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

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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.

# **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 if 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 indentical in which they cause circular, necrotic spots on the foliage, which leads to reduced photosynethic leaf area, as well as premature scenscence 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.

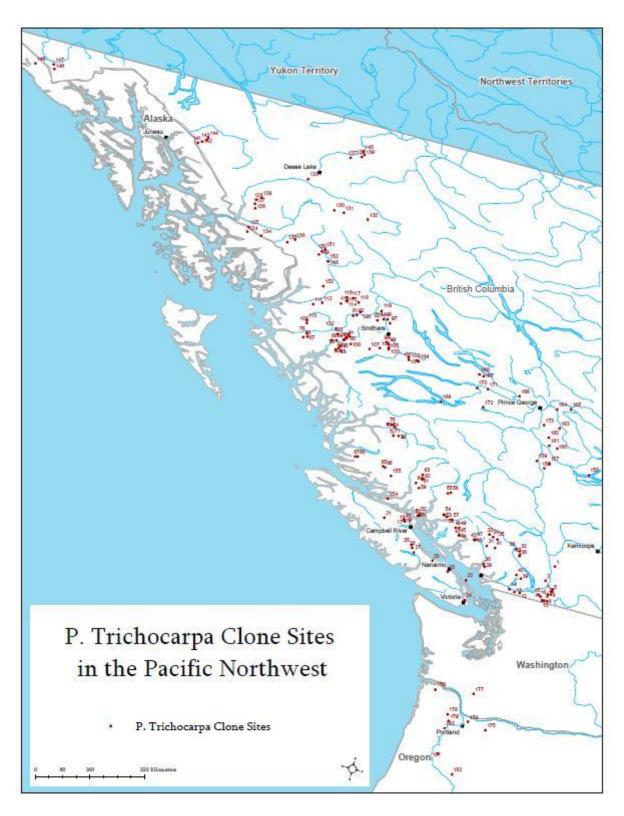


Figure 1 – A map of the origins of each *P. trichocarpa* within the plantation at Harrison Mills. Obtained from Dr. Harry H. Kope, Forest Practices and Investment Branch, British Columbia, Ministry of Forests, Lands, and Natural Resource Operations, Victoria, British Columbia)

# 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)

# <u>Leaf Sample Collection</u>

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 (<a href="http://www.qiagen.com/literature/render.aspx?id=201167">http://www.qiagen.com/literature/render.aspx?id=201167</a>) 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 x 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	S. musiva	CGGTATTTTCAGCCTGCAG
Smus_SSU_288R	S. musiva	GCC GTT ATC CGT ACA ACT GA
Smus_SSU243F_probe	S. musiva	AGGTAGATATTAGGACAATTGGTGTAAAGA TGAT
Spopulicola_SSU193F	S. populicola	CGGTATTTCAGCCTGCAC
Spopulicola_SSU288R	S. populicola	GCCGTTATCCGTACAACTAG
Spopulicola_SSU243F_probe	S. populicola	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 CFX96TM 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)

$$Z_{test} = \frac{(\widehat{p}_1 - \widehat{p}_2)}{S_{\widehat{p}1 - \widehat{p}2}}$$

(1.2)

$$S_{\widehat{p}1-\widehat{p}2} = \sqrt{\frac{(\widehat{p}_1\widehat{q}_1)}{n_1} + \frac{(\widehat{p}_2\widehat{q}_2)}{n_2}}$$

The Z-test was set at an alpha level of 0.05, where the  $Z_{0.05}$  value =  $\pm 1.96$ ; 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.

		201	.0	2011					
	No	orth	South		No	orth	South		
	S.musiva	S. populicola	S.musiva	S. populicola	S.musiva	S. populicola	S.musiva	S. populicola	
# Infected	11	92	9	118	9	9 104		106	
Total #(trees)	1	15	155		1	149		157	
ĝ	0.096	0.800	0.058	0.761	0.047	0.685	0.0385	0.679	
ĝ	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	S.musiva only	<i>S.populicola</i> only	S. musiva + S. populicola	Negative for Both Septoria spp.						
Dease Lake	7	2	3	0	2						
Far North	3	0	3	0	0						
Midcoast	40	0	31	2	7						
<b>Prince George</b>	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						
<b>Prince George</b>	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	S.musiva
570	A150-3	Dease Lake	Bell-Irving	5644	12944	579	S.musiva
1020	A56-3	Mid Coast	Homathko	5117	12450	152	S.musiva and S.populicola
1249	A75-4	Mid Coast	Dean	5249	12657	27	S.musiva and S.populicola
901	A165-7	Prince George	McGregor	5411	12200	579	S.musiva
1398	A171-5	Prince George	Nechako R.	5406	12426	655	S.musiva
618	A93-4	Smithers	Kitimat	5409	12835	61	S.musiva
52	A7-5	South Coast	Fraser R.	4924	12133	500	S.musiva
599	ATXD 15- 29	South Coast	-	-	-	-	S.musiva
724	A25-3	South Coast	EVIC	4957	12515	76	S.musiva
735	A23-2	South Coast	EVIS	4914	12404	60	S.musiva and S.populicola
958	A17-3	South Coast	EVIN	5013	12548	30	S.musiva
1212	ADTAC 7	South Coast	-	-	-	-	S.musiva and S.populicola

#### 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,  $Z_{0.05} = \pm 1.96 > Z_{test} = 1.353$ ; 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,  $Z_{0.05} = \pm 1.96 > Z_{test} = 0.946$ ; 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 leafs 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,  $Z_{0.05} = \pm 1.96 > Z_{test} = 0.499$ ; 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,  $Z_{0.05} = \pm 1.96 > Z_{test} = 0.237$ ; 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 of 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,  $Z_{0.05} = -1.96 < Z_{test} = -1.500$ ; 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,  $Z_{0.05} = -1.96 < Z_{test} = -0.739$ ; 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,  $Z_{0.05} = -1.96 < Z_{test} = -1.660$ ; 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,  $Z_{0.05} = -1.96 > Z_{test} = -2.166$ ; 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

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 nonsignificant 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.	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
320	71101 3	Timee George	11436114	6	101	3301	12231	.,	3.popunesia
				Cottonwood					
331	A160-4	Prince George	Fraser R.	QC	160	5302	12209	823	S.populicola
337	A94-2	Smithers	Kitimat	Kitimat R.IC. K	94	5403	12841	18	S.populicola
				Skeena R.IO.					negative for both
348	A88-2	Smithers	Skeena	SK	88	5413	12932	21	Septoria species
		0 111	a.	Skeena R.IN.					negative for both
349	A87-2	Smithers	Skeena	SK	87	5417	12922	30	Septoria species
350	A95-4	Smithers	Kitimat	Hirsch Cr.ID. H	95	5404	12827	335	negative for both Septoria species
330	A33-4	Sillitiers	Kitiiilat	Baker Cr.	93	3404	12027	333	Septoria species
356	A159-5	Prince George	Fraser R.	QBKR	159	5257	12252	823	S.populicola
				NassRtH.					
367	A116-5	Smithers	U. Nass	NASH	116	5543	12849	183	S.populicola
				6 Australian					
410	A156-7	Prince George	Quesnel R.	QA	156	5243	12228	442	S.populicola
424	47C F	Constale a ma	Classes	Exehamsiks	7.0	F 42F	42026	4.0	negative for both
424	A76-5	Smithers	Skeena	RIP 14	76	5425	12926	46	Septoria species
				Redmond Cr.					negative for both
448	A171-4	Prince George	Nechako R.	A.	171	5406	12426	655	Septoria species
				White RIA.					
453	A153-3	Smithers	U. Nass	WHTA	153	5556	12916	366	S.populicola
468	A98-4	Smithers	Bulkley	Bulkley R.IC. B	98	F4F7	12717	451	negative for both
408	A98-4	Smithers	викіеу	Hixon HIXN	98	5457	12717	451	Septoria species negative for both
475	A162-1	Prince George	Fraser R.	HIXON HIXIN	162	5324	12238	518	Septoria species
				Hirsch Cr.ID.					negative for both
505	A95-2	Smithers	Kitimat	Н	95	5404	12827	335	Septoria species
				6 Iskut R.IC.					negative for both
506	A136-2	Dease Lake	L. Stikine	I	136	5656	13020	317	Septoria species
		a	5 111	5 11 5 / 5				0.1.1	negative for both
519	A96-4	Smithers	Bulkley	Bulkley R./ A.	96	5515	12730	311	Septoria species
522	A41-3	South Coast	Squamish	GlacierlB Aha L.	41	5006	12300	579	negative for both Septoria species
322	7413	South Coust	Squarristi	WillowR.	71	3000	12300	373	negative for both
531	A163-4	Prince George	Fraser R.	WLOW	163	5355	12217	640	Septoria species
				7 Bell-					
570	A150-3	Dease Lake	Bell-Irving	Irving/C.	150	5644	12944	579	S.musiva
				Cassigy/J.		400	40055	•	
571	A22-4	South Coast	EVIS	CSYJ	22	4904	12352	20	S.populicola
573	A33-3	South Coast	Lillooet	a Lillooet R./ A.	33	5037	12323	411	S.populicola
5/5	,,,,,,,	Journ Coust	LINOUCE	11.1/ /\tau	J.J	5057	12323	-111	3.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

				WillowR.					
624	A163-3	Prince George	Fraser R.	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.lD. H	95	5404	12827	335	S.populicola
636	A76-5	Smithers	Skeena	Exehamsiks RIP	76	5425	12926	46	negative for both Septoria species
				6 Australian					negative for both
644	A156-1	Prince George	Quesnel R.	QA	156	5243	12228	442	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
			Bulkley	wa)lla					
663	A106-1	Smithers	(Telkwa)	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
				Nass RIE.					negative for both
685	A113-1	Smithers	U. Nass	NASE	113	5524	12859	207	Septoria species
702	A163-4	Prince George	Fraser R.	WillowR. WLOW	163	5355	12217	640	negative for both Septoria species
		5		Homathko					negative for both
705	A53-1	South Coast	Homathko	R./ A.	53	5056	12451	46	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
				Kitsumkalum					
717	A123-3	Smithers	Skeena	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.#I/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
, , , ,	7.05.5	304111 00431	1101113011	0	33	7,770	12213	07	3.populicolu
				W.Klinaklini					
723	A64-3	Mid Coast	Knight	R.	64	5118	12546	546	S.populicola
724	A25-3	South Coast	EVIC	Campbell R.IF. CM	25	4957	12515	76	S.musiva

				eanR./A.					
726	A72-1	Mid Coast	Dean	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	59	5107	12535	30	negative for both Septoria species
743	A64-1	Mid Coast	Knight	R./ A 0 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. #IIB. 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

				Salmon R.					
771	A19-5	South Coast	EVIN	#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
				Elaho R.ID.					
779	A32-3	South Coast	Squamish	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
				Squamish R./					
833	A29-2	South Coast	Squamish	A.	29	4952	12314	61	S.populicola
025	ACC 5	NA: d Cooot	N.A. alama all	Machmell	CC	F127	12625	122	Consuliante
835	A66-5	Mid Coast	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	wa)lla Telkwa R./	105	5440	12707	567	C nonulicala
041	A103-3	Similiers	(Telkwa)	McMillan	105	3440	12/0/	307	S.populicola
842	A14-3	South Coast	Fraser	Is.IN.	14	4911	12235	15	negative for both Septoria species
		1164	00024	Castle Rock,	477	4620	42255	400	6 11 1
844	A177-5	USA	GS024	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
043	7.52 4	Similar	Kitiiiat	Harrison	<u> </u>	3413	12031	122	Septoria species
846	A15-2	South Coast	Fraser	R./O. H	15	4917	12157	40	S.populicola
				Salmon R.					
848	A19-4	South Coast	EVIN	#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
		5		Bulkley R.IE.					negative for both
851	A100-4	Smithers	Bulkley	В	100	5445	12707	494	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
050	A 4 5 4			Skwawka	4 =	F04-	12404	222	Construction
856	A45-4	South Coast	Jervis	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
				GlacierlB Aha					
858	A41-5	South Coast	Squamish	L.	41	5006	12300	579	S.populicola
				23 TobaR.IB.					
860	A49-5	South Coast	Toba Inlet	TODAK.IB.	49	5034	12405	73	S.populicola
				1 Southgate					negative for both
861	A58-2	South Coast	Southgate	R.IB.	58	5053	12444	91	Septoria species

				Carson, WA.					
862	A174-3	USA	GS001	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./	17	5013	125/10	30	S.populicola
000	A17-2	30util Coast	EVIIV	A. ME eanR.IB.	17	3013	12548	30	3.populicola
881	A73-4	Mid Coast	Dean	DENB 7	73	5250	12642	152	S.populicola
000	433.5	6 11 6	6	Elaho R.ID.	22	5045	42225	205	6 11 1
882	A32-5	South Coast	Squamish	EL 23	32	5015	12235	305	S.populicola
				TobaR.IB.					
883	A49-2	South Coast	Toba Inlet	ТО	49	5034	12405	73	S.populicola
884	A181-5	USA	GS048	Halsey, OR. HALS	181	4425	12320	300	S.populicola
004	A101-3	USA	G3048	Yale West/D.	101	4423	12320	300	3.роринсова
886	A4-5	South Coast	Fraser	YA	4	4934	12128	549	S.populicola
				Homathko					
888	A53-2	South Coast	Homathko	R./ A.	53	5056	12451	46	S.populicola
889	A24-2	South Coast	EVIS	Chemainus R.IL. C	24	4853	12342	5	S.populicola
003	727 2	South Coust	EVIS	Harrison	24	4033	12342		negative for both
890	A15-5	South Coast	Fraser	R./O. H	15	4917	12157	40	Septoria species
				Lantzville/K.					
892	A23-4	South Coast	EVIS	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
	71200	Journ Jouet		Fanny Bayll.		.527	11107		<b>5.</b> popaco.u
896	A28-2	South Coast	EVIC	FNYI	28	4931	12451	46	S.populicola
				1 Southgate					negative for both
897	A58-2	South Coast	Southgate	R.IB.	58	5053	12444	91	Septoria species
898	A122-2	Smithers	Skeena	Cedar R./A (KITA)	122	5457	12855	274	S.populicola
				White R.IE.					negative for both
899	A21-4	South Coast	EVIN	WHTE,	21	5008	12603	213	Septoria species
000	4177.3	LICA	65024	Castle Rock,	177	4620	12255	100	Consuliante
900	A177-2	USA	GS024	W A. McGregorR.	177	4620	12255	100	S.populicola
901	A165-7	Prince George	McGregor	MCG	165	5411	12200	579	S.musiva
				Норе					
902	A6-1	South Coast	Fraser	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
304	A/0-3	Jiiidleis	SVEETIG	20a Atlatzi	70	2441	14043	144	Jeptoria species
905	A154-5	Mid Coast	KingcomeR.	R.IA.	154	5059	12607	0	S.populicola
				Jefferson,					negative for both
906	A180-4	USA	GS041	OR. JE	180	4444	12305	100	Septoria species
907	A12-1	South Coast	Fraser	Vender Cana1IL.	12	4909	12206	20	negative for both Septoria species
307	/ \12 1	30411 00431	114301	eanR.ID.	14	4303	12200		Septoria species
909	A75-1	Mid Coast	Dean	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	GlacierlB 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. 14	175	4535	12200	300	S.populicola
950	A171-1	Prince George	Nechako R.	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

1				Memekav R./		ĺ	]		
958	A17-3	South Coast	EVIN	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
				Lafayette,					
960	A183-4	USA	GS056	OR. LA	183	4512	12305	100	S.populicola
061	A150.6	Drings Coords	Cunningham	16 Kimball	150	F2F6	12110	022	Chanulicala
961	A158-6	Prince George	R.	Cr. Skeena RID.	158	5256	12110	823	S.populicola
962	A80-2	Smithers	Skeena	SKeena RID. SKN	80	5451	12820	140	negative for both Septoria species
				Skwawka					
963	A46-5	South Coast	Jervis	R.IE. S	46	5013	12357	231	S.populicola
				Nahatlatch					
964	A1-2	South Coast	Fraser	R./A.	1	5000	12134	213	S.populicola
0.55	.75.5		6	eanR.ID.		5240	40657	2-	6 11 1
966	A75-5	Mid Coast	Dean	DEND 7	75	5249	12657	27	S.populicola
967	A28-3	South Coast	EVIC	Fanny Bayll. FNYI	28	4931	12451	46	S.populicola
307	7120 3	South Coust	LVIC	GlacierIB Aha	20	4331	12431	70	3.populicolu
968	A41-3	South Coast	Squamish	L.	41	5006	12300	579	S.populicola
969	A36-2	South Coast	Lillooet	Lillooet R.ID.	36	5018	12245	222	S.populicola
				Jefferson,					
970	A180-3	USA	GS041	OR. JE	180	4444	12305	100	S.populicola
				Skeena R.IN.					
983	A87-2	Smithers	Skeena	SK	87	5417	12922	30	S.populicola
998	A165-7	Prince George	McGregor	McGregorR. MCG	165	5411	12200	579	S.populicola
				Homathko					S.musiva and
1020	A56-3	Mid Coast	Homathko	R.ID.	56	5117	12450	152	S.populicola
1040	4170.2	LICA	CCO22	Pittsburg, W	170	4550	12207	000	Consuliante
1049	A178-3	USA	GS033	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
				Hope					
1200	A7-3	South Coast	Fraser	South/G. H	7	4924	12133	500	S.populicola
	ADTAC								S.musiva and
1212	7	South Coast							S.populicola
1346		Cauth C.	J =	Skwawka	4-	F04-	13404	222	C manage to
1216	A45-5	South Coast	Jervis Cunningham	R.ID. SK 16 Kimball	45	5015	12401	323	S.populicola
1217	A158-5	Prince George	R.	Cr.	158	5256	12110	823	S.populicola
		-	Bulkley	wa)lla					
1218	A105-5	Smithers	(Telkwa)	Telkwa R./	105	5440	12707	567	S.populicola
				Lantzville/K.					
1225	A23-5	South Coast	EVIS	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
1230	A13-4	Journ Coast	riasei	Hope	13	431/	1213/	40	Septoria species
1233	A7-4	South Coast	Fraser	South/G. H	7	4924	12133	500	S.populicola
				Homathko	•		12		- 1-1-1-1-1-1
1235	A55-3	Mid Coast	Homathko	R.IC.	55	5114	457	88	S.populicola

				Skeena R/e.					
1245	A79-4	Smithers	Skeena	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
				Jasper, OR.					
1298	A182-3	USA	GS051	JASP Chilliwack	182	4400	12255	150	S.populicola
1302	A10-1	South Coast	Fraser	R.#3/J.	10	4905	12143	280	S.populicola
				5 Shelley					negative for both
1303	A164-2	Prince George	Fraser R.	SHEL	164	5402	12236	564	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
				Skwawka					
1326	A45-4	South Coast	Jervis	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
1329	A134-2	Iviiu Coast	Killgcolliek.	Chuckwalla	134	3039	12007	U	3.populicola
1330	A68-3	Mid Coast	Rivers	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
1337	A30-3	South Coast	Lillooet		30	2018	12245	222	5.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
1240	A 4 9 2	Courth Coost	Taba Inlat	23 Toba	40	F021	12414	67	Coopulicala
1348	A48-2 A142-3	South Coast Far North	Toba Inlet Taku	R./A. T Taku River/B	48 142	5031 5842	13324	67 49	S.populicola
1349 1350	A38-4	South Coast	Harrison	Lillooet R.IB.	38	5002	12232	213	S.populicola S.populicola
1350	A38-4	South Coast	Harrison	Lillooet R.IB.	38	5002	12232		S.populicola S.populicola
1332	A30-4	Joulii Coast	Bulkley	wa)lla	50	3002	12232	213	3.populicola
1353	A106-2	Smithers	(Telkwa)	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

				GlacierIB Aha					
1362	A41-5	South Coast	Squamish	L.	41	5006	12300	579	S.populicola
				6 Australian					
1380	A156-1	Prince George	Quesnel R.	QA	156	5243	12228	442	S.populicola
	_			Salmon R.	_				
1382	A18-4	South Coast	EVIN	#IIB. S Klinaklini	18	5013	12549	30	S.populicola
1383	A62-1	Mid Coast	Knight	R.ID.	62	5134	12530	427	S.populicola
				eanR./A.					ография
1384	A72-1	Mid Coast	Dean	DENA 7	72	5246	12637	213	S.populicola
				Klinaklini					
1386	A62-3	Mid Coast	Knight	R.ID.	62	5134	12530	427	S.populicola
1387	A22-5	South Coast	EVIS	Cassigy/J. CSYJ	22	4904	12352	20	S.populicola
1507	A22-3	30utii Coast	EVIS	Nass RIF.	22	4904	12552	20	3.populicola
1388	A114-2	Smithers	U. Nass	NASF	114	5534	12847	152	S.populicola
				Chuckwalla					- 11
1389	A67-1	Mid Coast	Rivers	R.IC.	67	5144	12719	67	S.populicola
				Chilliwack					
1390	A8-4	South Coast	Fraser	R.#I/H.	8	4906	12131	480	S.populicola
				Lafayette,					
1391	A183-1	USA	GS056	OR. LA	183	4512	12305	100	S.populicola
1392	A75-2	Mid Coast	Dean	eanR.ID. DEND 7	75	5249	12657	27	S.populicola
1592	A/3-2	IVIIU COast	Dean	20a Atlatzi	75	3249	12037	21	3.populicola
1397	A154-3	Mid Coast	KingcomeR.	R.IA.	154	5059	12607	0	S.populicola
			0	14					- 11
				Redmond Cr.					
1398	A171-5	Prince George	Nechako R.	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
1437	A103-1	Prince George	Fraser K.	5 Shelley	103	5355	12217	640	Septoria species
1440	A164-1	Prince George	Fraser R.	SHEL	164	5402	12236	564	S.populicola
				Skeena R.IN.					- 11
1442	A87-5	Smithers	Skeena	SK	87	5417	12922	30	S.populicola
				Skeena R.IO.					
1462	A88-2	Smithers	Skeena	SK	88	5413	12932	21	S.populicola
				Kitimat R.IC.					
1490	A94-3	Smithers	Kitimat	K	94	5403	12841	18	S.populicola
1491	A89-4	Smithers	Skeena	Skeena R.IP. SK	89	5433	12828	61	S.populicola
1431	A03-4	Similiers	Skeella	KitwangalF .	כט	3433	12020	OI	э.роринсова
1493	A82-6	Smithers	Skeena	Kitwangair . KT	82	5505	12811	177	S.populicola
				Kitimat R.IC.				-	negative for both
1495	A94-4	Smithers	Kitimat	K	94	5403	12841	18	Septoria species

			_,_		_Fras	er R	<u>iver</u>											<u>.</u>																				<u>Fra</u>	se
			trees trees trees	pos pos pos	itive fo itive fo itive fo	or S. or S. or S.	of Fun musiv musiv musiv a cank	a lea a lea a lea	fspot i fspot i fspot i	in 201 in 201	1 0		e)																										
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r30																							990		Х	871					750	X	X	631			570		
r29																				rep 2		992		932	929	872				752	749	692			629	X	569	512	
r28																						993	988	933	X		868	X		753		693	X	633		573	568	X	_
r27																					1	994	987	934	927	874		814		754	747	694	687	634	627	574	567	514	
r26	_							rep									1129		1079			995	986	935	926	X	866	Х	Х	Χ	Х	695	686	635	626	X	Х	515	_
r25	_	_	1466				1 1370															996	985	X	925		865	X	805	756	745	696	685	636	X		565	516	
r24		T	1465		_	X			1321			1228							1077			997	984	937	924			817	804	757	744	697	684	637	624	577	564	517	_
r23	1512		_		_	+	3 1368					1229							1076			998	983	938	923	878		818	803	758	743	698	683	638	623	578	X	518	_
r22	X		1463		_	_	4 1367		_													999	982	939	922	879		819	802	759	742	699	682	639	622	579	562	519	_
r21					_	+	5 1366			_									1074			X	981	940	921	880		820	801	760		700	681	640	621	580	561	520	-
r20	1509	14/2	1461	1424		_	1365				_			1184			-	1086	1073	1036	<u> </u>	1001		Х	920	881	860	821		761	740	701	680	Х	620	581	560		
_	1508			X			7 1364										1122	Х	1072	X	1029		Χ	942	919	882	X	822	799	762	X		679			582	X	522	
			_			_	8 1363			_	1267		1219			1138		Х	Х	1038	Х		978	943	918	883		823	798	Х	738		678	Х	618	583	Х	523	-
r17			+				9 1362		_		_	1235			Х	1139			1070		_		Х	944	917	884	857	824	797	764	737	704	677		617	584	557	524	
r16	1505	1476	1457	1428	1409	138	0 X	133	2 1313	1284	1265	1236	1217	1188	Х	Х	1119	1090	1069	1040	1026	Х	976	Х	916	Х	856	Х	796	765	736	705		645	616	585	556	X	-
r15	1504	L X	1456	1429	1408		1360											1091	1068	1041			975	946	Х		855	Х	Х	Χ		706			615	586	555		-
r14	1503	3 X	X	1430	) X	138	2 1359	X	X	_	_	1238						1092		1042		1007	974	Х	Х	X	854	827	794	X	X	X	674	647	614	587	554	527	_
r13			1454		_			X	X			1239								Х		Х	973	948	913		853	828	793	768	733	X	673			588	553	528	,
r12			_		_		4 1357	_	_										1065			Х	972	949	912	889		829	Х	Х	732		672		612	X	552	529	-
r11	_	1481	_		_		5 1356				+		1212							Х			971	950	911	890		830	791	770	Х	Х	Х	Х	Х	590	Х	530	
r10	1499	1482			_		6 1355	_	_		_								1063			1011	970	951	910	Х		831	790	771			670	651	610	591	550	531	
r9	X	X	1450				7 1354			_	+	1243							1062			1012	969	952	909		849			772	729	Х	669	652	609	592	549	_	_
r8	X	_					8 1353			_	_	1244									ı	1013		953	908		848			773			668	653	Х	593	548	533	-
r7	Х	1485	1448				9 1352														1					894	Х	Х	787	774	727	714	667	654	607	594	547	534	
r6	1495	1486	X	Х	1399	139	0 X	1342	2 1303	3 X	X	1246	1207	1198	1159	1150	1109	1100	1059	1050		1015	966	955	906	Х	846	835	786	775	726	715	666	655	Х	Х	546	535	
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r2	1491	1490	1443	1442	1395	139	4 1347	134	1299	1298	1251	1250	1203	1202	1155	1154	1105	Х	1055	1054	1022	1019	962	959	902	899	842	839	782	779	722	719	662	659	602	599	542	X	_
r1								rep	3												1021	1020	961	960	901	900	841	840	781	780	721	720	661	660	601	X	541	X	-
	c55	c54	c53	c52	c51	c50	c49	c48	c47	c46	c45	c44	c43	c42	c41	c40	c39	c38	c37	c36	c35	c34	c33	c32	c31	c30	c29	c28	c27	c26	c25	c24	c23	c22	c21	c20	c19	c18	į
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Fig.	041 r28 042 r27
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 C15 C14 C14 C15 C14 C14 C15 C14 C14 C14 C15 C14	032     2495     2496     X     X     2531     2532       033     2494     2497     X     2515     2530     2533       X     2493     2498     2511     X     2529     X       X     X     X     X     X     2528     X       X     X     2500     X     X     2527     2536       037     X     X     X     X     2526     2537       038     X     2502     X     2520     X     X       X     2488     2503     2506     2521     2524     X       040     2487     X     X     X     X     X     2540       041     r28       042     r27
Fig.	032     2495     2496     X     X     2531     2532       033     2494     2497     X     2515     2530     2533       X     2493     2498     2511     X     2529     X       X     X     X     X     X     2528     X       X     X     2500     X     X     2527     2536       037     X     X     X     X     2526     2537       038     X     2502     X     2520     X     X       X     2488     2503     2506     2521     2524     X       040     2487     X     X     X     X     X     2540       041     r28       042     r27
Fig.	033     2494     2497     X     2515     2530     2533       X     2493     2498     2511     X     2529     X       X     X     X     X     X     2528     X       X     X     2500     X     X     2527     2536       037     X     X     X     X     2526     2537       038     X     2502     X     2520     X     X       X     2488     2503     2506     2521     2524     X       040     2487     X     X     X     X     X     2540       041     r28       042     r27
Table   Tabl	X     2493     2498     2511     X     2529     X       X     X     X     X     X     2528     X       X     X     2500     X     X     2527     2536       037     X     X     X     X     2526     2537       038     X     2502     X     2520     X     X       X     2488     2503     2506     2521     2524     X       040     2487     X     X     X     X     2540       041     r28       042     r27
Fig.	X         X         X         X         Z528         X           X         X         2500         X         X         2527         2536           037         X         X         X         2526         2537           038         X         2502         X         2520         X         X           X         2488         2503         2506         2521         2524         X           040         2487         X         X         X         X         2540           041         r28         r27
C16 C15 C14 C13 C12 C11 C10 C9 C8 C7 C6 C5 C4 C3 C2 C1  451 450 391 390 331 330 271 270 211 210 151 150 91 90 31 30 452 449 392 389 332 329 272 X X 209 152 149 92 89 32 29 453 448 393 388 X 328 273 268 213 208 X X 93 88 33 28 X X X X X X X X X X X X X X X X X X	X X 2500 X X 2527 2536 037 X X X X 2526 2537 038 X 2502 X 2520 X X X 2488 2503 2506 2521 2524 X 040 2487 X X X X 2540 041 r28 042 r27
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c16         c15         c14         c13         c12         c11         c10         c9         c8         c7         c6         c5         c4         c3         c2         c1           451         450         391         390         331         330         271         270         211         210         151         150         91         90         31         30         r30         r	X         2502         X         2520         X         X           X         2488         2503         2506         2521         2524         X           040         2487         X         X         X         X         2540           041         r28         r27         r27         r28         r27         r27
C16       C15       C14       C13       C12       C11       C10       C29       C6       C5       C4       C5       C2       C1         451       450       391       390       331       330       271       270       211       210       151       150       91       90       31       30       r20       r29	X 2488 2503 2506 2521 2524 X 040 2487 X X X X 2540 041 r28 042 r27
452 449 392 389 332 329 272 X X 209 152 149 92 89 32 29 r29 453 448 393 388 X 328 273 268 213 208 X X 93 88 33 28	040 2487 X X X X 2540 041 r28 042 r27
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X       X       X       X       334       327       274       X       214       207       X       X       94       X       X       27       r27       r28       r2	042 r27
455 446 395 386 335 326 275 266 215 206 155 146 95 X 35 26 r26 r25 l539 X 1595 X 1651 1674 1723 1746 1797 1820 1871 1896 1945 1970 2019	· <u>-</u> ·
456 445 396 385 336 325 276 265 216 205 X 145 96 85 36 25 r25	
	x r26
457   444   397   384   337   324   277   264   217   204   157   144   97   84   X   X   r24   r24   1538   X   X   X   1650   1675   1722   1747   X   X   1870   1897   1944   1971   2018	x r25
	X r24
458 443 398 X 338 323 278 263 X X 158 143 98 X X 23 r23 r23 r23 r23 r23 r23 r23 r23 r23	X r23
459 442 399 382 X 322 279 X 219 202 X 142 99 X 39 22 122 I 122 1536 1549 1592 1605 X 1677 X 1749 1794 X 1868 1899 X 1973 2016 2	047 2090 2091 r22
X 441 400 381 340 321 280 261 220 201 X X X 81 40 21 r21 1535 1550 X 1606 1647 1678 1719 1750 1793 1824 X 1900 1941 1974 2015 2	121
461 X 401 380 341 320 281 260 221 200 X X X X X X 20 r20 1534 X 1590 1607 1646 1679 1718 1751 X 1825 1866 X 1940 X 2014 2	049 2088 2093 r <sub>20</sub>
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464 X 404 377 344 317 284 257 224 197 164 X 104 77 44 X r17 1531 1554 X 1610 1643 1682 X 1754 1789 1828 1863 X 1937 1978 2011 2	052 2085 2096 <sub>r17</sub>
X X 405 376 345 316 285 X 225 196 X 136 105 76 45 16 r16 r16 X 1555 1586 1611 1642 X 1714 1755 1788 1829 1862 1905 X 1979 2010	X 2084 2097 2128 X 2160 2161
X 435 406 375 346 315 286 255 226 195 166 135 106 75 46 15 r15 r15 r15 r15 r15 r15 r15 r15 r15	054 2083 2098 2127 2130 2159 2162
467 434 407 374 347 314 287 254 X 194 167 134 107 74 47 14 r14 1528 X 1584 1613 1640 1685 1712 1757 1786 X 1860 1907 1934 1981 2008	X 2082 X 2126 2131 2158 2163
468 433 408 373 348 313 288 253 228 193 168 133 108 73 48 13 r13 r13 r13 x 1558 1583 1614 x 1686 1711 1758 1785 1832 1859 1908 1933 1982 2007	X 2081 X 2125 2132 2157 X
469 432 409 372 349 X X 252 229 192 169 132 109 72 49 12 r12 r12 1526 1559 1582 1615 1638 X 1710 1759 1784 1833 1858 X 1932 1983 2006 2	057 2080 2101 2124 2133 X 2165
470 431 410 X 350 311 290 251 230 X 170 131 110 71 50 11 r11 i r11 X 1560 1581 1616 1637 X 1709 X 1783 1834 1857 X 1931 1984 2005 2	058 2079 2102 2123 2134 2155 2166
471 430 411 370 351 310 291 250 231 190 X 130 111 70 51 10 r10 1524 1561 1580 1617 1636 X X 1761 X 1835 1856 1911 X 1985 2004 2	059 2078 2103 2122 2135 2154 2167
X 429 X 369 X 309 292 X X 189 X 129 112 X 52 9 r9 r9 X 1562 1579 1618 1635 X 1707 1762 X X 1855 1912 1929 X X	X X X 2121 2136 2153 X
473 428 X 368 353 308 293 X X 188 173 X 113 68 53 8 r8 r8 r8 1522 1563 1578 X 1634 X 1706 1763 X X 1854 1913 1928 X 2002 2	061 X X X 2137 X 2169
474 X 414 367 354 307 294 X 234 187 X X 114 67 54 7 7 6 6 7 1521 1564 1577 X X X 1705 1764 1779 1838 1853 1914 1927 X 2001	X 2075 2106 2119 2138 X 2170
475 X X X X X 295 X 235 186 175 126 115 66 55 X r6 5 T T F T T T T T T T T T T T T T T T T	063 2074 X 2118 X X X
476 425 X 365 356 305 X 245 236 185 176 X 116 65 56 X r5 X 1566 1575 X 1631 X 1703 X 1777 1840 X 1916 1925 1990 1999 2	064 2073 2108 X X 2149 2172
X 424 X 364 357 304 297 X 237 184 177 124 117 X 57 4 r4 r4 r4 r4 r5 r4 1518 1567 1574 1623 1630 1695 1702 X 1776 1841 1850 1917 1924 X 1998 2	065 2072 X 2116 2141 2148 X
478 X 418 363 X 303 X 243 238 183 X 123 118 63 58 X r3 I 517 X 1573 1624 1629 X 1701 X 1775 1842 X 1918 1923 1992 X 2	066 X 2110 2115 2142 2147 2174
479 X 419 362 X 302 299 X 239 182 X 122 X X 59 2 r2 1516 X 1572 1625 1628 1697 X 1769 1774 1843 X X 1922 1993 X 2	067 X 2111 X X X X
480 421 420 361 360 301 X 241 X 181 180 121 120 61 60 1 r1 X X X X X 1627 1698 X 1770 X X 1847 1920 X 1994 1995 2	068 2069 2112 2113 2144 X X
c16 c15 c14 c13 c12 c11 c10 c9 c8 c7 c6 c5 c4 c3 c2 c1	16 c17 c18 c19 c20 c21 c22
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c23	c24	c25	c26	c27	c28	c29	c30	c31	c32	c33	c34	c35	c36	c37	c38	c39	c40	c41	c42	c43	c44	c45	c46	c47	c48	c49	c50	c51	c52	c53	c54	c55	c56	c57	c58	c59	c60	c61	c62	c63
X	2550	2567	2568	2585	2586	2603	Χ	2621	2622	2639	2640	2657	2658	2675	Χ	2693	2694	2711	2712	2729	2730	Х	Х	2768	2769	Χ	Х	Χ	2817	Χ	Χ	2864	2865	2888	2889	2912	Χ	2936	Х	Χ
2548	2551	2566	Χ	Χ	Х	2602	2605	Х	2623	2638	Х	2656	Х	2674	Χ	2692	2695	2710	2713	Х	2731	Х	2749	2767	2770	Х	Х	Χ	Х	Х	Χ	Х	2866	2887	Х	Х	2914	X	Χ	X
2547	Х	Χ	2570	Х	2588	2601	2606	2619	2624	2637	2642	2655	2660	2673	2678	Х	Х	Х	2714	Х	2732	Х	2750	2766	Х	2790	Х	2814	2819	Х	Χ	2862	Х	Х	Х	Х	Х	2934	Χ	2958
2546	Χ	2564	2571	2582	2589	2600	2607	2618	2625	2636	Х	2654	2661	Х	Х	Χ	Χ	Х	Χ	2726	2733	Х	Х	2765	Х	2789	Х	2813	2820	Х	2844	Х	Х	2885	X	2909	Χ	2933	Х	2957
2545	Χ	Χ	2572	Χ	2590	2599	2608	2617	Х	Х	Х	Х	2662	2671	2680	2689	Χ	2707	2716	2725	2734	2743	2752	2764	Х	Х	2797	2812	2821	Х	Χ	2860	Х	Х	2893	2908	2917	X	Х	X
2544	Х	Χ	Х	2580	2591	2598	2609	2616	X	2634	2645	2652	Х	Χ	Χ	2688	2699	2706	Х	2724	2735	Х	2753	Х	2774	Х	X	Χ	2822	Х	Χ	2859	2870	2883	2894	X	2918	X	Х	X
X	Χ	Χ	2574	Х	2592	2597	2610	Х	2628	2633	2646	2651	Х	Х	2682	Х	Χ	2705	2718	Х	Х	Х	2754	Х	Х	Х	Х	Х	Х	2834	2847	Х	2871	Х	Х	2906	Χ	2930	Х	X
2542	2557	2560	2575	2578	Х	2596	Х	2614	Х	2632	Х	2650	2665	2668	2683	Х	Х	2704	2719	Х	2737	2740	2755	2761	Х	Х	Х	Х	Х	2833	2848	2857	Х	2881	2896	2905	2920	Х	Х	2953
2541	Χ	2559	Х	2577	2594	2595	2612	2613	2630	2631	2648	Х	2666	Х	2684	2685	Χ	2703	Χ	Х	Х	Х	Х	Х	2777	2784	2801	2808	Х	Х	2849	2856	2873	2880	2897	2904	Χ	Х	2945	2952
						rep 6																		Х	2778	2783	2802	2807	Х	2831	2850	Х	2874	Х	2898	2903	2922	2927	Х	X
																								2758	2779	Х	2803	Х	2827	2830	2851	2854	2875	2878	2899	2902	2923	2926	X	X
																								2757	2780	Х	2804	Х	2828	2829	2852	2853	2876	Х	2900	Х	2924	2925	2948	2949

rep 7

2192	2193	2224	2225	2256	2257	2288	2289	2320	2321	Х	Х	Х	r16											
2191	2194	2223	2226	2255	Х	2287	2290	2319	2322	2351	2354	2383	r15											
2190	Х	2222	2227	2254	Х	Х	2291	2318	2323	2350	2355	2382	r14											
2189	Х	2221	2228	2253	2260	Х	2292	2317	2324	2349	2356	2381	r13											
2188	2197	2220	2229	2252	2261	2284	2293	2316	2325	2348	2357	2380	r12											
X	2198	2219	2230	2251	2262	2283	2294	2315	2326	2347	2358	Х	r11											
Х	2199	2218	2231	Х	2263	2282	2295	Х	Χ	2346	2359	2378	2385	2404	2405	2424	Χ	2444	2445	2464	2465	2484	r10	'
2185	2200	2217	Х	2249	2264	2281	2296	Х	2328	2345	2360	2377	2386	2403	2406	2423	2426	2443	2446	2463	2466	Х	r9	
2184	2201	2216	2233	2248	2265	2280	Χ	Х	2329	2344	2361	Х	2387	Х	2407	Х	2427	2442	2447	2462	Х	Х	r8	
2183	2202	2215	2234	2247	Х	Х	Х	2311	Х	2343	Х	Х	2388	2401	2408	2421	2428	Х	2448	2461	2468	2481	r7	
2182	2203	2214	2235	2246	2267	2278	2299	2310	Х	2342	2363	2374	2389	2400	Х	2420	2429	2440	2449	2460	Х	2480	r6	rep 4
Х	2204	2213	2236	Х	Х	2277	2300	2309	2332	Х	Х	2373	2390	2399	2410	Х	2430	2439	2450	2459	Х	2479	r5	
2180	2205	2212	2237	2244	2269	2276	2301	2308	Х	Х	2365	2372	2391	2398	2411	2418	2431	2438	2451	2458	2471	2478	r4	
2179	2206	Х	Х	2243	2270	2275	Х	Х	Х	2339	Х	2371	2392	Х	2412	2417	2432	2437	2452	2457	2472	2477	r3	
Х	Х	2210	Х	Х	Х	2274	Х	Х	Х	Х	Х	2370	2393	2396	2413	2416	2433	2436	Х	2456	Х	Х	Х	r2
2177	2208	2209	Х	2241	2272	2273	Х	Х	2336	2337	2368	Х	2394	2395	2414	Х	2434	2435	2454	2455	2474	Х	2486	r1
c23	c24	c25	c26	c27	c28	c29	c30	c31	c32	c33	c34	c35	c36	c37	c38	c39	c40	c41	c42	c43	c44	c45	c46	•

#### 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 3032 X 3104 X X X 3176 X X X 3224 X X r37 X X 3007 3010 X X X 3058 X 3202 X 2987 X X 3030 X X X X X X 3155 X X 3012 X 3036 X X X X X X X X X X X X X X 3197 3204 X 3228 X X 3100 3109 X X X X X 3181 X 3205 X 3229 3244 X X X 3037 3052 X 3076 r33 X 2990 X X X X X X 3075 X X 3110 3123 3134 3147 X X 3195 X X 3230 X 3254 X X X 3015 X X 3050 X X 3087 X X 3135 X X 3170 X 3194 3207 X 3231 3242 r31 X X X X X X X X X X X X X X X 3169 X X X 3217 X X X X X 3001 3016 X r30 X 2976 X 3000 3017 3024 3041 X 3065 X X 3096 X X X 3161 3168 X X 3209 3216 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 r28 2971 2974 2995 2998 3019 X 3043 X 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 r26

rep 8

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