was screened for leaf spots originating from the lower branches that resembled *S. musiva* or *S. populicola*; 3-5 leaves with *Septoria*-like leaf spots were collected from each individual, with each leaf originating from a different side of the tree. Leaves containing *Septoria* and other foliage diseases which had been found in Beauseigle et al. 2010, were omitted to avoid contamination of other leaf samples and complications during the DNA amplification. The collected leaves were placed in a brown paper lunch bag and stored in 4°C while awaiting leaf spot extraction. Trees were also inspected for branch or stem cankers that resemble *S. musiva* infection symptoms. Cankers were also collected to extract *S. musiva* isolates, which would support the samples identified in the leaves. However, the cankers, although extracted for *S. musiva* isolates, were not cross-referenced in this study.

Leaf Spot Extraction

The leaf spot extraction protocol from Beauseigle et al. 2010 was modified from selecting one leaf of the three leaves and selecting a single leaf spot from the leaf to extracting three leaf spots per leaf for each of the leaves collected from the plantation and pooling the spots into a microcentrifuge tube. The leaf spots were excised using a cork borer due to its circular shape, which allowed a more accurate excision while reducing the amount of uninfected plant tissue. All extraction tools were sterilized under a flame torch and 100% ethanol after each sample was processed to avoid cross sample contamination.

DNA Extraction

For each sample, 3-5 leaf spots were selected from and pooled into a new microcentrifuge tube for DNA extraction; this was another change from the protocol used by Beauseigle et al. 2010, where several leaf spots are pooled together for DNA extraction, as opposed to one leaf spot selected from the sample. Several DNA pooling experiments were conducted by spiking *S. musiva* DNA from petri dish cultures with plant and *S. populicola* DNA to determine the maximum number of leaf spots that could be pooled with distinctively observable *S. musiva* readings. This pooling of several leaf spots from one individual tree increases the chance of detecting *S. musiva* by obtaining a broader representation of all the leaf spots on an infected tree.

Metal beads were added to each microcentrifuge tubes and submerged in liquid nitrogen to weaken the cell membranes and allow the extraction of high quality DNA required for the real-time PCR. The microcentrifuge tubes were then placed in a mechanical shaker and ground up into a fine powder. The DNA from the grounded leaf spot samples were then extracted using the DNeasy Plant Mini Kit from Qiagen Company); the protocol (<http://www.qiagen.com/literature/render.aspx?id=201167>) enclosed with the extraction kit was used with the following amendments:

11. Add 100µl **autoclaved water** for elution. Incubate for 5 min at room temperature (15–25°C). Centrifuge for 1 min at $\geq 6000 \times g$.
12. Repeat step 11 - this step was omitted from the extraction protocol.

Extracted DNA samples were then stored in -20°C conditions until the RT-PCR is conducted.

Real-time Polymerase Chain Reaction (RT-PCR)

The efficacy of real-time polymerase chain reaction (RT-PCR) has seen a high degree of success in fungal pathogen detection (Gottsberger, 2010; Qu, Wanner, & Christ, 2011; Schaad, et al., 2003; Callan, Leal, Foord, Dennis, & van Oosten, 2007). RT-PCR also allows for a large number of samples to be processed with the possibility of a quantitative analysis DNA within each sample. The *ITS* sequence showed distinct polymorphisms between *S. musiva* and *S. populicola* (Feau, Weiland, Stanosz, & Bernier, 2005), and as such, *ITS* was targeted using sequence specific oligonucleotides and probes were used to amplify the region. Such methods were also used in Beauseigle, et al. (2010) to detect and differentiate between *S. musiva* and *S. populicola* DNA. An RT-PCR procedure based on the TaqMan probe was used; the primers and probes used is summarized below:

Table 1 - Summary of *S. musiva* and *S. populicola* primers and probes used in RT-PCR to detect and differentiate between the pathogens' DNA. (Beauseigle, Feau, & Hamelin, 2010)

Primer Name	Specificity	Sequence 5'-3'
Smus_SSU193F	<i>S. musiva</i>	CGGTATTTTCAGCCTGCAG
Smus_SSU_288R	<i>S. musiva</i>	GCC GTT ATC CGT ACA ACT GA
Smus_SSU243F_probe	<i>S. musiva</i>	AGGTAGATATTAGGACAATTGGTGTAAGA TGAT
Spopulicola_SSU193F	<i>S. populicola</i>	CGGTATTTTCAGCCTGCAC
Spopulicola_SSU288R	<i>S. populicola</i>	GCCGTTATCCGTACAACACTAG
Spopulicola_SSU243F_probe	<i>S. populicola</i>	AGGAAGATATTAGGACGATGGGTGTAAAGATGAAA

The primers and probes were designed based on the small subunit (SSU) gene using IDT Sci Tools Oligo Analyzer 3.1 software and were custom ordered from Biosearch Technologies (Novato, CA, USA). The probe FAM-BHQ 1 was an oligonucleotide containing fluorescent dye 6-carboxyfluorescein (FAM) as a reporter at the 5' end, and a non-fluorescent quencher dye at the 3' end.

Real-time PCR reactions were performed in MicroAmp Optical 96-well plates using a Bio-rad CFX96™ thermal cycler system (Bio-Rad. Hercules, CA, USA). Each reaction contained 3.6µl of sample DNA, 10µl of 1X SsoFast probes supermix (Bio-Rad. Hercules, CA, USA), with 0.7µl of each forward and reverse primer, 5µl of probe for a total volume of 20µl. Each primer and probe was used at 350nM and 250nM final concentration, respectively. Each sample was duplicated on the same plate.

The thermal cycling parameters used were 2 min at 95°C for enzyme activation followed by 40 cycles of denaturation at 95°C for 5s and 5s of annealing/extension at 60°C. The threshold cycles (CT) were determined by plotting the relative fluorescence Units (RFU) against the cycle number. The results of the amplifications were analyzed using the CFX Manager software (version 1.0, Bio-Rad. Hercules, CA., USA).

Statistical Analysis

Two-tailed Z-test comparisons of the difference between two proportions were conducted on the 2011 and 2010 dataset, using the formulae:

(1.1)

$$Z_{test} = \frac{(\hat{p}_1 - \hat{p}_2)}{S_{\hat{p}_1 - \hat{p}_2}}$$

(1.2)

$$S_{\hat{p}_1 - \hat{p}_2} = \sqrt{\frac{(\hat{p}_1 \hat{q}_1)}{n_1} + \frac{(\hat{p}_2 \hat{q}_2)}{n_2}}$$

The Z-test was set at an alpha level of 0.05, where the $Z_{0.05}$ value = ± 1.96 ; P-values for $Z_{0.05} = 0.256$. \hat{p} = proportion of trees infected with a specific pathogen over the total number of trees uninfected by the pathogen; the pathogen could be *Septoria musiva* or *Septoria populicola*. \hat{q} = number of uninfected trees/total number of trees in region. n_1 and n_2 are the total number of uninfected individuals, which includes samples that were positive for *S. populicola* and samples that were negative for both diseases. Using these formulae, the proportions of infected trees/uninfected trees from the Northern provenances were compared against the Southern populations for separately for both 2010 and 2011 datasets; a summary of the values used listed in the results section below:

Table 2 – Summary of disease infection substituted into equations 1.1 and 1.2. The total number of trees uninfected by either *Septoria musiva* or *Septoria populicola* is excluded from this table.

	2010				2011			
	North		South		North		South	
	<i>S.musiva</i>	<i>S. populicola</i>	<i>S.musiva</i>	<i>S. populicola</i>	<i>S.musiva</i>	<i>S. populicola</i>	<i>S.musiva</i>	<i>S. populicola</i>
# Infected	11	92	9	118	9	104	6	106
Total # (trees)	115		155		149		157	
\hat{p}	0.096	0.800	0.058	0.761	0.047	0.685	0.0385	0.679
\hat{q}	0.904	0.200	0.942	0.239	0.953	0.315	0.962	0.321

Results

Results Overview

The 2010 data set was collected by Beauseigle et al. (2010) and is summarized below in table 3, along with the 2011 results. Beauseigle et al. (2010) had grouped the 35 regions into 7 locations; in this study, the 7 locations (Dease Lake, Far North, Midcoast, Prince George, Smithers, South Coast and USA) were divided into a Northern and Southern population. The

north consists of: Dease Lake, Far North, Midcoast, Prince George and Smithers, while the south is composed of South Coast and USA. The division of these two groups is illustrated in table 3 where the non-shaded regions represent the north, while the shaded cells represent the south. A total of 940 trees were sampled in 2010, but only 270 samples representing 100 families had been analyzed (Beauseigle et al. 2010).

In 2011, trees from all 8 repetitions were sampled from provenance trial EP1123.02, with a total sample size of over 3000 trees; however, a sub-sample of 306 trees were processed, with 149 samples from the north and 157 samples representing the south. A summary of the data collected in 2011 and 2010 are shown in table 3 below; the *S. musiva* infected trees in 2011 are summarized in table 4.

Table 3 - Summary of *Septoria* spp. infection from 2011 and 2010. The pink shaded rows are collectively the Northern region, while the blue shaded rows represent the Southern region. Trees that were positive for both *Septoria* species are not counted in the "*S. musiva* only" and "*S. populicola* only" columns. 2010 data from Beauseigle et al. (2010)

2011					
Area	Number of trees sampled	<i>S.musiva</i> only	<i>S.populicola</i> only	<i>S. musiva</i> + <i>S. populicola</i>	Negative for Both <i>Septoria</i> spp.
Dease Lake	7	2	3	0	2
Far North	3	0	3	0	0
Midcoast	40	0	31	2	7
Prince George	41	2	28	0	11
Smithers	58	1	35	0	22
South Coast	135	4	100	2	29
USA	22	0	4	0	18
Total	306	9	204	4	89
2010					
Dease Lake	11	2	9	-	0
Far North	3	1	2	-	0
Midcoast	46	5	35	-	5
Prince George	22	1	17	-	4
Smithers	33	2	29	-	2
South Coast	136	7	101	-	28
USA	19	2	17	-	1
Total	270	20	210	-	40

Table 4 – Summary of trees that tested positive for *S. musiva* in 2011. The grey shaded rows indicate trees that were also positive for *S. musiva* infection in 2010. The green shaded rows indicate *S. musiva* infected trees in 2011 that were directly adjacent or in close proximity to *S. musiva* positive trees in 2010. Un-shaded rows indicate newly infected trees that are spatially segregated from *S. musiva* infected trees in 2010. The tree tag refers to the actual label of the tree within the plantation, while the tree number denotes the geographical origin and replicate number. Lat = Latitude, Long = Longitude, Elev = Elevation.

Tree Tag	Tree Number	Area	Main Drainage	Lat	Long	Elev	Identification
311	A150-3	Dease Lake	Bell-Irving	5644	12944	579	<i>S.musiva</i>
570	A150-3	Dease Lake	Bell-Irving	5644	12944	579	<i>S.musiva</i>
1020	A56-3	Mid Coast	Homathko	5117	12450	152	<i>S.musiva</i> and <i>S.populicola</i>
1249	A75-4	Mid Coast	Dean	5249	12657	27	<i>S.musiva</i> and <i>S.populicola</i>
901	A165-7	Prince George	McGregor	5411	12200	579	<i>S.musiva</i>
1398	A171-5	Prince George	Nechako R.	5406	12426	655	<i>S.musiva</i>
618	A93-4	Smithers	Kitimat	5409	12835	61	<i>S.musiva</i>
52	A7-5	South Coast	Fraser R.	4924	12133	500	<i>S.musiva</i>
599	ATXD 15-29	South Coast	-	-	-	-	<i>S.musiva</i>
724	A25-3	South Coast	EVIC	4957	12515	76	<i>S.musiva</i>
735	A23-2	South Coast	EVIS	4914	12404	60	<i>S.musiva</i> and <i>S.populicola</i>
958	A17-3	South Coast	EVIN	5013	12548	30	<i>S.musiva</i>
1212	ADTAC 7	South Coast	-	-	-	-	<i>S.musiva</i> and <i>S.populicola</i>

Septoria musiva and *Septoria populicola* infection in 2010

The proportion of infected individuals in Beauseigle et al. (2010) was not divided between north and south populations, but rather a total number of infected individuals across the entire plantation. When divided into north and south, the north had 11 *S.musiva* infected individuals out of a 115 leaf samples (9.6%), while the south had 9 trees that tested positive for *S.musiva* out of 155 trees (5.8%). With *S. populicola* infected individuals, the north had 92 infected trees out of 115 (80.0%) and the south 118 out of 155 (76.1%) that tested positive. The number of trees infected with both diseases is unknown, as it was not reported in Beauseigle et

al. (2010). 40 trees tested negative for either *Septoria* species; however, it should be noted that these 40 individuals were not free of lesions, but simply did not test positive for either disease under the identification protocol.

Z-test comparison of proportions between the number of trees infected with *S. musiva* in the northern population was not significantly different from the number of individuals infected with *S. musiva* in southern populations [Two-tailed Z-test, $Z_{0.05} = \pm 1.96 > Z_{\text{test}} = 1.353$; P-value = 0.256]. Similarly, the proportion of trees infected with *S. populicola* from the north was not significantly different from proportion of infected trees in the south [Two-tailed Z-test, $Z_{0.05} = \pm 1.96 > Z_{\text{test}} = 0.946$; P-value = 0.256].

Septoria musiva and *Septoria populicola* infection in 2011

Analogous to the 2010 analyses, the 7 geographical regions from Beauseigle et al. 2010 were separated into a north and south population and tested for proportion of infected trees over the total number of trees. The samples analyzed in 2011 contained a total of 13 individuals infected with *Septoria musiva* out of 306 processed trees (4.2%). *Septoria populicola* had a higher disease incidence of 210 infected individuals out of 306. Of the infected individuals, 4 trees were positive for both *Septoria musiva* and *Septoria populicola*. 89 trees were negative for infection of either *Septoria* species on leaf with lesions on it.

As in 2010, the proportion of trees infected with *S. musiva* in the north was not significantly different from the proportion of trees infected with *S. musiva* in the south [Two-tailed Z-test, $Z_{0.05} = \pm 1.96 > Z_{\text{test}} = 0.499$; P-value = 0.256]. The proportion of trees infected with

S. populicola in the north was also statistically the same as the proportion of *S. populicola* infected trees in the south. [Two-tailed Z-test, $Z_{0.05} = \pm 1.96 > Z_{\text{test}} = 0.237$; P-value = 0.256].

Septoria spp. infection in 2010 vs. 2011

Trees 570, 901 and 1020 had tested positive in 2010 for *S. musiva* infection; these same three trees have once again tested positive for *S. musiva* in 2011. In addition, trees: 311, 736 and 1398 have tested positive in 2011 and are situated directly adjacent to trees (tree 310 735 and 1400 respectively) that had tested positive in the previous year. Generally, trees that tested positive for *S. musiva* in 2011 are in close proximity to trees that were positive for *S. musiva* in 2010. The exceptions to this are trees 618, 1212 and 1249, which are confirmed for *S. musiva* infection in 2011, but are spatially segregated from previously infected trees in 2010.

A Z-test comparison of proportions was also conducted to compare the proportion of infected individuals in 2010 versus 2011 for each *Septoria* disease, with 2010 infection in the north compared with 2011 infection in the north. In 2010, 9.6% of the 115 samples from the north were infected with *S. musiva*, compared to the 4.7% of the 149 samples in 2011. However, the proportion of *S. musiva* infected trees in the north in 2011 did not statistically differ from the trees that were positive for *S. musiva* in 2010 [Two-tailed Z-test, $Z_{0.05} = -1.96 < Z_{\text{test}} = -1.500$; P-value = 0.256]. *S. musiva* infection was present in 3.8% of the 157 leaf samples from southern trees that were processed in 2011; however, this did not differ significantly from the 5.8% of *S. musiva* infection reported in the south in 2010 [Two-tailed Z-test, $Z_{0.05} = -1.96 < Z_{\text{test}} = -0.739$; P-value = 0.256].

76.1% of the 149 trees sampled from the southern population in 2010 were positive for *S. populicola*, compared to the 67.9% of the 157 trees southern trees in 2011; these proportions were not statistically different [Two-tailed Z-test, $Z_{0.05} = -1.96 < Z_{\text{test}} = -1.660$; P-value = 0.256]. However, the proportion of northern trees infected with *S. populicola* in 2010 (80.0%) did differ significantly from the proportion of infected individuals in 2011 (68.5%) [Two-tailed Z-test, $Z_{0.05} = -1.96 > Z_{\text{test}} = -2.166$; P-value = 0.256].

Discussion

Sampling in 2010 vs. 2011- Methods and Protocol Differences

Although nearly 3000 trees are present within the 8 repetitions at Harrison Mills, leaf samples and statistical analyses were only conducted on a subset of 306 trees located within repetitions 1-3. The purpose of analyzing a subsample of tree leaves is to detect any trends that could be observable at a coarse level scale and reduce the time and effort required to process over 3000 leaf spot samples. In addition, data from the previous year was available for repetitions 1-3 from Beauseigle et al. (2010); this allowed a temporal comparison of available data to detect any apparent trends in *S. musiva* and *S. populicola* infection within the plantation. Tree mortality was also a concern with respect to resampling trees from 2010, more specifically, the trees along the southern edge of replicates 2 and 3, where most of the trees had died, possibility due to maladaptation to the local environment. Trees such as tree 929, which was positive for a *S. musiva* canker in 2010 (Beauseigle et al. 2010), could not be resampled as it was one of the trees along the southeastern edge that had been removed. The 2010 data set from Beauseigle et al. (2010) also represented 100 families within the

provenance trial with a total sample size of 269 individual trees. In the 2011 data set, 107 families are represented over 306 tree samples; however, as the 2011 samples were randomly selected, there is no direct overlap between the families analyzed in 2010 versus those analyzed in 2011. The plantation contains families from 138 provenances; although not all of these families are represented in the 2011 data, a large proportion of the families are covered (107 out of 138). The time of sampling in 2011 was also slightly earlier in the year compared to the collection time of the 2010 dataset. The 2011 leaf samples, more specifically, replicates 1 – 3, were collected in August, while the samples collected by Beauseigle et al. (2010) were collected in the latter half of September. Initial lesions usually appear 3 – 4 weeks after bud burst (Feau, Mottet, Perinet, Hamelin, & Bernier, 2010), which would have been the optimal time to collect samples before other diseases propagate within the plantation. However, flooding from the adjacent river restricted the accessibility of the site and thus, leaf collection was delayed to August.

A key difference between the 2010 and the 2011 dataset is the protocol in the DNA extraction protocol. In Beauseigle et al. (2010), only 1 leaf spot from a single leaf was excised from the infected leaves and extracted. This method was changed to extracting 3 leaf spots from at least 3 leaves per tree, for a total of 9 leaf spots; from these leaf spots, 3-5 are randomly selected and pooled during the DNA extraction and RT-PCR. The detectability *S. musiva* and *S. populicola* using the pooled leaf spot protocol were tested using known isolates of *S. populicola* and *S. musiva* and the primers and probes from table 1. After several pooling and detection experiments, it was determined that 3-5 leaf spots was the optimal number to use. In addition, a portion of the samples in 2010 were processed using a modified Zolan and

Pukkila (1986) extraction protocol; although this protocol allowed more flexibility and controlling DNA purity, the DNA yield is lower than the DNeasy Plant Mini Kit extraction protocol (Beauseigle et al. 2010). The data set from 2011 also contained more than two times the samples that were negative for *Septoria* spp. (40 negatives in 2010 vs. 89 negatives in 2011), which could have been the result of standardizing the extraction protocol that favors the DNA quantity over DNA quality.

Septoria spp. infected trees in 2011

Previous studies have suggested that the primary inoculum for *S. musiva* originates from the leaf litter (Luley & McNabb Jr., 1989; Ostry, 1987). This is reflected in the 2010/2011 data, as the trees that were previously infected in 2010 were once again infected in 2011; trees 570, 901 and 1020 are all positive for *Septoria musiva* in both years of data (table 4). Of the 13 *S. musiva* positive trees identified in 2011, 7 of the infected trees are directly adjacent or in close proximity to trees that were previously infected in 2010 (table 4). However, trees 618, 1212 and 1249 had no prior history of *S. musiva* infection. In addition, these three trees were at least 3 tree rows and columns away from the closest *S. musiva* positive tree in 2010. It is uncertain if the flood from the Fraser River contributed to the spread of the disease, as the extent of the flooding is unknown. However, the three newly infected trees are relatively distant from the riverbank itself. Nonetheless, the infection of *S. musiva* within the plantation appears to have become self-propagating by infecting previously uninfected areas. It is worth noting that tree family A150-3, which originates from the Dease Lake geographical region, appears to be the most highly susceptible to *S. musiva*. Trees 311, 570, 1443 are all within the A150-3 family with

tree 570 testing positive for *S. musiva* infection in 2010 and 2011. Tree 311, although negative for *S. musiva* in 2010, was directly adjacent to tree *S. musiva* positive tree 310 and has been tested positive in 2011. Tree 1443 was not included in the 2011 subsampled data from this study. Beauseigle et al. (2010) had attributed this infection of two trees within the same provenance to a 1) chance event or 2) local inoculum concentrated in the area or 3) they are more susceptible to *S. musiva* than other provenances. The reinfection of tree 570, along with the newly affected tree 311 suggests the infection could be more than a chance event. Trees 291 and 350, similar to tree 311, are also directly adjacent to tree 310; neither of the two trees tested positive for *S. musiva*, suggesting a relatively higher resistance compared to 311. However, the absence of *S. musiva* on 291 and 350 could simply be due to sampling error, where despite increasing the number and pooling the collected leaf spots, the true *S. musiva* infected spots are missed. The susceptibility of northern geographical regions cannot be statistically shown however, as low representation Dease Lake and Far North provenances and tree mortality have drastically reduced the number of available samples from the northern geographical regions and limiting statistical analyses.

Unlike *S. musiva*, the infection of *S. populicola* within the plantation is well established and has infected the majority of the trees in the plantation, infecting 208 out of the total 306 samples collected in 2011. There are no apparent trends with respect to *S. populicola* infection within the plantation, as the disease is native to British Columbia and is distributed wherever susceptible hosts are present (Newcombe, Ostry, Hubbes, Perinet, & Mottet, 2001).

Z-test Results Interpretation

Analyses of the proportion of infected individuals within each geographical region would be the most ideal way of identifying resistant provenances; however, processing over 3000 leaf samples representing all 7 geographical demands a vast amount of time and resources. By using a subset of data, coarse scale analyses can be conducted; the results of such analyses could dictate if the additional 2700 samples would need to be processed. Furthermore, there was an unequal representation of each provenance from when the provenance trial was established due to the availability of clonal material; the mortality of numerous trees have also contributed to the low representation of each geographical region, as reflected in table 3. In response to these limiting factors, the regions were divided into a northern and southern population where the north includes: Dease Lake, Far North, Smithers, Prince George and Midcoast, while the south includes: Southcoast and USA.

In the 2010-northern population, 11/115 (9.6%) of the total samples were positive for *S. musiva* infection, compared to the 9/155 (5.8%) infected in the southern region. Although the north has a greater proportion of individuals infected, this was not statistically different from the proportion of infected individuals found in the southern population. Similarly, in 2011, the 7/149 (4.7%) of *S. musiva* infected trees in the north was not statistically different from the 6/157 (3.8%) infected trees in the south. It is interesting to note that the total number of *S. musiva* infected individuals is approximately an even split between the north and south, with 11 and 9 respectively in 2010, and 7 and 6 respectively in 2011. A comparison between proportion of infected individuals in 2010-north and 2011-north, as well as 2010-south and 2011-south also indicates that the proportions are not statistically different. This could be

interpreted as a stable rate of infection within the plantation between 2010 and 2011. The non-significant variation in *S. musiva* infection between the north and the south interesting, as one would expect a degree of resistance in one region over another, given the large amount of diversity within natural populations of poplars (Newcombe & Bradshaw, Quantitative trait loci conferring resistance in hybrid poplar to Septoria Populicola, the cause of leaf spot, 1996). However, as *S. musiva* had never been reported in the Pacific Northwest until 2006 (Callan, Leal, Foord, Dennis, & van Oosten, 2007), it is unlikely that *P. trichocarpa* has coevolved with *S. musiva* to generate some level of adaptive resistance (Clay & Kover, 1996). This is also reflected in *Populus deltoides* exhibiting some degree of resistances to *S. musiva* cankers, as the two have coevolved together and selective pressures place on one another have yielded resistance and increased virulence to one another (Newcombe & Ostry, 2001). Given total number of trees within the provenance trial, the frequency of cankers on the poplar clones was low. Cankers from branches that were found during the sampling were negative for *S. musiva* and were attributed to other injury causal agents. Several trees appeared to have cankers in the main stem; however, extracting the canker for *S. musiva* was not possible without killing the tree and was thus avoided. Variability in *S. musiva* aggressiveness has been documented given different isolates of the pathogen (Krupinsky, 1989); as the plantation is infected by a newly colonized pathogen that is subjected to population bottlenecks, it is possible that the *S. musiva* present within the plantation is one of mild aggressiveness. However, as LeBoldus et al. (2007) reports that host genotype variability had a bigger influence on disease severity than pathogen genotype variability, the founder's effect the newly colonized *S. musiva* might not be restricting the aggressiveness and disease severity within the plantation. Analyses of neutral molecular

markers within the *S. musiva* genome have suggested that *S. musiva* has a high potential for local adaptation to sudden environmental changes (Feau, Hamelin, Vandecasteele, Stanosz, & Bernier, 2005). Furthermore, LeBoldus et al. (2010) found a significant clone-isolate interaction when conducting spray inoculations on *Populus* clones and *S. musiva* isolates to induce cankers. This clone-isolate interaction could account for the low frequency of cankers within the plantation, as the newly colonized population of *S. musiva* is unable to bypass the tree's natural defense systems to generate cankers without infection through tissue wounds (LeBoldus & Blenis, 2010). Although the aggressiveness and disease severity in the plantation appear to be rare events, overtime, the infection of *S. musiva* in the Fraser Valley could be much more detrimental.

S. populicola infection also showed no significant differences between the north and south population in 2010 and 2011. This result is expected, as *S. populicola* is native to British Columbia; as such, the pathogen and the host have coevolved together through the selective pressure they exert on one another (Clay & Kover, 1996). However, as pathogens have a higher generation turnover, any resistance to *S. populicola* could be by *P. trichocarpa* can be rapidly negated through several generations of genetic recombination. Finally, a significant difference was found in between the *S. populicola* infection in the 2011-north infection and the 2010-north infection. The same significance was not detected in the comparison between 2011-south and 2010-south populations. This year over year difference could be attributed to *S. populicola* being epidemic in some years and not epidemic in others (Newcombe & Bradshaw, 1996).

Experimental Improvements and Future Research

Although north and south populations showed no significant differences in *S. musiva* infection proportions, the apparent trend in the reinfection of the provenances in Dease Lake suggests that the region is relatively more susceptible to *S. musiva* than the geographical regions to the south. However, given the limited dataset, statistically analyses could not be conducted on each geographical region alone. As such, a better representation of each individual region, specifically from the northern provenances could strengthen this apparent relationship. As samples from the other 5 repetitions are available, it may be beneficial to process the samples to detect for the presence or absence of *S. musiva*. In addition, repeating the experiment for an additional year may be helpful in determining if the reinfections of specific regions are due to chance and high inoculum concentrations, or if there is a difference in quantitative resistance to *S. musiva*.

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Appendices

Tree No.	Tree ID	Area	Main Drainage	Location	Map #	Lat	Long	Elev	Pathogen Identity
40	A80-2	Smithers	Skeena	Skeena RID. SKN	80	5451	12820	140	S.populicola
44	A165-6	Prince George	McGregor	McGregorR. MCG	165	5411	12200	579	S.populicola
45	A105-5	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R./	105	5440	12707	567	S.populicola
52	A7-5	South Coast	Fraser R.			4924	12133	500	S.musiva
55	A146-7	Far North	Aisek	Tatshenshini	146	5926	13750	34	S.populicola
95	A162-1	Prince George	Fraser R.	Hixon HIXN 1	162	5324	12238	518	S.populicola
109	A158-3	Prince George	Cunningham R.	16 Kimball Cr.	158	5256	12110	823	S.populicola
115	A158-4	Prince George	Cunningham R.	16 Kimball Cr.	158	5256	12110	823	S.populicola
168	A55-4	Mid Coast	Homathko	Homathko R.IC.	55	5114	12 457	88	S.populicola
169	A12-4	South Coast	Fraser	Vender Cana1IL.	12	4909	12206	20	negative for both Septoria species
176	A178-4	USA	GS033	Pittsburg, W A. P	178	4550	12307	900	S.populicola
177	A1-5	South Coast	Fraser	Nahatlatch R./A.	1	5000	12134	213	S.populicola
184	A87-4	Smithers	Skeena	Skeena R.IN. SK	87	5417	12922	30	S.populicola
185	A153-3	Smithers	U. Nass	White RIA. WHTA	153	5556	12916	366	S.populicola
190	A27-3	South Coast	EVIC	Courtenay/H. CNYH	27	4940	12504	76	S.populicola
192	A161-3	Prince George	Fraser R.	Fraser R. QFRS	161	5304	12231	472	S.populicola
231	A158-2	Prince George	Cunningham R.	16 Kimball Cr.	158	5256	12110	823	S.populicola
257	A163-7	Prince George	Fraser R.	WillowR. WLOW	163	5355	12217	640	negative for both Septoria species
274	A88-2	Smithers	Skeena	Skeena R.IO. SK	88	5413	12932	21	S.populicola
281	A164-6	Prince George	Fraser R.	5 Shelley SHEL	164	5402	12236	564	negative for both Septoria species
282	A156-7	Prince George	Quesnel R.	6 Australian QA	156	5243	12228	442	S.populicola
285	A94-4	Smithers	Kitimat	Kitimat R.IC. K	94	5403	12841	18	negative for both Septoria species
291	A164-1	Prince George	Fraser R.	5 Shelley SHEL	164	5402	12236	564	S.populicola
311	A150-3	Dease Lake	Bell-Irving	7 Bell-Irving/C.	150	5644	12944	579	S.musiva

321	A92-1	Smithers	Kitimat	Kitimat R./ A.	92	5415	12831	122	S.populicola
323	A82-2	Smithers	Skeena	KitwangaIF . KT	82	5505	12811	177	S.populicola
325	A156-6	Prince George	Quesnel R.	6 Australian QA	156	5243	12228	442	S.populicola
326	A161-3	Prince George	Fraser R.	Fraser R. QFRS	161	5304	12231	472	S.populicola
331	A160-4	Prince George	Fraser R.	6 Cottonwood QC	160	5302	12209	823	S.populicola
337	A94-2	Smithers	Kitimat	Kitimat R.IC. K	94	5403	12841	18	S.populicola
348	A88-2	Smithers	Skeena	Skeena R.IO. SK	88	5413	12932	21	negative for both Septoria species
349	A87-2	Smithers	Skeena	Skeena R.IN. SK	87	5417	12922	30	negative for both Septoria species
350	A95-4	Smithers	Kitimat	Hirsch Cr.ID. H	95	5404	12827	335	negative for both Septoria species
356	A159-5	Prince George	Fraser R.	Baker Cr. QBKR	159	5257	12252	823	S.populicola
367	A116-5	Smithers	U. Nass	NassRtH. NASH	116	5543	12849	183	S.populicola
410	A156-7	Prince George	Quesnel R.	6 Australian QA	156	5243	12228	442	S.populicola
424	A76-5	Smithers	Skeena	Exehamsiks RIP	76	5425	12926	46	negative for both Septoria species
448	A171-4	Prince George	Nechako R.	14 Redmond Cr. A.	171	5406	12426	655	negative for both Septoria species
453	A153-3	Smithers	U. Nass	White RIA. WHTA	153	5556	12916	366	S.populicola
468	A98-4	Smithers	Bulkley	Bulkley R.IC. B	98	5457	12717	451	negative for both Septoria species
475	A162-1	Prince George	Fraser R.	Hixon HIXN 1	162	5324	12238	518	negative for both Septoria species
505	A95-2	Smithers	Kitimat	Hirsch Cr.ID. H	95	5404	12827	335	negative for both Septoria species
506	A136-2	Dease Lake	L. Stikine	6 Iskut R.IC. I	136	5656	13020	317	negative for both Septoria species
519	A96-4	Smithers	Bulkley	Bulkley R./ A.	96	5515	12730	311	negative for both Septoria species
522	A41-3	South Coast	Squamish	GlacierB Aha L.	41	5006	12300	579	negative for both Septoria species
531	A163-4	Prince George	Fraser R.	WillowR. WLOW	163	5355	12217	640	negative for both Septoria species
570	A150-3	Dease Lake	Bell-Irving	7 Bell- Irving/C.	150	5644	12944	579	S.musiva
571	A22-4	South Coast	EVIS	Cassigy/J. CSYJ	22	4904	12352	20	S.populicola
573	A33-3	South Coast	Lillooet	a Lillooet R./ A.	33	5037	12323	411	S.populicola

574	A89-8	Smithers	Skeena	Skeena R.IP. SK	89	5433	12828	61	S.populicola
576	A9-2	South Coast	Fraser	Chilliwack R.#2/I.	9	4906	12138	360	S.populicola
577	A105-1	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R./	105	5440	12707	567	S.populicola
578	A179-2	USA	GS031	North Plains, OR.	179	4534	12300	100	S.populicola
579	A35-4	South Coast	Lillooet	a Lillooet R.IC.	35	5030	12300	271	S.populicola
580	A25-2	South Coast	EVIC	Campbell R.IF. CM	25	4957	12515	76	negative for both Septoria species
581	A57-5	South Coast	Southgate	1 Southgate R./ A	57	5049	12429	239	S.populicola
583	A171-1	Prince George	Nechako R.	14 Redmond Cr. A.	171	5406	12426	655	S.populicola
584	A67-1	Mid Coast	Rivers	Chuckwalla R.IC.	67	5144	12719	67	S.populicola
585	A87-4	Smithers	Skeena	Skeena R.IN. SK	87	5417	12922	30	S.populicola
586	A179-5	USA	GS031	North Plains, OR.	179	4534	12300	100	S.populicola
587	A71-2	Mid Coast	Burke	Bella Coola R.IC.	71	5223	12636	135	S.populicola
588	A174-3	USA	GS001	Carson, WA. CARS	174	4545	12250	650	negative for both Septoria species
590	A47-5	South Coast	Jervis	Skwawka R.IF. SK	47	5015	12356	244	S.populicola
591	A74-1	Mid Coast	Dean	eanR./C. DENC 7	74	5250	12648	91	S.populicola
592	A1-1	South Coast	Fraser	Nahatlatch R./A.	1	5000	12134	213	negative for both Septoria species
593	A154-4	Mid Coast	KingcomeR.	20a Atlatzi R.IA.	154	5059	12607	0	S.populicola
594	A13-5	South Coast	Fraser	MatsQui Is.IM. M	13	4907	12220	8	S.populicola
596	A19-3	South Coast	EVIN	Salmon R. #2/C SL	19	5016	12550	30	S.populicola
598	A72-4	Mid Coast	Dean	eanR./A. DENA 7	72	5246	12637	213	S.populicola
599	ATXD 15-29	South Coast							S.musiva
615	A79-4	Smithers	Skeena	Skeena R/e. SKN	79	5446	12816	122	negative for both Septoria species
616	A84-5	Smithers	Skeena	HazeltonIH. HAZ	84	5513	12740	238	negative for both Septoria species
618	A93-4	Smithers	Kitimat	Kitimat R.IB. K	93	5409	12835	61	S.musiva
619	A106-2	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R.I	106	5437	12730	735	S.populicola

624	A163-3	Prince George	Fraser R.	WillowR. WLOW	163	5355	12217	640	S.populicola
627	A151-1	Dease Lake	Bell-Irving	7 Bell- Irving/D.	151	5651	12937	677	S.populicola
631	A95-4	Smithers	Kitimat	Hirsch Cr.ID. H	95	5404	12827	335	S.populicola
636	A76-5	Smithers	Skeena	Exehamsiks RIP	76	5425	12926	46	negative for both Septoria species
644	A156-1	Prince George	Quesnel R.	6 Australian QA	156	5243	12228	442	negative for both Septoria species
645	A123-3	Smithers	Skeena	Kitsumkalum R.IB.	123	5444	12847	155	negative for both Septoria species
661	A93-4	Smithers	Kitimat	Kitimat R.IB. K	93	5409	12835	61	negative for both Septoria species
663	A106-1	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R.I	106	5437	12730	735	S.populicola
667	A164-3	Prince George	Fraser R.	5 Shelley SHEL	164	5402	12236	564	S.populicola
685	A113-1	Smithers	U. Nass	Nass RIE. NASE	113	5524	12859	207	negative for both Septoria species
702	A163-4	Prince George	Fraser R.	WillowR. WLOW	163	5355	12217	640	negative for both Septoria species
705	A53-1	South Coast	Homathko	Homathko R./ A.	53	5056	12451	46	negative for both Septoria species
706	A92-1	Smithers	Kitimat	Kitimat R./ A.	92	5415	12831	122	S.populicola
709	A37-2	South Coast	Harrison	Lillooet R./ A.	37	5007	12232	314	S.populicola
711	A94-3	Smithers	Kitimat	Kitimat R.IC. K	94	5403	12841	18	S.populicola
713	A183-1	USA	GS056	Lafayette, OR. LA	183	4512	12305	100	S.populicola
714	A157-3	Prince George	Quesnel R.	6 Quesnel L. QL	157	5258	12219	488	S.populicola
715	A36-1	South Coast	Lillooet	Lillooet R.ID.	36	5018	12245	222	S.populicola
716	A44-3	South Coast	Jervis	Skwawka R.IC. SK	44	5015	12400	152	S.populicola
717	A123-3	Smithers	Skeena	Kitsumkalum R.IB.	123	5444	12847	155	S.populicola
718	A177-1	USA	GS024	Castle Rock, W A.	177	4620	12255	100	negative for both Septoria species
719	A182-2	USA	GS051	Jasper, OR. JASP	182	4400	12255	150	S.populicola
720	A8-3	South Coast	Fraser	Chilliwack R.#/H.	8	4906	12131	480	S.populicola
721	A3-3	South Coast	Fraser	Wellington Bar/C.	3	4940	12125	91	S.populicola
722	A39-3	South Coast	Harrison	Lillooet R.IC.	39	4946	12213	64	S.populicola
723	A64-3	Mid Coast	Knight	0 W.Klinaklini R.	64	5118	12546	546	S.populicola
724	A25-3	South Coast	EVIC	Campbell R.IF. CM	25	4957	12515	76	S.musiva

726	A72-1	Mid Coast	Dean	eanR./A. DENA 7	72	5246	12637	213	S.populicola
727	A9-2	South Coast	Fraser	Chilliwack R.#2/I.	9	4906	12138	360	S.populicola
729	A45-4	South Coast	Jervis	Skwawka R.ID. SK	45	5015	12401	323	S.populicola
730	A53-1	South Coast	Homathko	Homathko R./ A.	53	5056	12451	46	S.populicola
732	A4-2	South Coast	Fraser	Yale West/D. YA	4	4934	12128	549	S.populicola
733	A17-2	South Coast	EVIN	Memekav R./ A. ME	17	5013	12548	30	S.populicola
735	A23-2	South Coast	EVIS	Lantzville/K. LNZ	23	4914	12404	60	S.musiva and S.populicola
736	A69-5	Mid Coast	Burke	Bella Coola R./ A.	69	5225	12610	152	S.populicola
737	A13-2	South Coast	Fraser	MatsQui Is.IM. M	13	4907	12220	8	S.populicola
738	A27-2	South Coast	EVIC	Courtenay/H. CNYH	27	4940	12504	76	S.populicola
740	A172-6	Prince George	Nechako R.	14 Diamond Is. B.	172	5357	12426	671	S.populicola
741	A39-2	South Coast	Harrison	Lillooet R.IC.	39	4946	12213	64	S.populicola
742	A59-4	Mid Coast	Knight	W.Klinaklini R./ A	59	5107	12535	30	negative for both Septoria species
743	A64-1	Mid Coast	Knight	W.Klinaklini R.	64	5118	12546	546	negative for both Septoria species
748	A23-4	South Coast	EVIS	Lantzville/K. LNZ	23	4914	12404	60	negative for both Septoria species
756	A18-2	South Coast	EVIN	Salmon R. #IB. S	18	5013	12549	30	S.populicola
757	A28-5	South Coast	EVIC	Fanny Bayll. FNYI	28	4931	12451	46	S.populicola
758	A26-5	South Coast	EVIC	Black Creek/G. BL	26	4950	12511	91	negative for both Septoria species
759	A5-5	South Coast	Fraser	Yale East/E. YA	5	4934	12124	61	negative for both Septoria species
760	A52-1	South Coast	Phillips	Phillips R.IC.	52	5041	12515	58	S.populicola
761	A46-1	South Coast	Jervis	Skwawka R.IE. S	46	5013	12357	231	negative for both Septoria species
762	A9-4	South Coast	Fraser	Chilliwack R.#2/I.	9	4906	12138	360	negative for both Septoria species
764	A161-4	Prince George	Fraser R.	Fraser R. QFRS	161	5304	12231	472	negative for both Septoria species
765	A66-4	Mid Coast	Machmell	Machmell R.IB	66	5137	12635	122	negative for both Septoria species
768	A12-2	South Coast	Fraser	Vender Cana1IL.	12	4909	12206	20	negative for both Septoria species
770	A74-4	Mid Coast	Dean	eanR./C. DENC 7	74	5250	12648	91	negative for both Septoria species

771	A19-5	South Coast	EVIN	Salmon R. #2/C SL	19	5016	12550	30	S.populicola
772	A33-5	South Coast	Lillooet	a Lillooet R./ A.	33	5037	12323	411	S.populicola
774	A32-2	South Coast	Squamish	Elaho R.ID. EL	32	5015	12235	305	S.populicola
778	A128-1	Dease Lake	L. Stikine	L. Stikine	128	5731	13147	61	S.populicola
779	A32-3	South Coast	Squamish	Elaho R.ID. EL	32	5015	12235	305	S.populicola
781	A50-1	South Coast	Phillips	Phillips R./ A.	50	5036	12519	5	S.populicola
782	A48-2	South Coast	Toba Inlet	23 Toba R./A. T	48	5031	12414	67	negative for both Septoria species
833	A29-2	South Coast	Squamish	Squamish R./ A.	29	4952	12314	61	S.populicola
835	A66-5	Mid Coast	Machmell	Machmell R.IB	66	5137	12635	122	S.populicola
837	A50-4	South Coast	Phillips	Phillips R./ A.	50	5036	12519	5	S.populicola
838	A39-5	South Coast	Harrison	Lillooet R.IC.	39	4946	12213	64	S.populicola
840	A75-2	Mid Coast	Dean	eanR.ID. DEND 7	75	5249	12657	27	S.populicola
841	A105-5	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R./	105	5440	12707	567	S.populicola
842	A14-3	South Coast	Fraser	McMillan Is.IN.	14	4911	12235	15	negative for both Septoria species
844	A177-5	USA	GS024	Castle Rock, W A.	177	4620	12255	100	S.populicola
845	A92-4	Smithers	Kitimat	Kitimat R./ A.	92	5415	12831	122	negative for both Septoria species
846	A15-2	South Coast	Fraser	Harrison R./O. H	15	4917	12157	40	S.populicola
848	A19-4	South Coast	EVIN	Salmon R. #2/C SL	19	5016	12550	30	S.populicola
849	A159-2	Prince George	Fraser R.	Baker Cr. QBKR	159	5257	12252	823	S.populicola
851	A100-4	Smithers	Bulkley	Bulkley R.IE. B	100	5445	12707	494	negative for both Septoria species
852	A92-3	Smithers	Kitimat	Kitimat R./ A.	92	5415	12831	122	S.populicola
854	A43-4	South Coast	Jervis	Skwawka R.IB. SK	43	5014	12400	115	S.populicola
856	A45-4	South Coast	Jervis	Skwawka R.ID. SK	45	5015	12401	323	S.populicola
857	A92-2	Smithers	Kitimat	Kitimat R./ A.	92	5415	12831	122	negative for both Septoria species
858	A41-5	South Coast	Squamish	GlacierIB Aha L.	41	5006	12300	579	S.populicola
860	A49-5	South Coast	Toba Inlet	23 TobaR.IB. TO	49	5034	12405	73	S.populicola
861	A58-2	South Coast	Southgate	1 Southgate R.IB.	58	5053	12444	91	negative for both Septoria species

862	A174-3	USA	GS001	Carson, WA. CARS	174	4545	12250	650	S.populicola
863	A37-2	South Coast	Harrison	Lillooet R./ A.	37	5007	12232	314	S.populicola
880	A17-2	South Coast	EVIN	Memekav R./ A. ME	17	5013	12548	30	S.populicola
881	A73-4	Mid Coast	Dean	eanR.IB. DENB 7	73	5250	12642	152	S.populicola
882	A32-5	South Coast	Squamish	Elaho R.ID. EL	32	5015	12235	305	S.populicola
883	A49-2	South Coast	Toba Inlet	23 TobaR.IB. TO	49	5034	12405	73	S.populicola
884	A181-5	USA	GS048	Halsey, OR. HALS	181	4425	12320	300	S.populicola
886	A4-5	South Coast	Fraser	Yale West/D. YA	4	4934	12128	549	S.populicola
888	A53-2	South Coast	Homathko	Homathko R./ A.	53	5056	12451	46	S.populicola
889	A24-2	South Coast	EVIS	Chemainus R.I.L. C	24	4853	12342	5	S.populicola
890	A15-5	South Coast	Fraser	Harrison R./O. H	15	4917	12157	40	negative for both Septoria species
892	A23-4	South Coast	EVIS	Lantzville/K. LNZ	23	4914	12404	60	S.populicola
893	A133-1	Dease Lake	U. Stikine	Tanzilla R./A.	133	5818	13028	567	S.populicola
894	A15-3	South Coast	Fraser	Harrison R./O. H	15	4917	12157	40	S.populicola
896	A28-2	South Coast	EVIC	Fanny Bayll. FNYI	28	4931	12451	46	S.populicola
897	A58-2	South Coast	Southgate	1 Southgate R.IB.	58	5053	12444	91	negative for both Septoria species
898	A122-2	Smithers	Skeena	Cedar R./A (KITA)	122	5457	12855	274	S.populicola
899	A21-4	South Coast	EVIN	White R.IE. WHITE,	21	5008	12603	213	negative for both Septoria species
900	A177-2	USA	GS024	Castle Rock, W A.	177	4620	12255	100	S.populicola
901	A165-7	Prince George	McGregor	McGregorR. MCG	165	5411	12200	579	S.musiva
902	A6-1	South Coast	Fraser	Hope North/F. H	6	4926	12126	61	S.populicola
904	A78-3	Smithers	Skeena	Skeena RIB. SKN	78	5441	12823	122	negative for both Septoria species
905	A154-5	Mid Coast	KingcomeR.	20a Atlatzi R.IA.	154	5059	12607	0	S.populicola
906	A180-4	USA	GS041	Jefferson, OR. JE	180	4444	12305	100	negative for both Septoria species
907	A12-1	South Coast	Fraser	Vender Cana1IL.	12	4909	12206	20	negative for both Septoria species
909	A75-1	Mid Coast	Dean	eanR.ID. DEND 7	75	5249	12657	27	S.populicola

910	A32-4	South Coast	Squamish	Elaho R.ID. EL	32	5015	12235	305	negative for both Septoria species
911	A58-1	South Coast	Squamish	GlacierIB Aha L.	41	5006	12300	579	negative for both Septoria species
912	A67-4	Mid Coast	Southgate	1 Southgate R.IB.	58	5053	12444	91	negative for both Septoria species
916	A71-4	Mid Coast	Burke	Bella Coola R.IC.	71	5223	12636	135	negative for both Septoria species
917	A2-3	South Coast	Fraser	Nahatlatch L.IB.	2	4958	12149	335	S.populicola
918	A54-4	South Coast	Homathko	Homathko R.IB.	54	5057	12454	37	S.populicola
919	A7-1	South Coast	Fraser	Hope South/G. H	7	4924	12133	500	negative for both Septoria species
920	A106-2	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R.I	106	5437	12730	735	S.populicola
921	A154-2	Mid Coast	KingcomeR.	20a Atlatzi R.IA.	154	5059	12607	0	S.populicola
922	A178-2	USA	GS033	Pittsburg, W A. P	178	4550	12307	900	S.populicola
923	A52-2	South Coast	Phillips	Phillips R.IC.	52	5041	12515	58	negative for both Septoria species
937	A161-2	Prince George	Fraser R.	Fraser R. QFRS	161	5304	12231	472	negative for both Septoria species
938	A15-3	South Coast	Fraser	Harrison R./O. H	15	4917	12157	40	negative for both Septoria species
939	A54-3	South Coast	Homathko	Homathko R.IB.	54	5057	12454	37	S.populicola
940	A122-2	Smithers	Skeena	Cedar R./A (KITA)	122	5457	12855	274	negative for both Septoria species
942	A72-1	Mid Coast	Dean	eanR./A. DENA 7	72	5246	12637	213	negative for both Septoria species
943	A38-2	South Coast	Harrison	Lillooet R.IB.	38	5002	12232	213	S.populicola
944	A54-2	South Coast	Homathko	Homathko R.IB.	54	5057	12454	37	negative for both Septoria species
946	A53-4	South Coast	Homathko	Homathko R./ A.	53	5056	12451	46	S.populicola
948	A20-2	South Coast	EVIN	Salmon R. #3ID. S	20	5017	12552	30	S.populicola
949	A175-5	USA	GS009	N. Bonneville WA.	175	4535	12200	300	S.populicola
950	A171-1	Prince George	Nechako R.	14 Redmond Cr. A.	171	5406	12426	655	S.populicola
951	A22-3	South Coast	EVIS	Cassigy/J. CSYJ	22	4904	12352	20	S.populicola
953	A11-2	South Coast	Fraser	Chilliwack R.#4/K.	11	4905	12144	280	negative for both Septoria species
957	A50-5	South Coast	Phillips	Phillips R./ A.	50	5036	12519	5	negative for both Septoria species

958	A17-3	South Coast	EVIN	Memekav R./ A. ME	17	5013	12548	30	S.musiva
959	A42-4	South Coast	Jervis	Skwawka R./ A. S	42	5013	12359	61	negative for both Septoria species
960	A183-4	USA	GS056	Lafayette, OR. LA	183	4512	12305	100	S.populicola
961	A158-6	Prince George	Cunningham R.	16 Kimball Cr.	158	5256	12110	823	S.populicola
962	A80-2	Smithers	Skeena	Skeena RID. SKN	80	5451	12820	140	negative for both Septoria species
963	A46-5	South Coast	Jervis	Skwawka R.IE. S	46	5013	12357	231	S.populicola
964	A1-2	South Coast	Fraser	Nahatlatch R./A.	1	5000	12134	213	S.populicola
966	A75-5	Mid Coast	Dean	eanR.ID. DEND 7	75	5249	12657	27	S.populicola
967	A28-3	South Coast	EVIC	Fanny Bayll. FNYI	28	4931	12451	46	S.populicola
968	A41-3	South Coast	Squamish	GlacierIB Aha L.	41	5006	12300	579	S.populicola
969	A36-2	South Coast	Lillooet	Lillooet R.ID.	36	5018	12245	222	S.populicola
970	A180-3	USA	GS041	Jefferson, OR. JE	180	4444	12305	100	S.populicola
983	A87-2	Smithers	Skeena	Skeena R.IN. SK	87	5417	12922	30	S.populicola
998	A165-7	Prince George	McGregor	McGregorR. MCG	165	5411	12200	579	S.populicola
1020	A56-3	Mid Coast	Homathko	Homathko R.ID.	56	5117	12450	152	S.musiva and S.populicola
1049	A178-3	USA	GS033	Pittsburg, W A. P	178	4550	12307	900	S.populicola
1068	A128-3	Dease Lake	L. Stikine	L. Stikine	128	5731	13147	61	negative for both Septoria species
1200	A7-3	South Coast	Fraser	Hope South/G. H	7	4924	12133	500	S.populicola
1212	ADTAC 7	South Coast							S.musiva and S.populicola
1216	A45-5	South Coast	Jervis	Skwawka R.ID. SK	45	5015	12401	323	S.populicola
1217	A158-5	Prince George	Cunningham R.	16 Kimball Cr.	158	5256	12110	823	S.populicola
1218	A105-5	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R./	105	5440	12707	567	S.populicola
1225	A23-5	South Coast	EVIS	Lantzville/K. LNZ	23	4914	12404	60	S.populicola
1230	A15-4	South Coast	Fraser	Harrison R./O. H	15	4917	12157	40	negative for both Septoria species
1233	A7-4	South Coast	Fraser	Hope South/G. H	7	4924	12133	500	S.populicola
1235	A55-3	Mid Coast	Homathko	Homathko R.IC.	55	5114	12 457	88	S.populicola

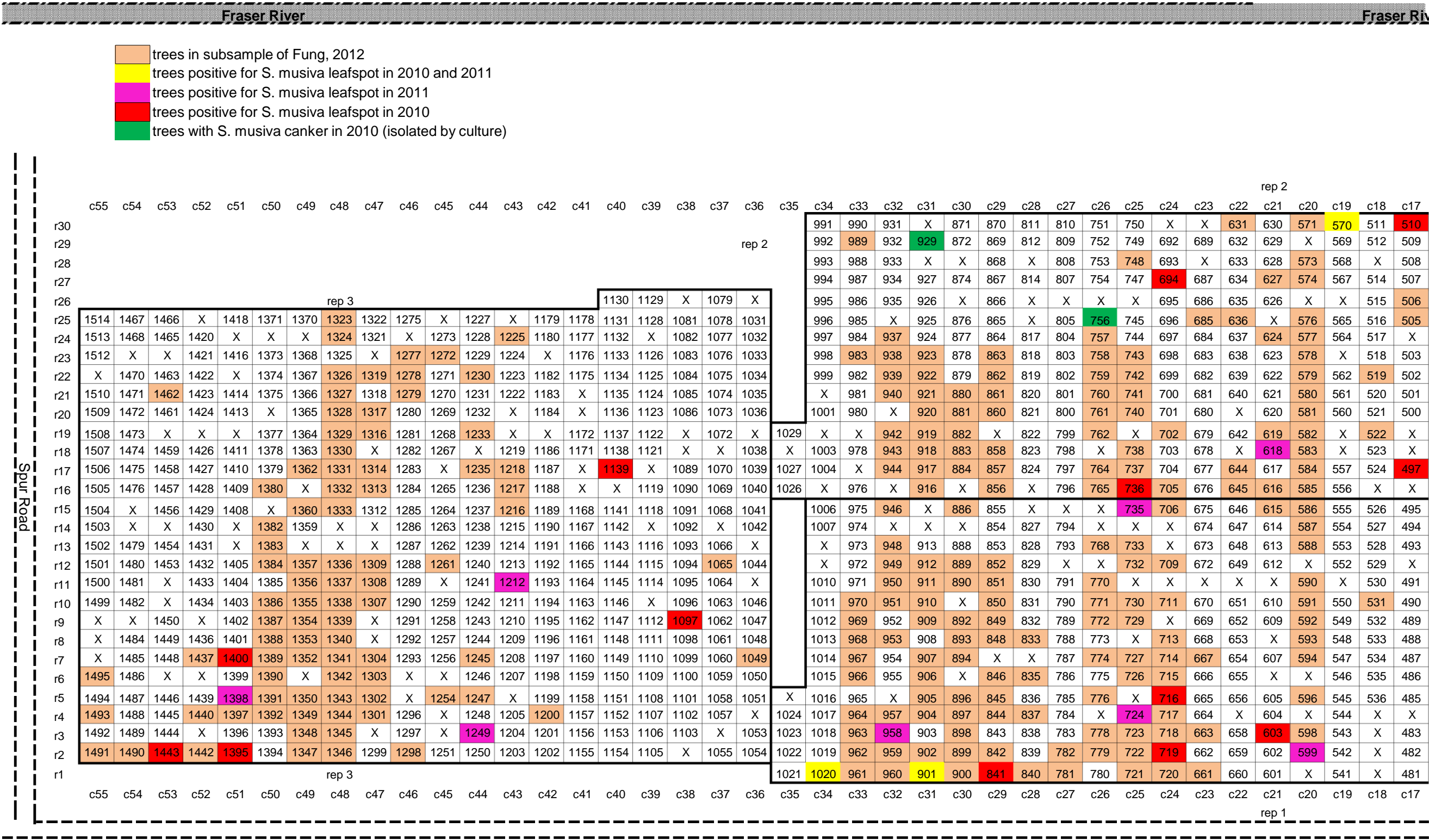
1245	A79-4	Smithers	Skeena	Skeena R/e. SKN	79	5446	12816	122	S.populicola
1247	A35-1	South Coast	Lillooet	a Lillooet R.IC.	35	5030	12300	271	S.populicola
1249	A75-4	Mid Coast	Dean	eanR.ID. DEND 7	75	5249	12657	27	S.musiva and S.populicola
1254	A173-2	Prince George	Nechako R.	14 Chilako R. C	173	5353	12259	183	S.populicola
1261	A64-2	Mid Coast	Knight	0 W.Klinaklini R.	64	5118	12546	546	S.populicola
1272	A28-4	South Coast	EVIC	Fanny Bayll. FNYI	28	4931	12451	46	S.populicola
1277	A50-2	South Coast	Phillips	Phillips R./ A.	50	5036	12519	5	S.populicola
1278	A11-1	South Coast	Fraser	Chilliwack R.#4/K.	11	4905	12144	280	S.populicola
1279	A178-1	USA	GS033	Pittsburg, W A. P	178	4550	12307	900	negative for both Septoria species
1298	A182-3	USA	GS051	Jasper, OR. JASP	182	4400	12255	150	S.populicola
1302	A10-1	South Coast	Fraser	Chilliwack R.#3/J.	10	4905	12143	280	S.populicola
1303	A164-2	Prince George	Fraser R.	5 Shelley SHEL	164	5402	12236	564	negative for both Septoria species
1304	A17-2	South Coast	EVIN	Memekav R./ A. ME	17	5013	12548	30	S.populicola
1307	A53-1	South Coast	Homathko	Homathko R./ A.	53	5056	12451	46	S.populicola
1308	A32-4	South Coast	Squamish	Elaho R.ID. EL	32	5015	12235	305	S.populicola
1309	A26-2	South Coast	EVIC	Black Creek/G. BL	26	4950	12511	91	S.populicola
1313	A165-6	Prince George	McGregor	McGregorR. MCG	165	5411	12200	579	S.populicola
1314	A25-1	South Coast	EVIC	Campbell R.IF. CM	25	4957	12515	76	S.populicola
1316	A142-5	Far North	Taku	Taku River/B	142	5842	13324	49	S.populicola
1317	A54-3	South Coast	Homathko	Homathko R.IB.	54	5057	12454	37	S.populicola
1319	A181-1	USA	GS048	Halsey, OR. HALS	181	4425	12320	300	S.populicola
1323	A16-2	South Coast	Fraser	Harrison Mill/P.	16	4914	12151	30	negative for both Septoria species
1324	A92-3	Smithers	Kitimat	Kitimat R./ A.	92	5415	12831	122	negative for both Septoria species
1326	A45-4	South Coast	Jervis	Skwawka R.ID. SK	45	5015	12401	323	S.populicola
1327	A154-5	Mid Coast	KingcomeR.	20a Atlatzi R.IA.	154	5059	12607	0	S.populicola
1328	A76-5	Smithers	Skeena	Exehamsiks RIP	76	5425	12926	46	S.populicola

1329	A154-2	Mid Coast	KingcomeR.	20a Atlatzi R.IA.	154	5059	12607	0	S.populicola
1330	A68-3	Mid Coast	Rivers	Chuckwalla R.ID.	68	5146	12712	79	S.populicola
1331	A9-5	South Coast	Fraser	Chilliwack R.#2/I.	9	4906	12138	360	S.populicola
1332	A4-2	South Coast	Fraser	Yale West/D. YA	4	4934	12128	549	S.populicola
1333	A27-3	South Coast	EVIC	Courtenay/H. CNYH	27	4940	12504	76	S.populicola
1336	A43-3	South Coast	Jervis	Skwawka R.IB. SK	43	5014	12400	115	S.populicola
1337	A36-3	South Coast	Lillooet	Lillooet R.ID.	36	5018	12245	222	S.populicola
1338	A22-4	South Coast	EVIS	Cassigy/J. CSYJ	22	4904	12352	20	S.populicola
1339	A46-1	South Coast	Jervis	Skwawka R.IE. S	46	5013	12357	231	S.populicola
1340	A181-5	USA	GS048	Halsey, OR. HALS	181	4425	12320	300	S.populicola
1341	A19-2	South Coast	EVIN	Salmon R. #2/C SL	19	5016	12550	30	S.populicola
1342	A16-5	South Coast	Fraser	Harrison Mill/P.	16	4914	12151	30	S.populicola
1343	A31-3	South Coast	Squamish	Squamish R.IC.	31	5006	12322	177	S.populicola
1344	A165-8	Prince George	McGregor	McGregorR. MCG	165	5411	12200	579	S.populicola
1345	A69-3	Mid Coast	Burke	Bella Coola R./ A.	69	5225	12610	152	S.populicola
1346	A3-1	South Coast	Fraser	Wellington Bar/C.	3	4940	12125	91	S.populicola
1347	A69-4	Mid Coast	Burke	Bella Coola R./ A.	69	5225	12610	152	S.populicola
1348	A48-2	South Coast	Toba Inlet	23 Toba R./A. T	48	5031	12414	67	S.populicola
1349	A142-3	Far North	Taku	Taku River/B	142	5842	13324	49	S.populicola
1350	A38-4	South Coast	Harrison	Lillooet R.IB.	38	5002	12232	213	S.populicola
1352	A38-4	South Coast	Harrison	Lillooet R.IB.	38	5002	12232	213	S.populicola
1353	A106-2	Smithers	Bulkley (Telkwa)	wa)lla Telkwa R.I	106	5437	12730	735	S.populicola
1354	A89-8	Smithers	Skeena	Skeena R.IP. SK	89	5433	12828	61	S.populicola
1355	A13-3	South Coast	Fraser	MatsQui Is.IM. M	13	4907	12220	8	S.populicola
1356	A64-1	Mid Coast	Knight	0 W.Klinaklini R.	64	5118	12546	546	S.populicola
1357	A50-4	South Coast	Phillips	Phillips R./ A.	50	5036	12519	5	S.populicola
1360	A56-4	Mid Coast	Homathko	Homathko R.ID.	56	5117	12450	152	S.populicola

1362	A41-5	South Coast	Squamish	Glacier/B Aha L.	41	5006	12300	579	S.populicola
1380	A156-1	Prince George	Quesnel R.	6 Australian QA	156	5243	12228	442	S.populicola
1382	A18-4	South Coast	EVIN	Salmon R. #IB. S	18	5013	12549	30	S.populicola
1383	A62-1	Mid Coast	Knight	Klinaklini R.ID.	62	5134	12530	427	S.populicola
1384	A72-1	Mid Coast	Dean	eanR./A. DENA 7	72	5246	12637	213	S.populicola
1386	A62-3	Mid Coast	Knight	Klinaklini R.ID.	62	5134	12530	427	S.populicola
1387	A22-5	South Coast	EVIS	Cassigy/J. CSYJ	22	4904	12352	20	S.populicola
1388	A114-2	Smithers	U. Nass	Nass RIF. NASF	114	5534	12847	152	S.populicola
1389	A67-1	Mid Coast	Rivers	Chuckwalla R.IC.	67	5144	12719	67	S.populicola
1390	A8-4	South Coast	Fraser	Chilliwack R.#/H.	8	4906	12131	480	S.populicola
1391	A183-1	USA	GS056	Lafayette, OR. LA	183	4512	12305	100	S.populicola
1392	A75-2	Mid Coast	Dean	eanR.ID. DEND 7	75	5249	12657	27	S.populicola
1397	A154-3	Mid Coast	KingcomeR.	20a Atlatzi R.IA.	154	5059	12607	0	S.populicola
1398	A171-5	Prince George	Nechako R.	14 Redmond Cr. A.	171	5406	12426	655	S.musiva
1437	A163-1	Prince George	Fraser R.	WillowR. WLOW	163	5355	12217	640	negative for both Septoria species
1440	A164-1	Prince George	Fraser R.	5 Shelley SHEL	164	5402	12236	564	S.populicola
1442	A87-5	Smithers	Skeena	Skeena R.IN. SK	87	5417	12922	30	S.populicola
1462	A88-2	Smithers	Skeena	Skeena R.IO. SK	88	5413	12932	21	S.populicola
1490	A94-3	Smithers	Kitimat	Kitimat R.IC. K	94	5403	12841	18	S.populicola
1491	A89-4	Smithers	Skeena	Skeena R.IP. SK	89	5433	12828	61	S.populicola
1493	A82-6	Smithers	Skeena	KitwangaIF . KT	82	5505	12811	177	S.populicola
1495	A94-4	Smithers	Kitimat	Kitimat R.IC. K	94	5403	12841	18	negative for both Septoria species

Provenance Trial EP.1123.02.05 Layout

Kilby - Harrison Mills Ct



EP.1123.02.05

Kilby - Harrison Mills Ct

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Fraser River

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c64	c65	c66	c67	c68	c69	c70	c71	c72	c73	c74	c75	c76	c77	c78	c79	c80	c81	c82	c83	c84	c85	c86	c87	c88	c89	
X	X	X	X	X	3032	X	X	X	X	X	3104	X	X	X	X	X	3176	X	X	X	3224	X	X	X	X	r37
X	X	X	3007	3010	X	X	X	3058	X	X	X	X	X	X	X	X	X	X	X	3202	X	X	3247	X	X	
X	X	2987	X	X	3030	X	X	X	X	X	X	X	X	X	X	3155	X	X	X	X	X	X	X	X	X	r35
X	X	X	X	3012	X	3036	X	X	X	X	X	X	X	X	X	X	X	X	3197	3204	X	3228	X	X	X	
X	X	2989	X	X	X	3037	3052	X	3076	X	3100	3109	X	X	X	X	X	3181	X	3205	X	3229	3244	X	X	r33
X	X	2990	X	X	X	X	X	X	3075	X	X	3110	3123	3134	3147	X	X	X	3195	X	X	3230	X	3254	X	
X	X	X	X	3015	X	X	3050	X	X	3087	X	X	X	3135	X	X	3170	X	3194	3207	X	3231	3242	X		r31
X	X	X	3001	3016	X	X	X	X	X	X	X	X	X	X	X	X	3169	X	X	X	3217	X	X	X		
X	2976	X	3000	3017	3024	3041	X	3065	X	X	3096	X	X	X	X	3161	3168	X	X	3209	3216	X	X	X		r29
X	2975	2994	2999	X	3023	X	X	3066	X	X	X	X	3119	3138	X	3162	X	X	3191	3210	X	3234	3239	X		
2971	2974	2995	2998	3019	X	3043	X	X	X	X	X	3115	3118	3139	3142	3163	3166	X	3190	X	3214	3235	X	X		r27
2972	X	2996	2997	3020	X	3044	X	3068	3069	3092	X	3116	3117	X	3141	X	3165	3188	3189	X	3213	3236	X	3260		

rep 8
