University of Alberta

Evaluating and attenuating the risk of Septoria canker in the *Septoria musiva*-hybrid poplar pathosystem

by

Jared Michael LeBoldus 

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Abstract

Septoria musiva Peck., a fungal pathogen of hybrid poplar, has caused plantation failures in Canada and the United Sates. To help develop disease management strategies, two experiments were conducted. The first evaluated the interactions of four hybrid poplar clones with four isolates of *S. musiva* under water-stressed and water-unstressed conditions. The absence of significant interactions between water stress and the other variables indicated that results from greenhouse inoculations would likely be applicable under field conditions. In the second experiment, 14 clones of hybrid poplar from three parent types were inoculated with 19 isolates of *S. musiva* from three geographic locations. The results indicated that (i) clones, rather than hybrid types, should be the focus of resistance screening, (ii) a small number of isolates from a single geographic location should be sufficient for resistance screening, and (iii) resistance should be relatively stable, given the small clone x isolate interaction.

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Chapter 1: General Introduction

1.1 Hybrid poplar in North America

The genus *Populus* is made up of six taxonomic sections comprising approximately 30 species distributed throughout Europe, Asia, and North America (Eckenwalder 1996). Species from these six sections are able to interbreed to varying degrees producing what are generically referred to as 'hybrid poplars' (Dickmann 2001; Zsuffa 1975). Traditionally, in Canada, hybrid poplar plantings have been restricted to shelterbelts and windbreaks (Isebrands and Karnosky 2001). In fact, *Populus* have historically been considered 'weed trees' and are often removed from cutblocks and regenerating stands. However, with the advent of oriented strand board (OSB) plants, wafer board plants, laminated strand lumber (LSL) plants, and hardwood pulp mills, the utilization of *Populus* has shown a marked increase over the last 25 years (Balatinecz *et al.* 2001). For example, in 1989, 14,500 hectares of hybrid poplar were planted in Eastern Ontario (Strobl and Fraser 1989). To date, in Alberta, Alberta-Pacific Forest Industries Inc. (Al-Pac) has 4000 hectares of hybrid poplar plantations (Alberta-Pacific Forest Industries Inc. 2006). It is in fact the life history of these tree species that has driven their emergence as an increasingly important fiber resource.

1.2 Hybrid poplar biology

All *Populus* species are efficient invaders of disturbed sites and typically occupy riparian or upland habitats (Dickmann 2001). Species from the section Populus, aspen and white poplar, generally prefer upland sites, whereas species from Leucoides, Aigeiros, and Tacmahaca usually prefer riparian areas (Dickmann 2001). All species of poplar are dioecious, with the occasional clone producing hermaphroditic flowers, and are able to reproduce either sexually, through pollination and seed dispersal, or asexually through root suckers or branches (Dickmann 2001). The methods of asexual reproduction are diverse and vary according to taxonomic section. All sections are able to produce stump sprouts and root suckers, whereas members of Aigeiros can reproduce via buried branches (Zsuffa 1975; Peterson and Peterson 1992; Eckenwalder 1996; Dickmann 2001; Riemenschneider *et al.* 2001). Furthermore, Tacamahaca and Aigeiros, and to a lesser

degree Populus and Leucoides, can reproduce via cuttings (Zsuffa 1975; Peterson and Peterson 1992; Eckenwalder 1996; Dickmann 1996). The ease with which the sections Aigeiros and Tacamahaca reproduce clonally by cuttings is largely the reason why hybrids from these *Populus* sections are favored by forest managers (Peterson and Peterson 1992).

A clone is any collection of root suckers, stem sprouts, or cuttings originating from a single genetic source (Dickmann 2001). In a species with extremely high variability in most traits, this characteristic is invaluable (Dickmann 2001). It allows large numbers of genetically identical and phenotypically desirable individuals to be planted over a large area. In addition, the intra- and inter-sectional hybridization of *Populus* species produces a phenomenon known as heterosis or hybrid vigor. Hybrid vigor is defined in one of two ways: (i) the expression of the trait exceeds that of the superior parent; or (ii) the expression of the trait exceeds that of the average of both parents (Stettler 1996). Hybrid vigor is widely recognized throughout the plant breeding world but is poorly understood (Hinckley *et al.* 1989). In natural populations, hybrid vigor is likely to exist between sympatric species but is more commonly recognized in anthropogenically produced hybrids (Zsuffa 1975; Stettler 1996). The expression of hybrid vigor often seems to exist in the first generation (F1) hybrids, and decrease in subsequent generations (Stettler 1996; Wu *et al.* 1992).

1.3 Natural vs. anthropogenic hybridization

Natural hybridization may occur wherever different species of *Populus* are sympatric (Stettler *et al.* 1996). For example, in areas where the ranges of *Populus angustifolia* James and *Populus deltoides* Bartr. ex Marsh. overlap, the hybrid of these two species, *Populus* x *acuminate* Rydb., can be found (Dickmann 2001). Another common natural hybrid, *P.* x *jackii* Sarg., is found throughout Canada where the ranges of *P. deltoides* and *Populus balsamifera* L. overlap (Peterson and Peterson 1992). Various other natural inter- and intra-sectional hybrids exist throughout North America (Barnes 1961; Brayshaw 1965).

Anthropogenic hybridization refers to the hybridization of allopatric *Populus* species adapted to different ecological environments. There are several reasons why this

type of hybridization is carried out by poplar breeders. The combination of desirable traits, the capture of hybrid vigor, and developmental homeostasis are the main motives (Stettler *et al.* 1996). As the number of plantations of anthropogenic hybrids increases, a third category of hybridization may become more common. This is the hybridization between hybrid plantations and natural stands of *Populus*. Significant geneflow between native and non-native hybrid poplars could alter the native population by producing progeny that express superior growth rates relative to native poplars (Stettler *et al.* 1996). Alternatively, the susceptibility of these hybrids to native insects and plant pathogens may increase to such a degree that the hybrids will be unable to outcompete the native populations (Fritz *et al.* 1999). In fact, Fritz *et al.* (1999) found that natural hybrid zones across a wide range of host taxa appear to be limited by increased susceptibility to disease.

Several hypotheses have been proposed for this limitation: (i) the 'hybrids as sinks' hypothesis, (ii) the 'phenological sink' hypothesis, and (iii) the hybrid susceptibility hypothesis (Strauss 1994). The 'hybrids as sinks' hypothesis suggests that the presence of susceptible hybrid genotypes may draw pests and pathogens away from more resistant pure genotypes (Whitham 1989). The second hypothesis contends that hybrid zones may provide an extended period of tissue availability for pest and pathogen utilization when compared to pure hosts (Floate *et al.* 1993). For example, in stands of pure hosts leaf flush is synchronous, whereas this is not the case in hybrid zones where different hybrids may undergo leaf flush at different times (Floate *et al.* 1993). This, in turn, extends the period of time where juvenile tissue is present, benefiting pests or pathogens which require juvenile tissue to complete their life-cycle (Floate *et al.* 1993). The third hypothesis states simply that resistance mechanisms are broken in the hybridization process (Strauss 1994).

1.4 Pathogens of hybrid poplar

To date, in North America, most hybrid poplar clones of economic importance are inter- or intra-sectional hybrids of Aigeiros and Tacamahaca descent. Fortunately, there are only four major fungal diseases of these hybrids: leaf rust caused by *Melampsora* spp., Septoria canker, leaf and shoot blight caused by *Venturia* spp., and Marssonina leaf spot

(Peterson and Peterson 1992; Newcombe *et al.* 2001). Of these four diseases, leaf and shoot blight and Marssonina leaf spot cause severe damage on only a few highly susceptible clones (Newcombe *et al.* 2001). In contrast, Septoria canker and Melampsora rust have caused widespread damage in plantations of hybrid poplar and are considered the two most important diseases of hybrid poplars in Canada and the United States (Newcombe *et al.* 2001). In Alberta, neither of these diseases have caused unacceptable losses to hybrid poplar plantations. In the case of *Melampsora* spp., heavy foliar infections are necessary for this disease to reduce growth and yield. The relatively dry climate and short growing season in Alberta make it unlikely that the conditions necessary for heavy infection will occur (Crane 2002, "Poplar leaf rusts in central Alberta-assessing the risks to agroforestry" an unpublished final report to Alberta-Pacific Forest Industries). However, a single stem infection by Septoria canker is sufficient to kill a tree of any age. Furthermore, in the past, Septoria canker has caused extensive infection on shelterbelts made up of Aigeiros and Tacamahaca hybrids in the Prairie Provinces (Bier 1939).

1.5 Septoria canker biology

Septoria canker is caused by the coelomycete *Septoria musiva* Peck. (teleomorph = *Mycosphaerella populorum* Thompson). Fig 1.1 depicts a generalized life-cycle of this pathogen (adapted from Sutton 1980). This fungus overwinters on dead leaves, producing pseudothecia and ascospores in the spring (Bier 1939). Mature ascospores are hyaline, straight or slightly curved, one-septate with two nearly equal cells, and have a central pore (Niyo *et al.* 1986). The ascospores are slightly constricted at the septum, and measure 13-24 x 4-6 μm (Niyo *et al.* 1986). Ascospores are wind dispersed and their release coincides with the bud swell peaking in mid to late May and continuing for 3-4 months after initial release (Luley *et al.* 1989). Ascospores seem to be present for the majority of the growing season and a strong correlation between peak ascospore dispersal and the degree of primary stem and leaf infection seems to exist (Luley *et al.* 1989). Luley *et al.* (1989) found that clone NE–19 (*Populus nigra* L. var *charkowiensis* x *Populus nigra* L. var *caudina*), typically resistant, was susceptible during weeks of peak ascospore release.

Ascospores are not, however, the only source of inoculum. Conidia, the secondary inoculum, are dispersed by rain splash and are produced throughout the growing season on leaf and stem infections (Bier 1939; Waterman 1954). Conidia have 3-6 septations, are hyaline, and measure 15-28 x 4-6 μm (Bier 1939; Waterman 1954). Under the appropriate environmental conditions, many generations of pycnidia and conidia are produced, greatly increasing inoculum levels (Bier 1939; Waterman 1954). At the end of the growing season, spermagonia, found only on senescent leaves in the fall, precede the formation of pseudothecia (Waterman 1954). In the spermagonia, continous hyaline spermatia 4-6 x 1.5 μm in size, are present (Waterman 1954).

Both leaf spots and stem infections are caused by ascospores and conidia. Anecdotal evidence suggests that leaf infection occurs via stomatal penetration (Luley *et al.* 1989). Typical *S. musiva* leaf-spots have whitish centers surrounded by black necrotic tissue (Waterman 1954). Pycnidia are often visible as black dots at the center of leaf-spots and exude pink tendrils of conidia (Waterman 1954). The impact that foliar infections have on growth and yield of hybrid poplar is unclear. However, heavy leaf-spot infection has been shown to cause premature defoliation and subsequent yield loss (Ostry 1987). The mechanism by which stem infections occur is unclear (Ostry and McNabb 1985). Once infected, cankers form on the stem; however, the wide variation in clonal response to stem infection prevents the description of a characteristic *S. musiva* canker (Waterman 1954). Rapid invasion of infected stems by other fungi tends to mask the presence of *S. musiva* (Waterman 1954). The damage caused by stem infections is severe, well documented, and typically results in stem breakage at the canker (Bier 1939; Waterman 1954; Filer *et al.* 1971; Cooper and Filer 1976; Long *et al.* 1985; Ostry and McNabb 1985; Spielman 1986; Strobl and Fraser 1989; Mottet *et al.* 1991; Newcombe 1998).

1.6 The Septoria canker-hybrid poplar pathosystem

A pathosystem is defined as any sub-system of an ecosystem that involves parasitism (Robinson 1976). The four components that make up a pathosystem are the (i) host population, (ii) pathogen population, (iii) environment, (iv) and their interactions.

1.6.1 Evaluating disease severity

In order to evaluate the variation in host and pathogen populations it is necessary to evaluate disease severity. There are two key terms that are important in this evaluation, pathogenicity and virulence. Pathogenicity is defined as the ability of an isolate to cause disease on a host (Day 1974). This term is binary, either an isolate has the ability to cause disease or it does not (Day 1974). The second term is virulence and is defined as the amount of disease caused by a pathogenic isolate (Day 1974). In general, virulence can be measured in two ways, incidence or severity. Incidence is measured as the proportion of plants or plant parts infected, whereas severity is evaluated on a continuous scale in which the amount of tissue damage is measured (Gaunt 1987). Many authors refer to isolate aggressiveness rather then virulence and for the purpose of this thesis these two terms will be considered synonymous.

1.6.2 Variation in the host population

In native stands of *Populus* species (e.g. *P. balsamifera* and *P. deltoides*) *S. musiva* has been shown to cause foliar, but not stem, infections. (Bier 1939; Waterman 1954; Newcombe 1996). However, inoculation studies have shown that *S. musiva* will cause stem infection on non-native *Populus* species and their inter- and intra-sectional hybrids (Newcombe 1996). In particular, hybrids with Tacamahaca parentage are highly susceptible to this disease (Ostry 1987). In fact, the variation in disease resistance of hybrid poplars to *S. musiva* is quite large and has been documented in many different studies in both Canada and the United States (Bier 1939; Waterman 1954; Filer *et al.* 1971; Cooper and Filer 1976; Long *et al.* 1985; Ostry and McNabb 1985; Spielman 1986; Strobl and Fraser 1989; Krupinsky 1989; Mottet *et al.* 1991; Newcombe 1998).

In an attempt to understand the impact that hybridization has on Septoria canker resistance, Newcombe and Ostry (2001) analyzed a three generation pedigree of hybrids between *P. deltoides,* a resistant species, and *P. trichocarpa* Torr. and Gray., a susceptible species. These hybrids are economically important in the Pacific Northwest and results from previous field trials have hinted that a single recessive gene might control Septoria canker resistance (Newcombe and Ostry 2001). Although the empirical

evidence supporting this hypothesis was not observed in this study (Newcombe and Ostry 2001), qualitatively, it appeared that the F2 generation of (*P. trichocarpa* x *P. deltoides*) x *P. deltoides* hybrids had increased resistance to Septoria canker (Newcombe and Ostry 2001). This suggests that backcrossing resistant clones of the pure species with susceptible hybrids may increase resistance in the next generation (Newcombe and Ostry 2001). However, the lack of a single recessive gene controlling resistance implies that successful incorporation of resistance will vary from progeny to progeny (Newcombe and Ostry 2001).

1.6.3 Variation in the pathogen population

Although the body of work surrounding the variation in *S. musiva* populations is not as extensive as that evaluating clonal resistance in the poplars, it is still significant (Krupinsky 1989; Mottet *et al.* 1991; Feau *et al.* 2005). Krupinsky (1989) evaluated variation in the aggressiveness of leaf-spot infections, by comparing isolates collected in different geographic areas and compared isolates from leaf spots with those from cankers. The results indicated that isolates collected from any geographic location were just as aggressive as isolates collected from a more widespread geographic area (Krupinsky 1989). Furthermore, isolates collected from leaf spots and cankers were equally aggressive on leaves (Krupinsky 1989). Mottet *et al.* (1991) compared canker severity on 725 clones from three sections, Aigeiros, Leuce and Tacamahaca, inoculated with four isolates of *S. musiva*. These isolates exhibited differences in aggressiveness (Mottet *et al.*1991). Finally, in a study of randomly amplified polymorphic DNA (RAPD) Feau *et al.* (2005) analysed the genetic structure of seven populations, made up of several sub-populations, of *S. musiva*. Subpopulations of *S. musiva* sampled from *P. deltoides* and hybrid poplar were not significantly differentiated (Feau *et al.* 2005). Geographic and genetic distances were highly correlated, suggesting that populations may be isolated by distance (Feau *et al.* 2005). Furthermore, gametic equilibrium for RAPD loci indicated that recombination events seem to occur and contribute to local levels of genetic diversity (Feau *et al.* 2005). Finally, 20% of the genetic differentiation was accounted for by differences at the sub-population level (Feau *et al.* 2005). Although this information is

valuable in terms of understanding the genetic structure of the pathogen population, it is unclear how these differences relate to isolate virulence.

1.6.4 The interaction between host and pathogen populations

An essential component of any pathosystem is the interaction between the host and pathogen populations. The presence of such an interaction may indicate the existence of specificity between the two populations and consequently, affect stability of resistance in the pathosystem (Robinson 1976). In the case of the *S. musiva*-hybrid poplar pathosystem, the stability of resistance could be determined by evaluating the presence of a differential interaction between clones and isolates. In the absence of a strong differential interaction, clones of hybrid poplar would exhibit constant ranking. That is, differences in disease severity among clones would be the same irrespective of the isolate to which the clones were exposed (Robinson 1976). Although the average disease severity might increase or decrease, the most resistant clone would always remain the most resistant (Robinson 1976). On the other hand, if a differential interaction existed, the difference in disease severity among different clones would vary with isolate and the phenomenon of constant ranking might not occur (Robinson 1976). In other words, the clone that was the most resistant when exposed to one isolate might become the most susceptible when exposed to a different isolate (Robinson 1976). From this description, resistance would be far more stable in a system lacking a differential interaction than in a system with a strong differential interaction.

In the case of the *S. musiva*-hybrid poplar pathosystem, conclusive evidence regarding the presence of a significant interaction between clone and isolate has not been observed. Although Krupinsky (1989) found statistical evidence for the presence of a significant interaction between clones and isolates in terms of aggressiveness of leaf spot infection, the main effects of clone and isolate were much larger than the interaction effect. Maxwell *et al.* (1997), evaluating stem infections on two clones of hybrid poplar inoculated with two isolates of *S. musiva,* did not discover significant interactions. However, the small number of clones and isolates used in this latter experiment did not provide much power for detecting interaction effects. In a study where 725 clones were inoculated with four isolates under field conditions, no effort was made to evaluate the

clone x isolate interaction (Mottet *et al.* 1991). In order to develop a better understanding of the clone x isolate interaction in the *S. musiva*-hybrid poplar pathosystem, a large number of clones from different hybrid types need to be inoculated with a large number of isolates.

1.6.5 Environmental variation

In most pathosystems, environmental variation plays an important role in disease incidence and development. Understanding the role of environmental variation in the *S. musiva*-hybrid poplar pathosystem is important due to the apparent presence of 'Septoria suppressive' sites (Newcombe *et al.* 2001). In these sites, susceptible hybrids and *S. musiva* spores are present; however, bioclimatic or edaphic conditions seem to limit successful infection and disease development (Newcombe *et al.* 2001). It is clear that environmental conditions will affect the infection process; however, their effect on disease development after successful infection is not entirely obvious.

Of the many environmental factors affecting disease development, moisture seems to play an extremely important role. This role is highlighted in a study evaluating the impact of site factors on Hypoxylon canker (*Entoleuca mammatum* (Wahl.) Miller) incidence in stands of trembling aspen (*Populus tremuloides* Michx.) (Bruck and Manion 1980) where it was demonstrated that site factors affecting moisture availability were highly correlated with disease incidence (Bruck and Manion 1980). The relative importance of moisture demonstrates the need to understand its role in any pathosystem.

In general, lower host water potentials are associated with greater disease severity (Bier 1939; Bloomberg 1962; Bagga and Smalley 1969; Crist and Schoeneweiss 1975; Tao *et al.* 1984; Bruck and Manion 1980). Bloomberg (1962) demonstrated that hybrid poplar clones experiencing water stress were more susceptible to Cytospora canker (*Cytospora chrysosperma* (Pers.) Fr.) than unstressed clones. Increasing the soil moisture of clones showing disease symptoms reduced the rate of disease development when compared to clones remaining under stressed conditions (Bloomberg 1962). These results indicate that water stress interferes with host defense reactions and once this stress is removed, the host can initiate a defense response to inhibit or prevent further disease development (Schoeneweiss 1975). Reduced levels of host bark moisture increase disease

establishment of many plant pathogenic fungi (Bier 1939; Bloomberg 1962), perhaps because certain pathogens grow at relatively low water potentials, which inhibit antagonistic organisms (Cook 1973).

Given the well established role of water stress on disease establishment and development and the apparent existence of *S. musiva* suppressive sites discussed above, it is clear that environmental variation must play a role in the infection, disease development, and/or resistance response processes. To date, very little research has been conducted to evaluate that role. The most recent study, conducted by Maxwell *et al.* (1997), evaluated the impact of water stress on two clones of hybrid poplar and two isolates of *S. musiva*. Although water stress increased disease severity, there were no significant interactions between clone, isolate, or their interactions and water stress (Maxwell *et al.* 1997). The lack of interaction showed that varying moisture availability did not alter susceptibility rankings among clones. These results are a good first step; however, similar experiments need to be conducted with more clones, from different hybrid types, and more isolates, to determine the generality of these results.

1.7 Disease management

Four possible means of controlling *S. musiva* on hybrid poplar plantations include: cultural methods, chemical methods, biological control, and resistant clones. Cultural methods, such as the removal of dead leaves, are ineffective due to the ability of the pathogen to disperse over large distances from native hosts or neighboring plantations (Ostry and McNabb 1985). Chemical control is extremely expensive due to the number of applications necessary each year to control the disease (Ostry and McNabb 1985). Biological control, though explored, has not been thoroughly tested (Yang *et al.* 1994). Resistant clones, therefore, seem to be the most promising means of disease prevention (Ostry and McNabb 1985).

With many pathosystems, indirect selection is often used to screen for resistant clones. This process screens for disease resistance by controlled inoculation under greenhouse conditions. Historically, indirect selection has been used in the southeastern United States to screen for fusiform rust (*Cronartium quercuum* Berk. miyabe Shirai f. sp. *fusiforme*) resistance in loblolly pine (*Pinus taeda* L.) (Carson and Young 1987). The

results from these trials may be considered reliable if good correlations between greenhouse and field results exist and there are no large host x environment interactions (Carson and Young 1987). In fact, strong correlations between greenhouse and field trials have been observed (Carson and Young 1987). However, host resistance has been shown to interact with fertilization (Rowan 1977). Rather than halting the use of greenhouse screening, these interactions have been incorporated into the process by determining the levels of fertilization with the highest correlation to field results, and subsequently, using those levels in greenhouse screening (Carson and Young 1987). The success of this procedure in detecting resistant loblolly pine is promising for the *S. musiva*-hybrid poplar pathosystem.

As described above, much work has been done to identify clones resistant to Septoria canker. Most of these studies have been carried out by artificially inoculating juvenile tissue or through evaluation of disease severity through time on clones under field conditions (Filer *et al.* 1971; Cooper and Filer 1976; Long *et al.* 1985; Ostry and McNabb 1985; Spielman 1986; Strobl and Fraser 1989; Krupinsky 1989; Mottet *et al.* 1991; Newcombe 1998; Ares 2002). It has generally been assumed that the response of clones to artificial inoculation is a good predictor of long-term field performance. Weiland *et al.* (2002) tested this assumption by determining the probability that clones would receive the same relative disease severity rankings in the greenhouse and the field. Weiland *et al.* (2002) found that the probability of achieving the same ranking under field and greenhouse conditions varied with susceptibility. The most susceptible or resistant clones had a high probability of being ranked in the same categories under field and greenhouse conditions, whereas those clones in the mid range were often ranked in different categories under greenhouse and field conditions (Weiland *et al.* 2002). This suggests that greenhouse trials are a reasonable preliminary method for screening for disease resistance for the extreme responses.

Once resistant clones have been identified, the clonal nature of hybrid poplar allows for the relatively rapid production of large numbers of individuals (Peterson and Peterson 1992; Stettler *et al.* 1996). The temptation to find one or two highly resistant individuals and plant them widely is quite strong. However, the danger inherent in planting a small number of genetically identical individuals has been demonstrated repeatedly in

agriculture. Consequently, one of the most important questions presenting itself to plantation managers is how many clones should be deployed on the landscape to minimize the risk of plantation failure (Roberds and Bishir 1997). Researchers have explored this question extensively through modeling. In general, depending on the complexity of the model, the number of unrelated clones should be approximately 20-40 (Libby 1982; Roberds *et al.* 1990; 1990 Hühn 1992). The general agreement between these models is promising news for plantation managers. However, to increase the reliability of these predictions even further, specific information about clone response, isolate virulence, and the clone x isolate interaction needs to be incorporated into these models.

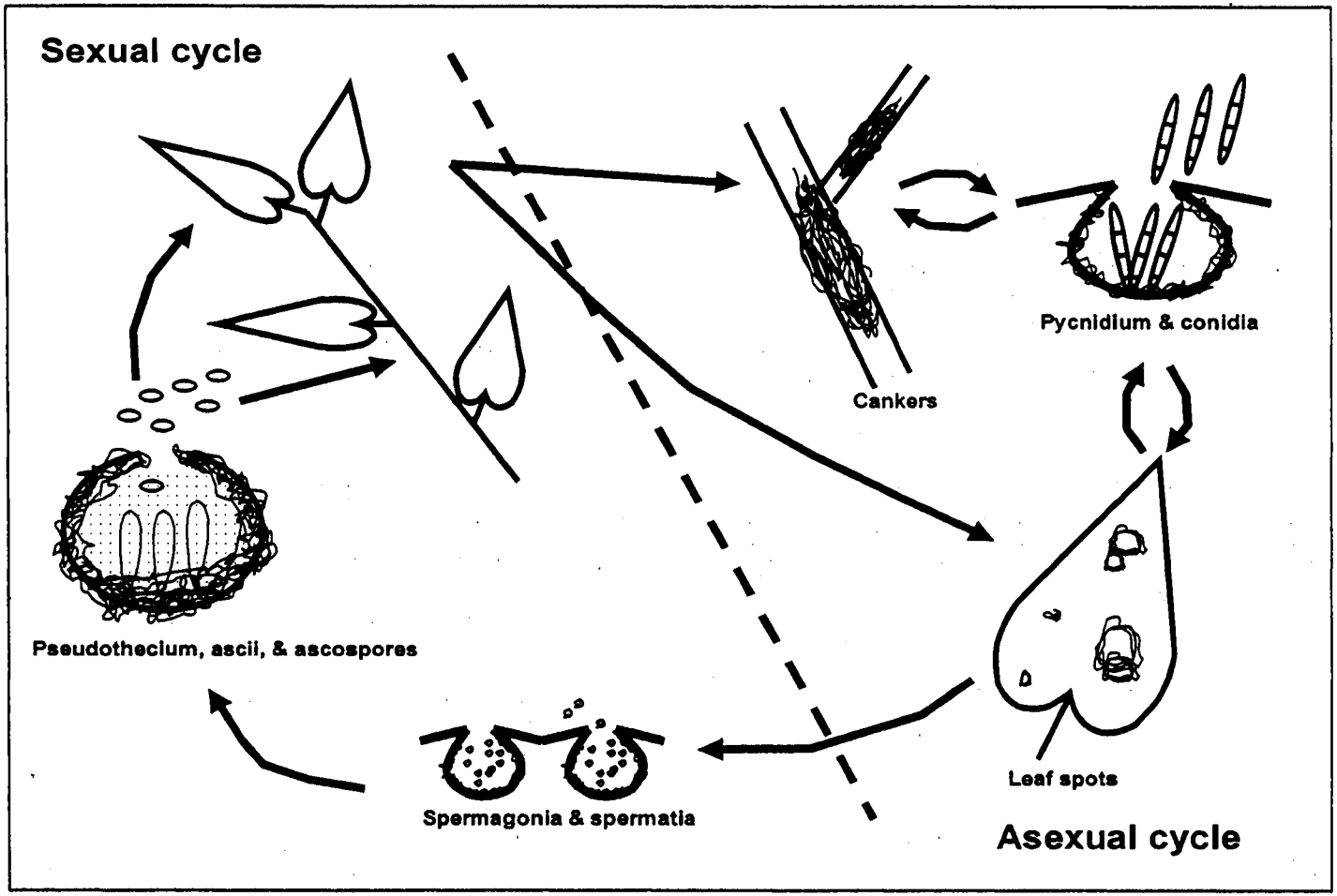
1.8 Study rationale and research objectives

From the literature reviewed above, it is evident that information on the interaction between *S. musiva* and hybrid poplar populations is lacking. The specificity that exists between clones of hybrid poplar and isolates of *S. musiva* is one important indicator of pathosystem stability. Consequently, the presence of an interaction will guide the disease management decisions of a plantation manager. Although much work has been conducted to evaluate the variation in the host and pathogen populations, an exploration of the pathosystem as a whole has not been undertaken. To investigate these topics, 14 clones of hybrid poplar from three hybrid types were inoculated with 19 isolates of *S. musiva* from three geographic areas under greenhouse conditions. The specific objectives of this study were to: (i) determine the magnitude of the clone, isolate, and the clone x isolate interaction effects; (ii) determine if the virulence of isolates varied with geographic location; and (iii) determine if disease severity varied among hybrids.

Resistance screening trials are often conducted in greenhouses to minimize disease escapes and inoculum variability. Given that clones grown under greenhouse conditions do not experience the range of environmental variation found under field conditions, the results from these studies are often called into question. To this end, an experiment was designed to evaluate the effect of soil moisture on disease severity. The objective of this study was to determine the reliability of greenhouse results under field conditions by evaluating the interaction between (i) clone, (ii) isolate, and (iii) the clone by isolate

interaction, if present, with environment. The rationale was that the absence of strong interactions between the environment and the other factors would indicate that greenhouse results should be reasonably good predictors of resistance under field conditions.

Fig. 1.1. Daigram depicting the *Septoria musiva* lifecycle. (Figure adapted from Sutton 1980) Dashed line divides sexual from asexual cycle.



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Chapter 2: Evaluating the Impact of Water Stress on the Hybrid Poplar-*Septoria musiva* Pathosystem

2.1 Introduction

As demands for pulp and timber increase, and the area of land available to help meet these demands decreases, plantations of intensively managed fast growing tree species are becoming ever more common. The clonal nature and quick growth of hybrid poplars make them ideal species for these plantations (Dickmann 2001). This shift towards an agricultural system of intensively managed plantations will provide unique challenges for forest managers. One of these challenges will be disease management.

In North America the two most important diseases of hybrid poplar are Melampsora rust and Septoria canker (Newcombe 1996). Neither disease has, as yet, caused serious damage to hybrid poplar plantations in Alberta. Given the short growing season and relatively dry climate, the conditions for heavy infection by *Melampsora* spp. may not exist (Crane 2002; "Poplar leaf rusts in central Alberta – assessing the risks to agroforestry" an unpublished report to Alberta-Pacific Forest Industries Inc.). Septoria canker, on the other hand, has historically caused damage to hybrid poplar in shelterbelts across the Prairie Provinces (Bier 1939). Furthermore, plantations of hybrid poplar in Eastern Canada and the United States have suffered extensive damage due to this pathogen (Waterman 1954; Ostry and McNabb 1985; Ostry *et al.* 1989; Strobl and Fraser 1989).

The causal agent of Septoria canker is *Septoria musiva* Peck. (teleomorph = *Mycosphaerella populorum* Thompson) a coelomycete. This pathogen overwinters on dead leaves, producing pseudothecia and ascospores which infect newly emergent leaves and shoots (Bier 1939). Throughout the growing season, leaf spots arise on infected leaves; pycnidia on these spots produce conidia which increase infection levels (Bier 1939). Cankers form on infected shoots and stems and often result in stem breakage in high winds (Bier 1939; Waterman 1954; Ostry and McNabb 1985). Chemical and cultural controls have proven expensive and largely ineffective (Ostry 1987). The planting of resistant clones appears to be the best means of controlling this disease

(Newcombe *et al.* 2001; Ostry and McNabb 1985; Ostry 1987; Gyenis 2003). It is therefore essential to develop a quick and efficient means of screening hybrid poplar clones for *S. musiva* resistance.

Unlike many pathogens, the bulking of multiple isolates in spore suspensions and subsequent exposure of one clone to many isolates is not practical for *S. musiva* (Bier 1939; Waterman 1954), because inoculation of unwounded shoots with spores rarely results in infection. Rather, artificial inoculation typically involves removal of a single leaf and placement of mycelium on the wound (Mottet *et al.* 1991; Long *et al.* 1986). Although evaluation of disease resistance under field conditions will always be necessary, greenhouse inoculation, where inoculum pressure and environmental conditions can be controlled, may be a useful preliminary screening strategy for identifying and culling extremely susceptible clones before expensive field studies are undertaken. For this process to be effective, the results from greenhouse screening must be reasonably correlated with long-term field results.

Determination of the correlation between field and greenhouse susceptibility is time consuming since it requires a large number of greenhouse-inoculated clones to be exposed to natural inoculum under field conditions for a number of years. A faster, albeit less reliable approach, would be to evaluate the consistency of greenhouse inoculation results under a range of controlled environmental conditions. The absence of any genotype by environment interaction under greenhouse conditions would increase one's confidence that the relative resistances of clones found in the greenhouse would also be expressed in the field.

The objective of this study was to evaluate the reliability of greenhouse results under varying environmental conditions. This objective was tested by exposing four clones of hybrid poplar to four isolates of *S. musiva* under water stressed and unstressed conditions.

An experiment was conducted to evaluate the interaction between (i) clone, (ii) isolate and (iii) the clone by isolate interaction, if present, and environment. Small interactions with environment would support the hypothesis that greenhouse results would be robust over a range of environments and thus likely good predictors of field

results. Large interactions with environment, on the other hand, would indicate that differences among clones would vary with environment.

2.2 Materials and methods

2.2.1 Poplar culture

In late February 2004, 20 dormant cuttings, 10 cm in length, from each of four hybrid poplar clones, were collected from stool beds and research trials at the Alberta-Pacific Forest Industries Inc. (Al-Pac) millsite in Northern Alberta (approximate location 54° 53' 35.1 N, 112° 51' 38.5 W, 575m elevation) (Table 2.1). The dormant cuttings were then returned to the University of Alberta and soaked in de-ionized water for 48 hours at 4°C (DesRochers and Thomas 2003). These cuttings were then planted in 12 cm rootrainers (Spencer-Lemaire® Rootrainers; Spencer-Lemaire Industries, Edmonton, AB) containing Metromix® 290 growing media (Terra-Lite 2000 series; WR Grace and Company, Ajax, ON).The planting was carried out so that only the top-most bud remained exposed above the surface of the growing medium (DesRochers and Thomas 2003).

After planting, the rootrainers were placed in a greenhouse maintained at 20/15°C (day/night) with an 18h photoperiod supplemented with 400 W high pressure sodium lamps. The irradiance varied with cloud cover, but was on average 450 μmol photons $m^{-2}s^{-1}$ PAR (photosynthetically active radiation) at the tree canopy level. The cuttings were grown in the greenhouse for 56 days prior to any transplanting or treatment application. During the first 28 days, the cuttings were fertilized once a week with a 500 ppm solution of 15-30-15 fertilizer (Plant Products Company Ltd., Brampton, ON). For the following 28 days, the cuttings were fertilized once every two weeks with a 500 ppm solution of 20-20-20 fertilizer (Plant Products Company Ltd., Brampton, ON).

At the end of the initial 56 day growth period, 20 trees from each clone were transplanted from the rootrainers into 20 cm fiber pots (Kord standard; Kord products Inc., Brampton, ON) containing Metromix® 290 growing media amended with 2.73g L^{-1} Nutricote® 100 day slow release 13-13-13 fertilizer (6.5% NO_3-N, 6.5% NH_4-N, 13% P_2O_5, 13% K_20, 1.2% Mg, 0.02% B, 0.05% Cu, 0.2% chelated Fe, 0.06% Mn, 0.02% Mo, 1.3% EDTA; Plant Products Company Ltd., Brampton, ON). These trees were then

allowed to grow for 14 days before being pruned, leaving only the stem and three vegetative buds; seven days after the buds had flushed, all but the top most branch was removed. These shoots were allowed to grow until they reached a minimum height of 10 cm.

Throughout the course of the experiment, various species of spider mite and thrip were controlled via the introduction of predatory mites. The spider mite eliminator (Applied Bio-nomics Ltd.; Sidney; BC) *Phytoseiulus persimilis* Athias-Henriot and the thrip predatory mite *Amblyseius cucumeris* Oudemans were spread evenly throughout the greenhouse every 2 weeks.

2.2.2 Pathogen culture

Six isolates, each a single spore culture of *S. musiva* (Table 2.2), were received from the University of Laval, in Quebec, for this experiment. The cultures were stored at -80°C in cryogenic vials (Nalgene® labware, Rochester, NY USA). There were six vials per culture, each containing a solution of 300 µL of 50% glycerol and 700 µL of potato dextrose broth (PDB; Difco ™ potato dextrose broth, Franklin Lakes, NJ USA). For each culture, two vials were removed from cold storage and allowed to warm to room temperature. Each tube was then poured onto a petri dish of KV-8 growth medium and sealed with Parafilm®. These plates were then placed on a light bench, under Gro-Lux© wide spectrum fluorescent bulbs (Sylvania; Osram Gmbh, Munich, Germany), at room temperature, where they received 24 hours of light for 5 days.

The four isolates exhibiting the best growth and sporulation were chosen for the experiment. One plate from each of these four isolates was then selected, and from this, four, 5 mm x 5 mm sporulating masses of mycelium were removed and placed on KV-8 growing media. Eight similar plates were created for each isolate. These plates were then sealed and placed on the light bench with the same growing conditions described above. After 14 days of growth, the cultures with the most growth and sporulation were used as inoculum in the experiment.

2.2.3 TDR probe construction and installation

Time Domain Reflectometry (TDR) probes were constructed using a procedure adapted from Robinson *et al.* (2003). Coaxial cable was cut to 1.5 m lengths and a Bayonet Neill Concelman (BNC) connector was attached to one end. The opposite end of the cable was stripped back approximately 10 cm and the two wires were separated. Crimp connectors were then used to attach each wire to a 12 cm length of thoriated welding rod and the remaining exposed wire was re-insulated with Plasti Dip® (Plasti Dip International©, Blaine, MN, USA; Fig. 2.1).

Probes were installed into pots so the distance between rods was 5 cm and the distance from the stem of the tree to either rod was approximately 2.5 cm. Each pot in the water stressed treatment had one probe installed. For the unstressed treatment, one tree per clone was randomly selected to receive a probe.

2.2.4 Experimental design

This experiment evaluated if clone, isolate, and clone by isolate interaction effects varied with water stress. The experimental design was a split-plot with four blocks. The treatment structure was a 2 x 4 x 4 factorial. The two moisture regimes were the main plot factors and were randomly assigned to one half of each of the four blocks. Each of the four blocks occupied separate positions on a greenhouse bench. The four clones and four isolates were sub-plot factors. Within each main plot, five trees from each of the four clones were randomly arranged and four isolates and one uninoculated control were then randomly assigned to the trees so that all clone and isolate combinations occurred once per main plot.

2.2.5 Inoculation

Inoculations were carried out once the mycelium began to sporulate (approximately 14 days after colony establishment) and the new growth of the stecklings (rooted cuttings) had reached a minimum height of 10 cm. One Petri-dish was used per isolate in each block. The stecklings were inoculated by removing the 3rd fully extended leaf from the shoot apex (Mottet *et al.* 1991). A 5 mm plug of sporulating mycelium, with the excess agar removed, was then placed over the wound and wrapped in Parafilm® (Mottet *et al.*

1991). Inoculated controls were carried out in the same manner, with the exception that sterile KV-8 agar, rather than sporulating mycelium, was placed on the wounds. Finally, the Parafilm® was removed from all stecklings 14 days after inoculation (Weiland *et al.* 2005).

2.2.6 Moisture regime application

Immediately after inoculation, trees were exposed to two different moisture treatments, stressed and unstressed. For the stressed treatment, a soil water potential of -1.0 MPa was chosen (Maxwell *et al.* 1997). Based on a water retention curve constructed for this soil, -1.0 MPa corresponded to a volumetric soil water content of 10 %. Water contents were monitored once daily using TDR. Whenever the water content of the pots dropped below 10 % they were watered to field capacity. Pots in the unstressed treatment were watered to field capacity every other day; the water content was at least 35 %.

2.2.7 Measurements

Height and root collar diameter of all the trees were measured using a measuring tape and calipers, respectively. Measurements were taken at the beginning of the experiment and once again at the conclusion, 56 days after the treatments were applied. The trees were then harvested and a 30 cm section of the stem, centered on the original wound, was kept for further assessment. These sections were labeled by clone, isolate, and block number, placed in paper bags, and stored at 4°C. The disease severity was then evaluated using a dissecting scope and assigned a number on a 1 to 5 scale (Table 2.3). All sections of stem were evaluated within 2 weeks of harvest.

2.2.8 Re-isolation

At the conclusion of the experiment, to ensure that the observed necrosis was caused by *S. musiva*, five cankers from each clone were selected and reisolations (Stanosz and Stanosz 2002) were attempted. The cankers were soaked in a 5 % NaOCl solution for 2 minutes and then rinsed in de-ionized water for 30 seconds. The periderm was then removed from the symptomatic area, exposing the margin between healthy and necrotic tissue. From this margin, a sliver of necrotic tissue, approximately 3 mm in length, was

removed and placed in a Petri-dish containing *Septoria musiva* media (SMM; KV-8 growing media amended with Chloramphenicol and Streptomycin at 300 mg/l and 25mg/l respectively). Four such slivers were placed in each dish, with three dishes for each canker. Once the fungal colonies had grown to sufficient size they were transferred to plates containing KV-8 media, allowed to grow for an additional 2 weeks, and then identified (Thompson 1941).

2.2.9 Statistical analysis

Statistical analyses were performed using SAS MIXED and GLM procedures (SAS Institute 2003), with significance assessed at $\alpha = 0.05$. The analysis began with the full model and Akaike's Information Criteria (AIC) and a chi-square test of the likelihood ratio were used to choose the most parsimonious model. The initial statistical model was the same for disease severity, height growth, and root collar diameter growth:

$$Y_{ijkl} = \mu + B_i + W_j + C_k + I_l + W_j*C_k + W_j*I_l + C_k*I_l + W_j*C_k*I_l + \varepsilon_{ijkl}$$

$$i = 1\text{-}4 \qquad j = 1\text{-}2 \qquad k = 1\text{-}4 \qquad l = 1\text{-}4$$

where:

Y_{ijkl} = the response to the j^{th} treatment in the i^{th} block of the k^{th} clone inoculated with the l^{th} isolate;
μ = the overall mean;
B_i = the random effect of the i^{th} block;
W_j = the fixed effect of the j^{th} treatment;
C_k = the random effect of the k^{th} clone;
I_l = the random effect of the l^{th} isolate;
W_j*C_k = the random effect of the interaction between the j^{th} treatment and k^{th} clone;
W_j*I_l = the random effect of the interaction between the j^{th} treatment and l^{th} isolate;
C_k*I_l = the random effect of the interaction between the k^{th} clone and l^{th} isolate;
$W_j*C_k*I_l$ = the random effect of the interaction between the j^{th} treatment and k^{th} clone and the l^{th} isolate;
ε_{ijkl} = the residual.

Height growth a root collar diameter growth were estimated by subtracting the initial measurment from the final measurment. These values were then used to conduct the statistical analyses. Significance of the clone and isolate effects was tested using PROC

GLM (SAS Institute 2003) after the final model was selected.The Best Linear Unbiased Predictors (BLUPs) and their associated 95% prediction intervals were calculated for each clone (SAS Institute 2003).

2.3 Results

2.3.1 Final model

After comparing several models with different combinations of explanatory variables (treatment, clone, isolate, and their interactions) the most parsimonious models for disease severity, height growth, and root collar diameter growth were chosen. The model for disease severity was:

$$Y_{ijkl} = \mu + B_i + W_j + C_k + I_l + C_k*I_l + \varepsilon_{ijkl}$$

$$i = 1\text{-}4 \qquad j = 1\text{-}2 \qquad k = 1\text{-}4 \qquad l = 1\text{-}4$$

where:

Y_{ijkl} = the response to the j^{th} treatment in the i^{th} block of the k^{th} clone inoculated with the l^{th} isolate;
μ = the overall mean;
B_i = the random effect of the i^{th} block;
W_j = the fixed effect of the j^{th} treatment;
C_k = the random effect of the k^{th} clone;
I_l = the random effect of the l^{th} isolate;
C_k*I_l = the random effect of the interaction between the k^{th} clone and l^{th} isolate;
ε_{ijkl} = the residual.

The model for the difference in height and root collar diameter (RCD) was:

$$Y_{ijkl} = \mu + B_i + W_j + C_k + I_l + C_k*I_l + \varepsilon_{ijkl}$$

$$i = 1\text{-}4 \qquad j = 1\text{-}2 \qquad k = 1\text{-}4 \qquad l = 1\text{-}4;$$

where:

Y_{ijkl} = the response to the j^{th} treatment in the i^{th} block of the k^{th} clone inoculated with the l^{th} isolate;
μ = the overall mean;
B_i = the random effect of the i^{th} block;
W_j = the fixed effect of the j^{th} treatment;

C_k = the random effect of the k^{th} clone;
I_l = the random effect of the l^{th} isolate;
C_k*I_l = the random effect of the interaction between the k^{th} clone and l^{th} isolate;
ε_{ijkl} = the residual.

For both height growth and disease severity, residual variances differed among clones; these differences were incorporated into the respective models.

2.3.2 Disease severity, height growth, and root collar diameter growth

The ANOVA indicated that there were no significant differences between the four isolates tested in this experiment (Table 2.4). There were however, significant differences between the four clones tested in this experiment (Table 2.5). Furthermore, there was no significant effect of water stress on disease severity (Fig. 2.2) and none of the interactions between, clone, isolate, and treatment were significant (Table 2.4). On the other hand, the treatment means for height growth and RCD growth were significantly different, whereas the treatment means for disease severity were not (Fig. 2.2).

2.3.3 Re-isolations

S. musiva was the only plant pathogenic fungus re-isolated from the sampled cankers. *S. musiva* was successfully re-isolated from 12 of the 20 cankers sampled and the remaining eight were completely colonized by typical lab contaminants.

2.4 Discussion

2.4.1 Clones

The disease severity of each clone was determined using the disease severity index in Table 2.3. Any clone with a disease rating of 3 or less has either successfully contained the infection or avoided it altogether (Table 2.3). The ANOVA indicated that the clones were significantly different; consequently, clones with a disease severity BLUP less then 3 could be considered resistant. In the case of the four clones tested in this experiment clone SxB1 had a disease severity BLUP less then 3 where as the other three clones (DxB1, DxLxN1, and DxLxN2) had an average disease severity BLUP greater than 3

(Table 2.5). For this reason SxB1 could be considered resistant, whereas the other three clones could be considered susceptible (Table 2.3; Table 2.5). However, it is important to note that these clones were only exposed to four isolates of *S. musiva* and should be exposed to a larger number of isolates and subsequently field tested before certainty of resistance or susceptibility can be determined.

2.4.2 Water stress

Water stress decreased tree diameter and height growth, but had no effect on disease severity (Fig. 2.2). Similar studies examining the impact of water stress found that disease severity increased with decreasing host water potential (Bloomberg 1962; Bagga and Smalley 1968; Bruck and Manion 1980; Maxwell *et al.* 1997). In particular, Maxwell *et al.* (1997) found a significant treatment effect on disease severity when inoculating hybrid poplar with *S. musiva* and subsequently exposing the inoculated clones to two different watering regimes. The reason for the discrepancy between the current study and others is unclear; however, the timing of treatment application in combination with the length of the experiment may be a possible cause.

Predisposition is a term referring to "an internal degree of susceptibility resulting from external causes" (Yarwood 1959) and has been shown to play an important role in disease development (Schoeneweiss 1975). In this study, the hybrid poplar clones were not exposed to water stress until after they had been inoculated. The lack of a significant treatment effect on disease severity may have been due to the absence of predisposition in the host tissue. Furthermore, in Maxwell *et al.* (1997), the clones were exposed to water stress for 79 days whereas in this study they were only exposed to the treatment for 49 days. The 49-day period of this experiment may have been too short for disease development to be affected even though height and RCD were. It is likely that these two factors in combination were responsible for the lack of a significant difference in disease severity between the stressed and unstressed treatments.

2.4.3 The interactions between clones, isolates, and water stress

In general significant interactions between genotype and environment indicate that fluctuations in the environment may override host resistance (Kulkarni and Chopra

1982). In other words, the relative response of different clone by isolate combinations may change with environmental conditions. In both this study (Table 2.4) and that of Maxwell *et al.* (1997), there were no significant interactions between clone, isolate (or their interaction) and water stress. These results suggest that it may be practical to conduct greenhouse screening even though, in the greenhouse, the clones are unlikely to experience the wide range of environmental conditions they would in the field. However, there are two important caveats to this statement: (i) there was no significant treatment effect on disease severity in this experiment; and (ii) there was little power to detect interactions in Maxwell *et al.* (1997).

Of primary concern is the lack of a significant treatment effect on disease severity, described above. Given that the treatment effect on both height and RCD growth was highly significant, the trees must have undergone water stress. This stress did not affect the disease severity. Of secondary consideration is the lack of power in the study by Maxwell *et al.* (1997) to detect interactions. Two clones, two isolates, and two watering regimes provide only one degree of freedom to detect interactions. If a relatively small interaction between clone x isolate x water stress existed, then it is likely that their study did not have sufficient power to detect it. Either study considered individually does not provide strong evidence for the reliability of greenhouse results under field conditions. However, the absence of interactions in both studies together does support the conclusion that greenhouse results should be reasonably robust under field conditions.

2.4.4 The greenhouse vs. the field

As discussed above, the applicability of results from greenhouse screening trials to the field are often questioned. This is primarily because it is unknown how well greenhouse results will correlate with long term field performance and how various interactions between host, pathogen, and the environment will affect resistance. In the case of the *S. musiva*-hybrid poplar pathosystem, Weiland *et al.* (2003) found that for 14 of the 15 clones tested, the damage category assigned to trees in the greenhouse matched those assigned in the long term field study. Their results suggested that the prediction of long term canker damage from responses of greenhouse inoculated clones is most reliable at the extremes in field performance (Weiland *et al.* 2003). In other words, greenhouse

results are the most reliable for detecting highly resistant and highly susceptible clones (Weiland *et al.* 2003).

In terms of evaluating host x pathogen x environment interactions, the control of environmental conditions can have positive consequences, as seen in the case of fusiform rust (*Cronartium quercuum* Berk. miyabe Shirai f. sp. *fusiforme*) resistance in loblolly pine (*Pinus taeda* L.). When significant host x environment interactions were detected, they were used to increase the correlation between the greenhouse and field studies by evaluating loblolly pine resistance at the levels of environmental factors where the correlations between greenhouse and field studies were the highest (Carson and Young 1987). The success in developing fusiform rust resistant loblolly pine through the combination of field and greenhouse screening (Carson and Young 1987; Foster and Anderson 1989) can be a blueprint for disease management in the *S. musiva*-hybrid poplar pathosystem.

Table 2.1. Origin and hybrid type of inoculated clones. The prefix of the clone label refers to the hybrid type (DxB = *P.deltoides* x *P. balsamifera*, SxB = *P. simonii* x *P. balsamifera*, and DxLxN = *P. deltoides* x (*P. laurifolia* x *P. nigra*)). AP number is the Al-Pac number given to each clone in their breeding program. Clone name is the common name of each clone.

Clone label	AP number	Clone name	Female parent	Male parent
DxB1	AP 27	Northwest	*P. deltoides*	*P. balsamifera*
SxB1	AP 33	P38xP38	*P. simonii*	*P. balsamifera*
DxLxN1	AP 794	Brooks 6	*P. deltoides*	*P. x petrowskyana*[a]
DxLxN2	AP 2403	-	*P. deltoides*	*P. x petrowskyana*

[a] *P. x petrowskyana* = *P. laurifolia* x *P. nigra*

Table 2.2. Origin of isolates. The prefix of the isolate label refers to the geographic area. Quebec designation is the collection number given to isolates received from the University of Laval. Locations are approximate and based on location of the nearest distinguishing landmark.

Isolate label	Quebec designation	Legal location	Geographic area
S1	SO3002	45°N 73°W	St-Ours (QC)
B1	AB0102	47°N 79°W	Baby (QC)
T1	TH1502	46°N 71°W	Thetford mines (QC)
T2	TH0802	46°N 71°W	Thetford mines (QC)

Table 2.3. Disease severity classes and the descriptions of their associated symptoms.

Class	Description
1	Wound healed with no callus development
2	Callus
3	Necrosis contained by callus
4	Necrosis extending beyond callus
5	Complete encirclement

Table 2.4. Analysis of variance including source, degrees of freedom (df), p-value (p) and variance estimate showing the effects of water stress, clone, isolate, and their interactions on disease severity based on the full model.

Source	df	p	Variance estimate
Block	3	0.242	0.01
Treatment (W)	1	0.469	-[a]
Clone (C)	3	0.119	1.19
Isolate (I)	3	1.000	0
C x I	9	0.115	0.12
C x W	3	1.000[b]	0
I x W	3	1.000[b]	0
C x I x W	9	1.000[b]	0
Residual error	93	-	0.38
Total	127	-	-

[a] treatment is a fixed, not random, effect
[b] not included in the final model.

Table 2.5. Best Linear Unbiased Predictor (BLUP) of disease severity for each clone. PI refers to the 95% Prediction interval of each BLUP.

Clone	Clone name	BLUP +/- 95% PI
DxB1	Northwest	4.91 +/- 0.58
SxB1	P38xP38	2.48 +/- 0.50
DxLxN1	Brooks 6	4.50 +/- 0.58
DxLxN2	-	3.64 +/- 0.51

Fig. 2.1. Diagram of a Time Domain Reflectometry (TDR) Probe. The thoriated welding rods are inserted into the soil parallel to each other; the crimp connectors attach the welding rod to the coaxial cable; and the Bayonet Neill Concelman (BNC) connector is attached to a cable tester.

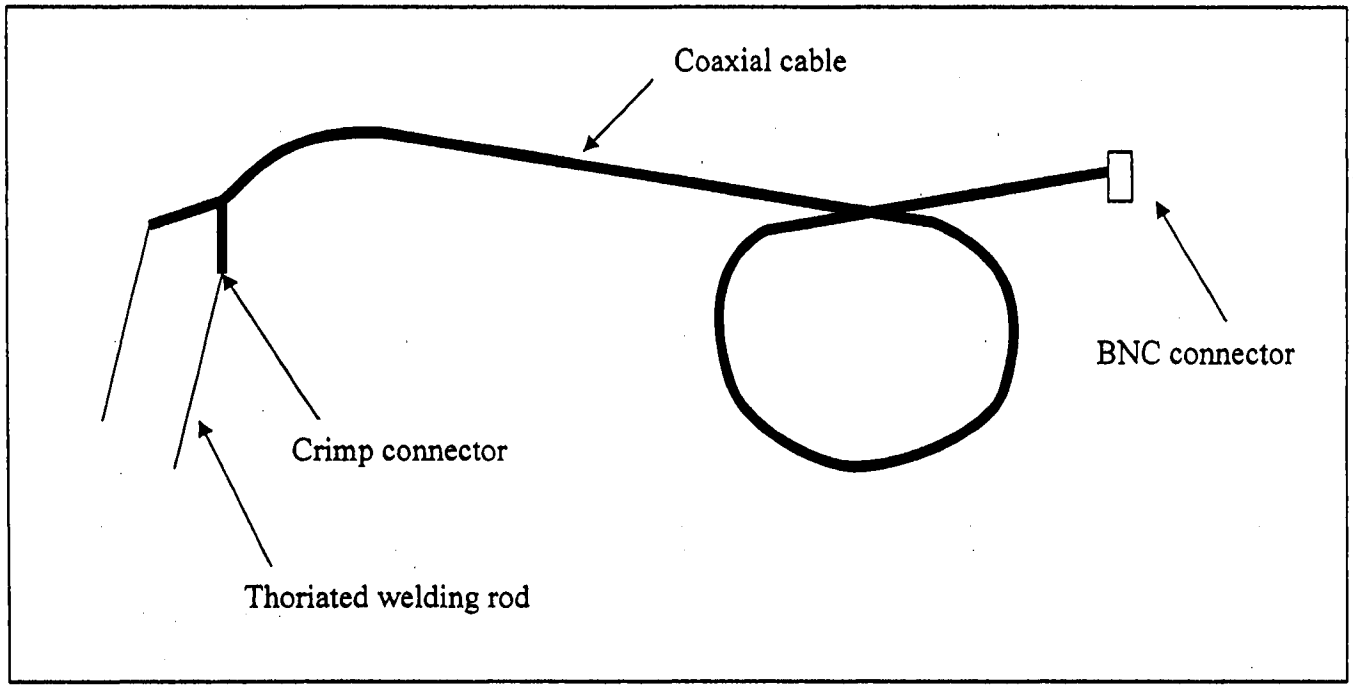
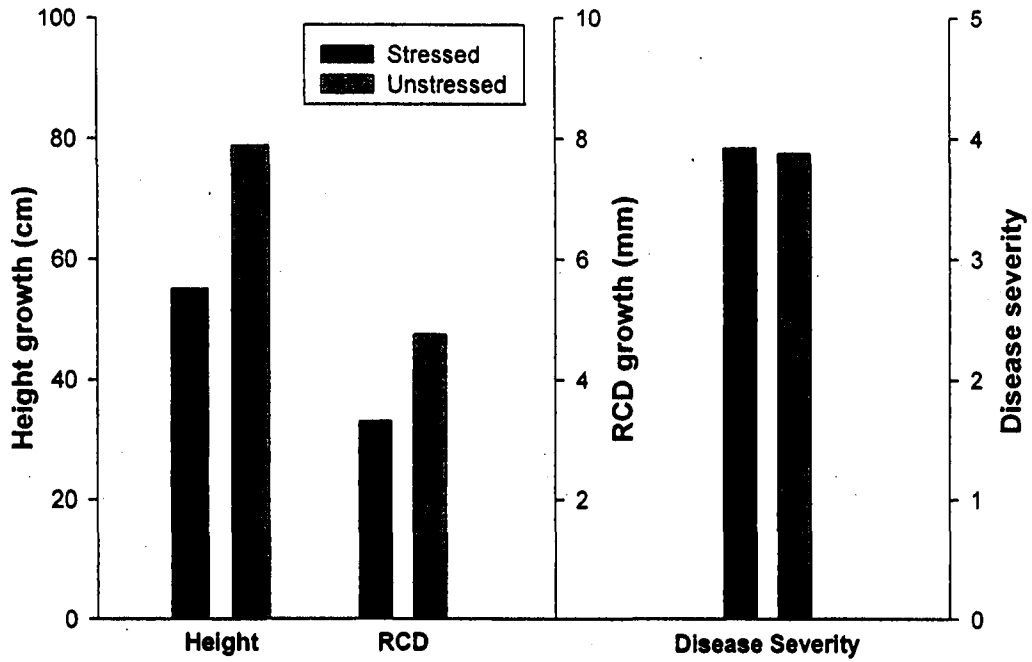


Fig. 2.2. Mean height growth, root collar diameter (RCD) growth, and disease severity for stressed and unstressed treatments (chart) and 95 % confidence intervals for the difference of the means (embedded table). Estimate refers to the estimated difference of the treatment means and c.i. refers to the confidence interval.



Dependent variable	Estimate +/- 95% c.i. on the difference of the means ($\bar{x}_{Stressed} - \bar{x}_{Unstressed}$)	p-value
Height growth	30.4 cm +/- 5.1 cm	< 0.0001
RCD growth	1.5 mm +/- 0.5 mm	< 0.0001
Disease severity	0.05 +/- 0.16	0.4867

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Chapter 3: Evaluating the Hybrid Poplar-*S. musiva* Pathosystem

3.1 Introduction

Species of *Populus* and their inter- and intra-specific hybrids are among the fastest growing tree species of the northern latitudes. Intensively managed plantations of genetically superior hybrid poplar clones have demonstrated impressive yields in recent years (Stettler *et al.*1996). As such, they represent an important resource in both Canada and the United States to help meet demands for wood, fiber, and biofuel (Stettler *et al.* 1996). Unfortunately, disease has become an issue on many of these plantations, calling their economic viability into question (Ostry and McNabb 1985; Newcombe 1998). Of the various pathogens affecting hybrid poplar in Canada and the United States, *Septoria musiva* Peck (teleomorph = *Mycosphaerella populorum* G. E. Thompson) has been known to cause severe damage and even plantation failure (Bier 1939; Waterman 1954; Ostry and McNabb 1985; Ostry 1987; Strobl and Fraser 1989; Newcombe *et al.* 2001).

The life-cycle of this pathogen is reasonably well understood. The fungus overwinters on dead leaves and produces pseudothecia in the spring (Bier 1939). Ascospores infect new leaves and branches as they grow (Bier 1939). Infected leaves develop necrotic lesions that may cause early-season defoliation, and in the case of severe infections, lead to a reduction in yield (Ostry and McNabb 1985). Pycnidia and conidia are produced on leaf spots throughout the growing season, giving rise to secondary infections (Bier 1939). Stem and branch infections cause cankers, making susceptible clones prone to breakage during heavy winds (Bier 1939; Waterman 1954; Ostry and McNabb 1985). Chemical and cultural controls of this pathogen have been attempted but remain either ineffective or are not economically viable (Ostry 1987). Consequently, the selection and deployment of resistant clones appears to be the best strategy for controlling this disease (Newcombe *et al.* 2001; Ostry and McNabb 1985; Ostry 1987; Gyenis 2003).

Many studies and surveys have evaluated the resistance of hybrid poplar clones (Bier 1939; Waterman 1954; Filer *et al.* 1971; Cooper and Filer 1976; Long and Bowersox 1985; Ostry and McNabb 1985; Spielman 1986; Strobl and Fraser 1989;

Mottet *et al.* 1991; Newcombe 1998). Inoculation experiments have also been conducted to determine the range of variation present in the pathogen population (Krupinsky 1989; Mottet *et al.* 2001). However, in order to ensure that clones being screened for resistance are exposed to the widest range of variation possible, isolates from different geographic areas need to be collected and compared in terms of virulence. This is necessary since isolates from different locations may be reproductively isolated and therefore exhibit differences in virulence due to differences in the host populations to which they were exposed (Leonard 1987). This was done by Feau *et al.* (2005) who used neutral markers to describe the genetic structure, at the landscape level, for populations of *S. musiva* from Quebec, Ontario, and Wisconsin. However, isolate virulence was not compared. A pedigree examining the inheritance of resistance through hybridization of a resistant and susceptible parent was assessed (Newcombe and Ostry 2001). An appraisal of the pathosystem as a whole, however, has not as yet been undertaken.

The specific objectives of this study were to: (i) determine the magnitude of the clone, isolate, and the clone x isolate interaction effects; (ii) determine if the virulence of isolates varied with geographic location; and (iii) determine if disease severity varied between hybrids.

3.2 Materials and methods

3.2.1 Poplar culture

Hybrid poplar material was collected from stool beds and research trials at the Alberta-Pacific Forest Industries Inc. (Al-Pac) millsite in Northern Alberta (approximate location 54°N 112°W; see chapter 2 for more detail) and native poplar material was collected from seven different clones along highways 36, 875, and 879 near Brooks, Alberta (approximate location 50°N 111°W) (Table 3.1). All collections were carried out in late February and early March 2005, from dormant material. The current year's growth was removed and cut into 10 cm sections that were returned to the University of Alberta and soaked in de-ionized water for 48 hours at 4°C (DesRochers and Thomas 2003). These cuttings were then planted in 20 cm rootrainers (Spencer-Lemaire® Rootrainers; Spencer-Lemaire Industries, Edmonton, AB) containing Metromix® 290 growing media (Terra-Lite 2000 series; WR Grace and Company, Ajax, ON) such that only the top-most bud

remained exposed above the surface of the growing medium (DesRochers and Thomas 2003).

After planting, the trees were placed into a greenhouse maintained at 20/15°C (day/night) with an 18h photoperiod supplemented with artificial lights. The irradiance varied with cloud cover, but was, on average, approximately 450 μmol photons $m^{-2}s^{-1}$ PAR (photosynthetically active radiation) at the pot level. During the first 28 days, plants were fertilized once a week with a 500 ppm solution of 15-30-15 fertilizer (Plant Products Company Ltd., Brampton, ON). For the following 28 days, they were fertilized once every two weeks with a 500 ppm solution of 20-20-20 fertilizer (Plant Products Company Ltd., Brampton, ON).

Trees were arranged in blocks, each with 14 rootrainers. A plastic vapor barrier, 6 mm thick, was placed under each block and secured to the sides of the rootrainers at a height of 20 cm to create a water-holding compartment for the block. Beginning 15 days after planting, trees were watered from below by filling this compartment to a height of approximately 10 cm, or half-way up the rootrainer, with water. No further water was added until the compartment was empty and the surface of the growing media appeared dry. The plants continued to be fertilized from above once every 2 weeks with a 500 ppm solution of 20-20-20 fertilizer (Plant Products Company Ltd., Brampton, ON).

Throughout the course of the experiment, various species of spider mite and thrip were controlled via the introduction of predatory mites. The spider mite eliminator (Applied Bio-nomics Ltd.; Sidney; BC) *Phytoseiulus persimilis* Athias-Henriot and the thrip predatory mite *Amblyseius cucumeris* Oudemans were spread evenly throughout the greenhouse every 2 weeks.

3.2.2 Pathogen culture

Isolates of *S. musiva* were collected from three geographic locations in Quebec (Table 3.2). Eleven of the isolates were received from the University of Laval as single spore cultures in Petri dishes. The eight isolates of *S. musiva* collected by myself were obtained from leaves with *S. musiva* symptoms collected from plantations of native and hybrid poplar. Leaf spots with pycnidia visible under a dissecting microscope were used for isolation and only one leaf spot per leaf was used. Isolations were performed by placing

and then removing approximately 5 µl of sterile distilled water onto a leaf spot with a micro-pipette. This drop of water, containing any conidia that may have been present on the surface of the leaf, was placed onto a Petri-dish containing Corn Meal Agar (CMA; Difco ™ Corn Meal Agar, Franklin Lakes, NJ USA) amended with 300 mg/l of Chloramphenicol (Sigma-Aldrich Inc.™, St. Louis, MO USA) and 25mg/l of Streptomycin (Sigma-Aldrich Inc.™, St. Louis, MO USA). Approximately 1 ml of water was then added and each plate was swirled several times to spread the solution over the surface of the growing media. These plates were then sealed with Parafilm® (Structure Probe Inc., West Chester, PA USA) and placed on a light bench under Gro-Lux© wide spectrum fluorescent bulbs (Sylvania; Osram Gmbh, Munich, Germany) at room temperature where they received 24 hours of light. After 7 days, any sporulating fungal colonies were transferred to a second CMA plate. This process was repeated as necessary to remove all fungal and bacterial contaminants. Once a single fungal colony arose, it was transferred to KV-8 growth media (180 ml V-8 juice, Campbell Soup Company, Camden, NJ, USA; 2g calcium carbonate; 20g agar, Difco ™, Franklin Lakes, NJ, USA; and 820 ml de-ionized water) and allowed to grow until sporulation occurred (Krupinsky 1989). A slide of the sporulating mycelium was then prepared and examined under a compound microscope. The fungi were identified and single spore isolates were created for each colony of *S. musiva* (Thompson 1941; Peck 1884).

The single spore cultures of each isolate were stored at -80°C in cryogenic vials (Nalgene® labware, Rochester, NY USA) containing 300 µL of 50% glycerol and 700 µL potato dextrose broth (PDB; Difco ™ potato dextrose broth, Franklin Lakes, NJ USA). As needed, two vials of each isolate were removed from cold storage and allowed to warm to room temperature. Each tube was then poured onto a petri-dish of KV-8 growth media and sealed with Parafilm®. These plates were then placed on the light bench, described above, for 5 days.

For each isolate, four 5 mm sporulating masses of mycelium were excised from the plate with the most growth and placed on KV-8 growing media. Eight similar plates were created for each isolate. These plates were then sealed and placed on the light bench, described above. After 14 days, the cultures exhibiting the most growth and sporulation were used as inoculum for the experiment.

3.2.3 Experimental design

The experimental design was a randomized complete block with five blocks. Each block was made up of 14 rootrainers each having 40 cavities. No attempt was made to achieve any particular arrangement of clones in the rootrainers; rather the 560 pairs of trees (19 pairs per clone) were randomly dispersed within the blocks. After the initial 56 days of growth, the least robust of the pair was culled leaving 280 trees per block, 20 individuals in each rootrainer, and one empty cavity between individuals in the same row.

Of the seven clones collected in the Brooks area, only two were of the desired native species, *Populus deltoides* Bartr. ex Marsh and the remainder could not be identified to species. Hence the data from these other five clones were discarded. A further 157 individuals were lacking due to insufficient plant material at the Al-Pac millsite. In each of the five blocks 14 clones, from three hybrid groups (Table 3.1), were inoculated with 19 isolates of *S. musiva*, from three geographic areas (Table 3.2). Inoculations occurred in all possible combinations and each clone by isolate combination occurred once per block. Furthermore, one uninoculated control for each clone occurred in each block, giving a total of 1243 trees.

3.2.4 Inoculation

Inoculations were carried out 56 days after the trees had been planted. One Petri dish was used per isolate per block. The plants were inoculated by first removing the third fully extended leaf from the shoot apex (Mottet *et al.* 1991). A 5 mm plug of mycelium, with excess agar removed, was then placed over the wound and wrapped in Parafilm® (Mottet *et al.* 1991). Inoculated controls were treated in the same manner except that sterile KV-8 agar, rather than mycelium, was used. Finally, the Parafilm® was removed from all stems 14 days after inoculation (Weiland *et al.* 2005).

3.2.5 Measurement

Forty-nine days after inoculation, a 30 cm stem section, centered at the inoculation site, was removed. These sections were labeled by clone, isolate, and block number, placed in

paper bags, and stored at 4°C for up to 4 weeks. Disease severity was evaluated on a 1 to 5 scale using a dissecting scope (Table 3.3; Figure 3.3).

3.2.6 Re-isolation

Five cankers from each clone were selected for re-isolation, using a protocol developed by Stanosz and Stanosz (2002). The cankers were soaked in a 5 % NaOCl solution for 2 min and then rinsed in de-ionized H_2O for 30 sec. The periderm was then removed from the symptomatic area, exposing the margin between healthy and necrotic tissue. From this margin, a sliver of necrotic tissue, approximately 3 mm in length, was removed and placed in a Petri dish containing *Septoria musiva* media (SMM; KV-8 growing media amended with Chloramphenicol and Streptomycin at 300 mg/l and 25 mg/l respectively). Four such slivers were placed in each dish, three dishes per canker. Once the fungal colonies had grown to sufficient size they were transferred to plates containing KV-8 media, allowed to grow for two weeks, and then identified (Thompson 1941).

3.2.7 Statistical analysis

All statistical analyses were carried out using the SAS MIXED procedure (SAS Institute 2003) with significance assessed at $\alpha = 0.05$. The initial statistical model for the experiment was:

$$Y_{ijklm} = \mu + B_i + H_j + G_k + C_l(H_j) + I_m(G_k) + C_l(H_j)*I_m(G_k) + \varepsilon_{ijklm}$$

$$i = 1\text{-}5; \quad j = 1\text{-}3; \quad k = 1\text{-}3; \quad l = 1\text{-}14; \quad m = 1\text{-}19$$

where:

Y_{ijklm} = Disease severity for the i^{th} block in which the l^{th} clone nested within the j^{th} hybrid was inoculated with the m^{th} isolate nested within the k^{th} geographic area;
μ = the overall mean;
B_i = the random effect of the i^{th} block;
H_j = the fixed effect of the j^{th} hybrid;
G_k = the random effect of the k^{th} geographic area;
$C_l(H_j)$ = the random effect of the l^{th} clone nested within the j^{th} hybrid;
$I_m(G_k)$ = the random effect of the m^{th} isolate nested within the k^{th} geographic area;

$C_l(H_j)*I_m(G_k)$ = the random effect of the interaction between the l^{th} clone nested within the j^{th} hybrid and the m^{th} isolate nest within the k^{th} geographic area; and

ε_{ijklm} = the residual error.

Beginning with the initial model, non-significant factors were removed; Akaike's Information Criteria (AIC) and a chi-square test of the likelihood ratio were used to choose the most parsimonious model. Because the residual error variances differed among clones, the SAS MIXED procedure was used to model these heterogeneous errors.

The significance of hybrid and geographic area were assessed based on their significance in the final model. However, an additional post hoc test was conducted to determine if the hybridization of resistant *Populus deltoides* Bartr. ex Marsh. with susceptible *Populus laurifolia* Ledeb. x *Populus nigra* L. produced progeny of intermediate resistance. One-tailed tests were used to test the hypotheses that (i) the average disease severity of *P. deltoides* would be less than that of *P. deltoides* x (*P. laurifolia* x *P. nigra)* and (ii) the average disease severity of *P. deltoides* x (*P. laurifolia* x *P. nigra)* would be less than that of *P. laurifolia* x *P. nigra.*

The final model was used to estimate the variance components associated with the clone, isolate, and clone by isolate effects. In addition, the distributions among clone effects within hybrids and among isolate effects within geographic areas were tested for normality and equality of variance. First, the Best Linear Unbiased Predictors (BLUPs) were calculated (SAS Institute 2003) for the different clones and isolates. The Shapiro-Wilk test was then used to evaluate the normality of the distribution of BLUPs for clones within each of the three hybrids and for isolates within each of the three geographic areas. To assess whether the variability among clones within hybrids was the same for the three hybrids, a chi-squared test of the likelihood ratio for two models, clones within hybrids have equal variance and clones within hybrids have unequal variances was conducted. A similar procedure was conducted for the isolates.

For each isolate, the correlation coefficient between (i) the disease severity of that particular isolate on each of the clones, and (ii) the average disease severity of all isolates on each of the clones was calculated. To determine which individual isolates might best predict the average response of individual clones to all of the isolates, the correlations for the different isolates were then ranked. The ranked isolates were then divided into two

groups, "correlated" and "uncorrelated", depending on whether their correlation (based on disease severity across 14 clones) with the average severity induced by all isolates, was greater or lesser than 0.65, respectively. Assuming that the proportion of "correlated" and "uncorrelated" isolates on the landscape is the same as that determined by this experiment it would be possible to use the following equation to calculate the probability of having at least one "correlated" isolate as a function of the number of isolates randomly sampled from the population to test for resistance.

$$p(detect)=1-P^{y}$$

where:

p(detect) = the probability of having at least one "correlated" isolate;
P = the proportion of uncorrelated isolates; 5/19 in this experiment;
y = the number of isolates used to evaluate resistance.

Assuming that the purpose of screening was to identify highly susceptible clones, p(detect) reflects the probability that there would be at least one isolate that would cause relatively severe cankering on any susceptible clone.

3.3 Results

3.3.1 Final model

After the comparison of several models with different combinations of explanatory variables (hybrid, clone, geographic area, isolate, and their interactions) the most parsimonious model was chosen. The model was:

$$Y_{ijkl} = \mu + B_i + H_j + I_k + C_l(H_j) + I_k*H_j + C_l(H_j)*I_k + \varepsilon_{ijkl}$$

$$i = 1\text{-}5; \quad j = 1\text{-}3; \quad k = 1\text{-}19; \quad l = 1\text{-}14$$

where:

Y_{ijkl} = Disease severity for the i^{th} block in which the l^{th} clone nested within the j^{th} hybrid was inoculated with the k^{th} isolate;
μ = the overall mean;
B_i = the random effect of the i^{th} block;
H_j = the fixed effect of the j^{th} hybrid;
I_k = the random effect of the k^{th} isolate;

$C_l(H_j)$ = the random effect of the l^{th} clone nested within the j^{th} hybrid;
I_k*H_j = the random effect of the interaction between the k^{th} isolate and the j^{th} hybrid;
$C_l(H_j)*I_k$ = the random effect of the interaction between the l^{th} clone nested within the j^{th} hybrid and the k^{th} isolate; and
ε_{ijkl} = the residual error.

3.3.2 Hybrid types and Geographic areas

The least squares means of disease severity for the three parent types were 2.39, 3.33, and 2.97 for *P. deltoides*, *P. deltoides* x (*P. laurifolia* x *P. nigra*), and *P. laurifolia* x *P. nigra*, respectively. Although ANOVA did not show these values to be different, one-tailed post-hoc tests indicated that disease severity for *P. deltoides* was significantly less than for *P. deltoides* x (*P. laurifolia* x *P. nigra*) ($p = 0.047$), whereas *P. deltoides* x (*P. laurifolia* x *P. nigra*) and *P. laurifolia* x *P. nigra* were similar ($p = 0.271$). The ANOVA indicated that geographic areas were not significantly different.

3.3.3 Clones (hybrids), isolates, and their interactions

The BLUPs for isolates within geographic areas were normally distributed and did not vary with geographic area ($p = 0.670$).The BLUPs for clones within hybrid types were normally distributed and the chi-squared test of the likelihood ratio found no significant differences in the variances between clones within hybrid types ($p = 0.778$). The ANOVA indicated that there were significant differences between the average disease severities for clones within hybrids ($p = 0.017$, Table 3.4) and for isolates ($p = 0.027$, Table 3.4). The isolate x hybrid interaction was not significant ($p = 1.000$; Table 3.4). The clone x isolate interaction was significant ($p = 0.033$, Table 3.4 Fig 3.1). Of the factors of interest, the clone (hybrid) effect was largest ($\sigma_c^2 = 0.25$; 95% ci = 0.14 – 0.6). The isolate variance ($\sigma_i^2 = 0.06$; 95% ci = 0.12 – 0.04) and clone (hybrid) by isolate variance ($\sigma_{ci}^2 = 0.03$; 95% ci = 0.08 – 0.06) were approximately equal to each other and smaller than the clone (hybrid) variance (Table 3.4).

When the correlation over all 14 clones between the average disease severity following inoculation with all isolates and the average disease severity following inoculation with each individual isolate was calculated, 14 of the 19 isolates had a correlation coefficient greater then 0.65 (Fig 3.2). As a consequence, if screening was

done with five isolates, the probability of at least one of them would be adequately representative of most isolates to identify a susceptible clones would be 0.99 (Fig. 3.3).

3.3.4 Re-isolations

S. musiva was the only plant pathogenic fungus re-isolated from the sampled cankers. *S. musiva* was successfully re-isolated from 35 of the 70 cankers sampled the remaining 35 were colonized by typical laboratory contaminants

3.4 Discussion

3.4.1 Geographic areas and hybrid types

There were no significant differences between the geographic areas for *S. musiva* isolates (Table 3.4). Krupinsky (1989) found similar results, when he tested the aggressiveness of *S. musiva* leaf spot infection using five different clones of hybrid poplar. He found that isolates from different regions were no more aggressive than locally collected isolates (Krupinsky 1989). These results differ from those of Feau *et al.* (2005) who found significant genetic differentiation among two of the three Quebec populations, Thetford-Mines and St-Ours, used in this study (Feau *et al.* 2005). It is important to note that Feau *et al.* (2005) used randomly amplified polymorphic DNA (RAPD), a selectively neutral marker, to test for genetic differentiation among populations. In contrast, this study and that of Krupinsky (1989) used isolate virulence, a highly selected trait, as a measure of variability. Although the results of Feau *et al.* (2005) provide evidence that sexual reproduction and recombination are occurring at the population level, neutral markers cannot detect the effects of these processes on virulence and pathogenicity (Feau *et al.* 2005). Though the St-Ours and Thetford-Mines populations may be sufficiently reproductively isolated to permit genetic drift and limit geneflow (Feau *et al* 2005; McDermott and MacDonald 1993), it appears as if there has not been the necessary selection pressure to produce changes in pathogenicity and virulence.

The discrepancy between the results using neutral markers and those using virulence is difficult to explain. One possible explanation may be that the pattern of virulence on the landscape is the same in the three geographic areas where collections

occurred, consequently no differences in virulence were detected. However, the areas were reproductively isolated and genetic drift to caused differences in neutral markers to occur.

The screening process for *S. musiva* resistance is quite laborious and thus the results described above have important implications. It is not possible to bulk isolates in a spore suspension and expose one clone to multiple isolates because infection rates are extremely low if spores are placed on unwounded tissue (Bier 1941). Rather, clones must be mechanically wounded and inoculated with one isolate at a time as was done in this study. As a consequence, inoculation with multiple isolates would be very onerous. However, if the statistical interaction between isolate and clone is minimal, it would imply that relative clone resistance would be quite similar, irrespective of which isolates were used for screening. If isolates collected from different populations of *S. musiva* have the same range of virulence as isolates collected from one population, then it would be possible to use a range of isolates from one population rather than many more isolates from several populations. The absence of a strong clone x isolate interaction and the lack of variability among populations of this pathogen indicates that a random selection of 5 isolates ought to be sufficient for identifying highly susceptible clones of *P. deltoides*, *P. deltoides* x (*P. laurifolia* x *P. nigra*) and *P. laurifolia* x *P. nigra* hybrid types.

The ANOVA indicated that hybrid types were not significantly different (Table 3.4). The LS means however, demonstrated that the average disease severity of the *P. deltoides* hybrid type was significantly less than the *P. deltoides* x (*P. laurifolia* x *P. nigra*) and *P. laurifolia* x *P. nigra* hybrid types, which were similar. It is widely accepted that *P. deltoides* is resistant to Septoria canker (Ostry and McNabb 1985) and that *P. deltoides* x (*P. laurifolia* x *P. nigra*) and *P. laurifolia* x *P. nigra* hybrids are susceptible (Bier 1941; Waterman 1954). Hypothetically, the hybridization of susceptible and resistant *Populus* species should confer some of the resistance to the offspring; however, this has not been the case. In fact, Newcombe and Ostry (2001) found that the F1 hybrid progeny of *Septoria* resistant *P. deltoides* and susceptible *P. trichocarpa* Torr. and Gray parents, were no more resistant to stem cankers than were the susceptible *P. trichocarpa* parent. Given these results it is not surprising that *P. deltoides* resistance was absent in the *P. deltoides* x (*P. laurifolia* x *P. nigra*) hybrid. The results for the relative

susceptibility of the three hybrid types are likely quite robust since the absence of an isolate x hybrid interaction indicates that the relative disease severity on these hybrid types would be independent of the isolates to which they were exposed.

3.4.2 Clones (hybrids), isolates, and their interactions

This study found that clones (hybrid) were significantly different ($p = 0.017$) in their disease severity rating, and that the majority of the explained variation in this model was accounted for by the clone (hybrid) effect (13%) (Table 3.4). The clonal variability demonstrated in this study (Table 3.4; Fig. 3.1) is typical of what has been demonstrated by others (Bier 1939; Waterman 1954; Filer *et al.* 1971; Cooper and Filer 1976; Long and Bowersox 1985; Ostry and McNabb 1985; Spielman 1986; Strobl and Fraser 1989 Mottet *et al.* 1991; Newcombe 1998). The variability among hybrid types (Fig. 3.1) and the large estimate of clone (hybrid) variance (Table 3.4) indicate that the selection process for resistance should focus on clone rather than hybrid type. Furthermore, other desirable traits such as form, fiber length, wood density, wood chemistry, growth rate, or rooting ability also vary greatly among clones (Dickman *et al.* 2001). The variation in Septoria canker resistance described above would allow for the incorporation of other desirable traits while maintaining disease resistance as long as there was no negative correlation between disease resistance and these traits.

The ANOVA indicated that the 19 isolates studied were significantly different ($p = 0.027$) and that the isolate effect accounted for 3 % of the variation explained by the model (Table 3.4). The relatively small amount of variation among isolates and the lack of a large clone by isolate interaction simplifies the screening process (Table 3.4). A small amount of variation assumes that a small number of isolates could be representative of the total variation present in the pathogen population and the lack of a strong clone by isolate interaction indicates that the relative ranking of clones in terms of resistance will remain approximately the same regardless of which isolates are used in the screening process. The question remains as to how many isolates should be used to screen for *S. musiva* resistance.

Based on correlation with the average results of all isolates, there appeared to be, two distinct groups of isolates (Fig 3.2). "Correlated" isolates resulted in a similar pattern

of disease severity over the tested clones as would have been obtained by using the average of all isolates; in contrast, "un-correlated" isolates behaved differently than did the average of all isolates. One might consider isolates L1-L3 to be "uncorrelated" and the remainder to be "correlated". However, to further minimize the probability of selecting only uncorrelated isolates in resistance screening, isolates L4 and S1 were also included in the "uncorrelated" group, making 0.65 the cutoff correlation value between the two groups. It is clear from Fig. 3.3 that five isolates are necessary to minimize the chance that only uncorrelated isolates are used in the screening process. The confidence that a relatively small number of isolates are required for screening will enable a reduction in the area, host tissue, and labor needed to reliably identify highly susceptible clones, while still exposing clones to isolates which are representative of the variation present on the landscape.

The clone by isolate interaction in this model was significant ($p = 0.033$) and accounted for 4 % of the variation (Table 3.4), approximately one third of the variability accounted for by the clone (hybrid) effect. This interaction is not as strong ($p = 0.033$) as one would expect to encounter in a gene for gene pathosystem such as some of the rusts. The high correlation between isolates (16 of 19 isolates $R^2 > 0.65$) is further evidence of a lack of a strong clone x isolate interaction. Theoretically, isolates from a pathosystem with strong clone x isolate interactions would be poorly correlated because differences between host responses among clones would vary greatly depending on the isolate with which they were challenged. The lack of a strong clone x isolate interaction has important implications for pathosystem stability, since pathosystems lacking strong clone x isolate interactions are more stable then those with strong interactions (Robinson 1976).

Constant ranking refers to how host resistance changes when exposed to different isolates. In a pathosystem exhibiting constant ranking, the difference in disease severity between different hosts will be the same irrespective of which isolate causes infection (Robinson 1976). The most resistant host will always be the most resistant (Robinson 1976). In a pathosystem with a strong clone x isolate interaction, this constant ranking is not present (Robinson 1976). Rather, the differences in disease severity between different hosts depend on which isolate infects the host. In other words, the most resistant host when infected with one isolate may actually be the most susceptible when infected by a

different isolate (Robinson 1976). Thus, a pathosystem with constant ranking would be more stable in terms of resistance than one without.

There is however, an important caveat to the apparent stability of this pathosystem. It is important to remember that the clone x isolate interaction was significant. A significant clone x isolate interaction implies that there is a certain degree of specificity between a host and a pathogen. The presence of this significant interaction allows for the possibility that an extremely virulent isolate of *S. musiva* may arise on the landscape and cause plantation failure if only one or a small number of clones are deployed. It is important therefore, to develop a comprehensive disease management strategy as part of a breeding program for screening clones for resistance and subsequently choosing an appropriate number of clones to plant on the landscape to minimize the risk of plantation failure.

Table 3.1.Origin and hybrid type of inoculated clones. The prefix of the clone label refers to the hybrid type (D= *P. deltoides*, DxLxN = *P. deltoides* x (*P. laurifolia* x *P. nigra*), and LxN = *P. laurifolia* x *P. nigra*). AP number is the Al-Pac number given to each clone in their breeding program. Clone name is the common name of each clone. Clones collected in the Brooks area (50°N 111°W), which could not be positively identified as *P.deltoides,* are not included in this table.

Clone label	AP number	Clone name	Female parent	Male parent	Legal location
D1	-	*P. deltoides*	*P. deltoides*	*P. deltoides*	50°N 111°W
D2	-	*P. deltoides*	*P. deltoides*	*P. deltoides*	50°N 111°W
DxLxN 1	AP 24	Walker	*P. deltoides*	*P. x petrowskyana*[a]	54°N 112°W
DxLxN 2	AP 2400	Hill	*P. deltoides*	*P. x petrowskyana*	54°N 112°W
DxLxN 3	AP 36	Brooks #1	*P. deltoides*	*P. x petrowskyana*	54°N 112°W
DxLxN 4	AP 2385	Brooks #2	*P. deltoides*	*P. x petrowskyana*	54°N 112°W
DxLxN 5	AP 2386	Brooks #4	*P. deltoides*	*P. x petrowskyana*	54°N 112°W
DxLxN 6	AP 2387	Brooks #5	*P. deltoides*	*P. x petrowskyana*	54°N 112°W
DxLxN 7	AP 794	Brooks #6	*P. deltoides*	*P. x petrowskyana*	54°N 112°W
LxN 1	AP 42	TACN 1	*P. laurifolia*	*P. nigra*	54°N 112°W
LxN 2	AP 51	Dunlop	*P. laurifolia*	*P. nigra*	54°N 112°W
LxN 3	AP 2383	P. x berolinensis	*P. laurifolia*	*P. nigra*	54°N 112°W
LxN 4	AP 2395	Russian	*P. laurifolia*	*P. nigra*	54°N 112°W
LxN 5	AP 2410	P. x berolinensis	*P. laurifolia*	*P. nigra*	54°N 112°W

[a] *P. x petrowskyana* = *P. laurifolia* x *P. nigra*

3.2. Origin of isolates. The prefix of the isolate label refers to the geographic area. Host indicates whether the isolate was collected on native or hybrid poplar. Quebec designation is the collection number given to isolates received from the University of Laval. Locations are approximate and based on location of nearest distinguishing landmark.

Isolate label	Host	Quebec designation	lat/long	Geographic area	Collector
L1	hybrid	SO4.43	46°N 71°W	Lotbinière	D. Lamontagne
L2	hybrid	SO4.24	46°N 71°W	Lotbinière	D. Lamontagne
L3	hybrid	SO4.26	46°N 71°W	Lotbinière	D. Lamontagne
L4	hybrid	SO4.48	46°N 71°W	Lotbinière	D. Lamontagne
L5	native	-	46°N 71°W	Lotbinière	J. LeBoldus
L6	native	-	46°N 71°W	Lotbinière	J. LeBoldus
L7	native	-	46°N 711°W	Lotbinière	J. LeBoldus
L8	native	-	46°N 711°W	Lotbinière	J. LeBoldus
L9	native	-	46°N 71°W	Lotbinière	J. LeBoldus
L10	hybrid	-	46°N 70°W	Lotbinière	J. LeBoldus
L11	hybrid	-	46°N 70°W	Lotbinière	J. LeBoldus
L12	native	-	46°N 71°W	Lotbinière	J. LeBoldus
S1	hybrid	SO7.02	45°N 73°W	St-Ours	N. Feau
S2	native	S49.02	45°N 73°W	St-Ours	N. Feau
S3	native	SO3002	45°N 73°W	St-Ours	N. Feau
S4	hybrid	SO2202	45°N 73°W	St-Ours	N. Feau
T1	hybrid	TH1502	46°N 71°W	Thetford mines	N. Feau
T2	hybrid	TH0802	46°N 71°W	Thetford mines	N. Feau
T3	hybrid	TH 0102	46°N 71°W	Thetford mines	N. Feau

Table 3.3. Disease severity classes and the description of their associated symptoms.

Class	Description
1	Wound healed with no callus development
2	Callus
3	Necrosis contained by callus
4	Necrosis extending beyond callus
5	Complete encirclement

Table 3.4. Analysis of variance with variance components partitioned among sources. CI refers to the 95% confidence intervals for the variance estimates.

Source	df	P	Variance estimate	% of Variance	95 % CI
Block	4	-	0.01	1	-
Hybrid (H)[a]	2	0.117	-	-	-
Clone (C) (C(H))	11	0.017	0.25	13	0.14 - 0.60
Isolate (I)	18	0.027	0.06	3	0.04 - 0.12
C(H) x I	234	0.033	0.07	4	0.06 - 0.08
I x H	36	1.000	0.00	0	-
Residual error	867	-	1.55	79	-
Total	**1172**	**-**	**-**	**100**	**-**

[a] Hybrid was a fixed effect and thus there was no variance estimate.

Fig. 3.1. Average disease severity for each clone x isolate combination. Disease severity was ranked on a 1 – 5 scale (no disease – completely girdled). The size of the black dot represents the average disease severity for each clone by isolate combination. The prefix for clone number indicates the hybrid type. The prefix for isolate number indicates the collection location. Hybrid types were ranked by increasing average disease severity from left to right. The isolate number "Average" indicates the average disease severity for each hybrid type across all isolates.

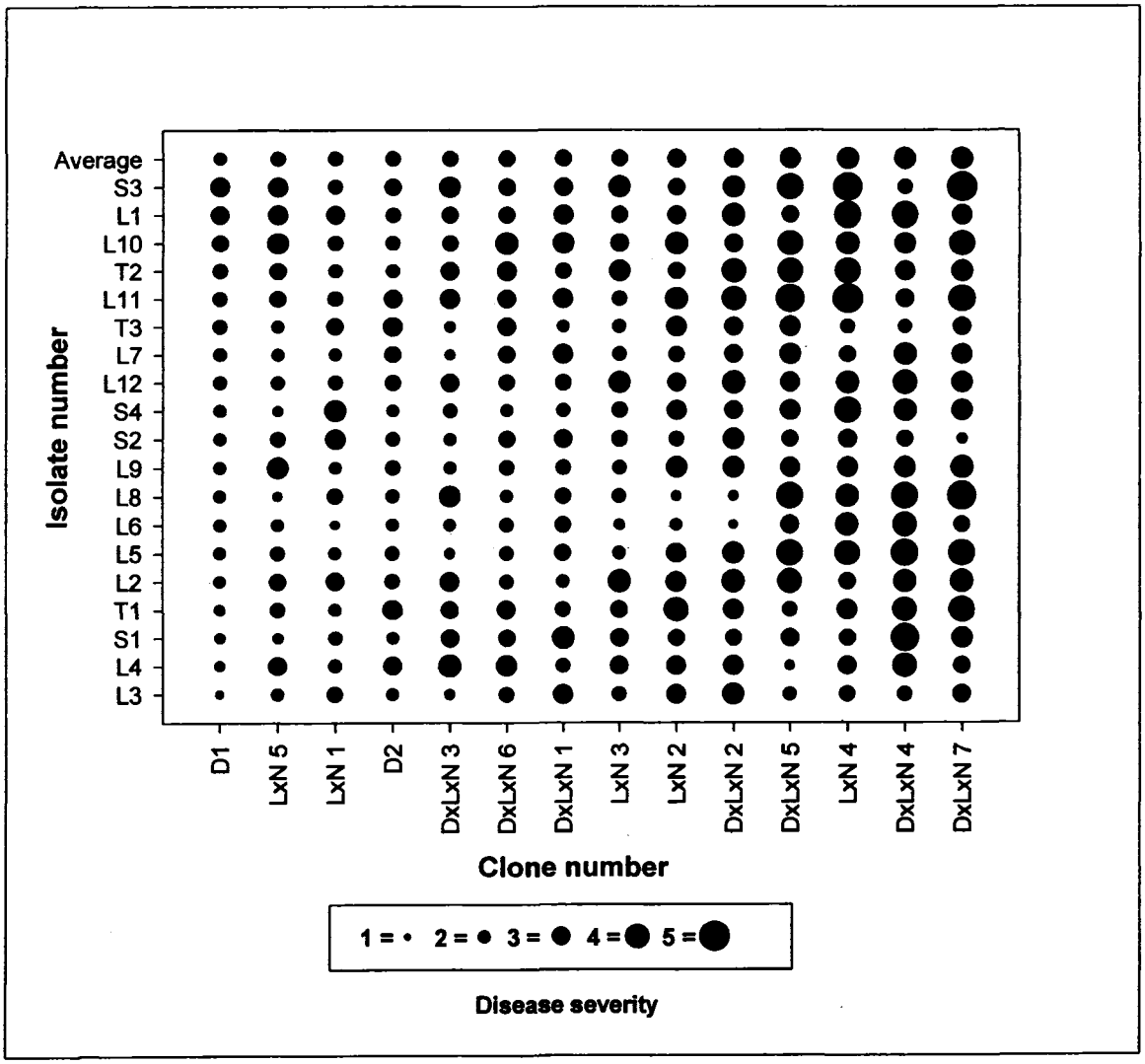


Fig. 3.2. Correlation coefficients over 14 clones between the average disease severity for each of 19 isolates and the average disease severity for all isolates. For example, the correlation based on n = 14 clones, between the average disease severity for all isolates and the disease severity of isolate S3, was 0.80.

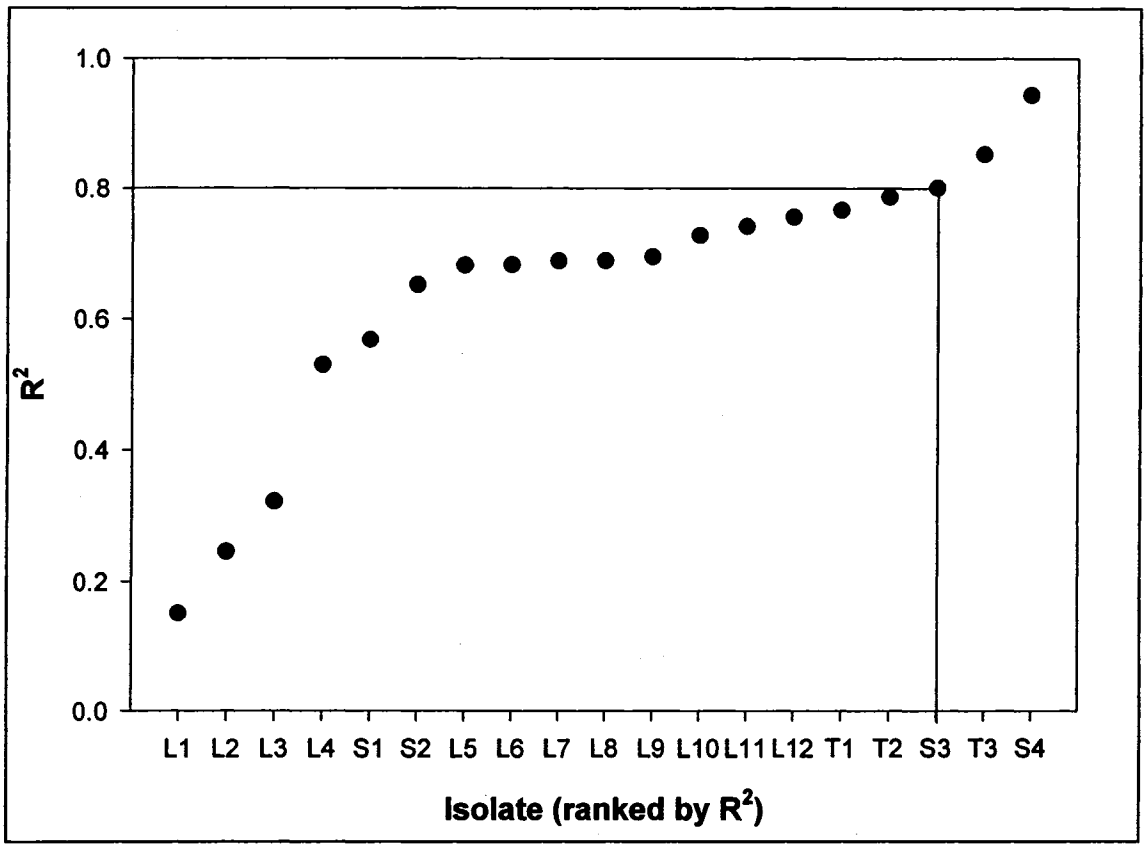
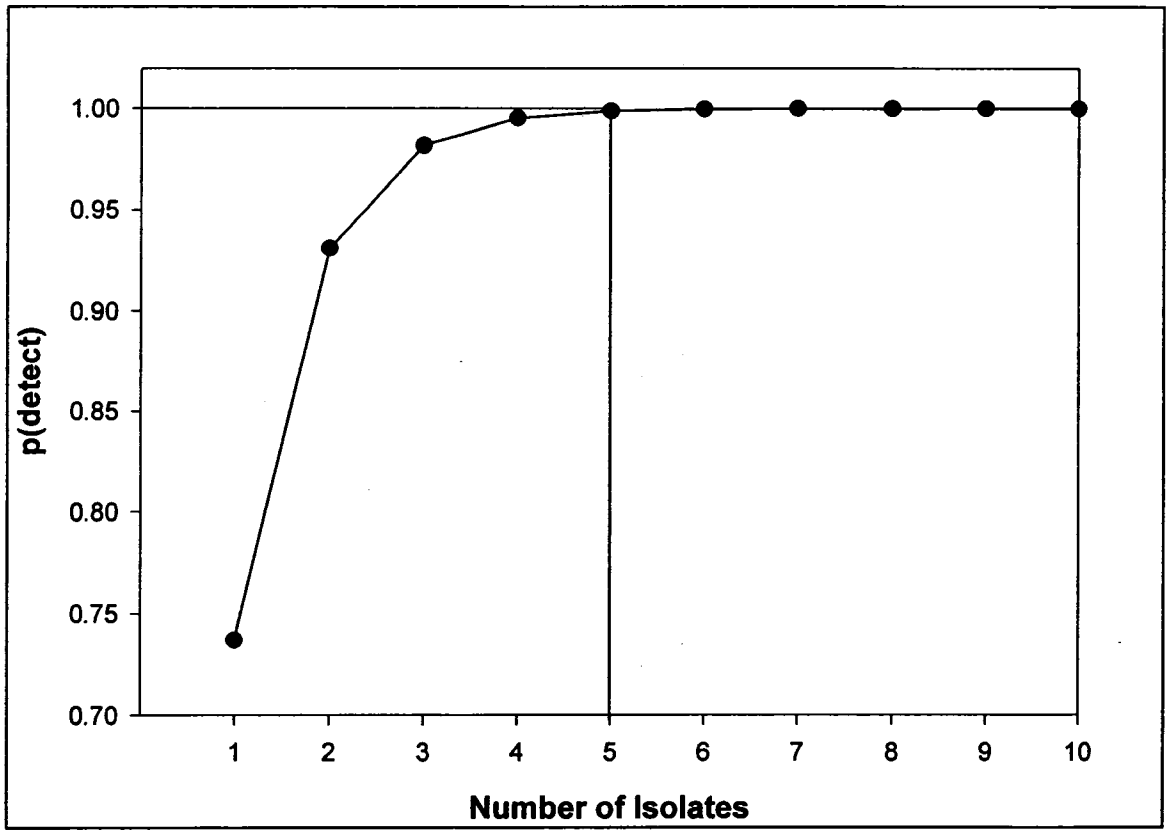


Fig. 3.3. Probability of choosing at least one correlated isolate as the number of chosen isolates increases. P(detect) is the probability of choosing at least one correlated isolate when the proportion of uncorrelated isolates is 5/19. For example, with five isolates the probability of choosing at least one correlated isolate is 0.99.



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Chapter 4: General Discussion and Conclusions

4.1 Introduction

The increase in short rotation high yield hybrid poplar plantation culture is being driven by the anticipation of shortages in forest products and the search for alternative energy sources (Phelps 1983). Unfortunately, there are several diseases of hybrid poplar which can greatly affect yield in these plantations. Of these diseases, Melampsora rust and Septoria canker are of greatest concern (Newcombe 1996). Due to environmental conditions, length of the growing season, and historical records, *Septoria musiva* Peck., the causal agent of Septoria canker, is likely to be the greatest threat (Bier 1939; Crane 2002 "Poplar leaf rusts in central Alberta – assessing the risks to agroforestry" an unpublished final report to Alberta-Pacific Forest Industries Inc.). To reduce the risk of this threat, an integrated approach to disease management is essential. This approach should combine information on the host population, pathogen population, and the interaction between the two to develop an integrated pathosystem management strategy.

4.2 Traditional disease management

4.2.1 Greenhouse screening for disease resistance

The identification of resistant individuals is essential to any disease management strategy. Often the first step in this screening process is conducted under greenhouse conditions. However, to evaluate the reliability of these results under field conditions, information on the effect of environmental variation on disease severity is essential. One way to evaluate the effect of environmental conditions on disease severity is to evaluate the clone x isolate x environment, clone x environment, and isolate x environment interactions. The size of these interactions will indicate the degree to which differences in disease severity among clones will remain constant irrespective of environmental conditions. The water stress experiment described in Chapter 2 was designed to evaluate these interactions and determine the feasibility of greenhouse screening as a preliminary means of estimating resistance in the field.

The results described in Chapter 2 indicate that neither the three-way nor the two-way interactions between environment and clone, isolate, and the clone x isolate interaction, were significant. Similar results were published by Maxwell *et al.* (1997), who found no significant interaction between clone and water stress. The combination of these results implies that rankings of clonal resistance from greenhouse trials should be reasonably robust across varying environmental conditions. This is not to say that field trials can be eliminated, but rather that greenhouse inoculations are a good place to begin searching for resistant clones. This process would save time and money by removing highly susceptible clones from the breeding program and concentrating efforts on those clones which show good preliminary resistance in the greenhouse.

It is important to note that the question still remains as to what effect tree age has on disease resistance. How robust will the results from the inoculation of young trees be when compared to long term field trials? Fortunately, Weiland *et al.* (2003) compared greenhouse resistance ratings with long term field resistance of 15 clones and found that damage categories assigned to the clones in the greenhouse matched the long term field data 14 out of 15 times. Their analysis also suggested that reliable prediction of long-term field performance was the most accurate for clones at the extremes of field performance (Weiland *et al.* 2003). Weiland's *et al.* (2003) study is further evidence that greenhouse screening can function as an efficient means of culling highly susceptible individuals from a breeding program before the expensive process of field testing begins, while remaining confident that those individuals will not become more susceptible with age or under a different set of environmental conditions.

One final piece of evidence supporting this statement is the relative success of other resistance screening programs that have used greenhouse inoculations for preliminary evaluation. Two successful programs are the *S. musiva* resistance screening program in Quebec (Newcombe *et al.* 2001) and the fusiform rust (*Cronartium quercuum* Berk. miyabe Shirai f. sp. *Fusiforme*) resistance program in the southern United States (Carson and Young 1987). Both of these programs have used greenhouse screening to select resistant individuals for incorporation into field trials and breeding programs, and in both cases the disease incidence has not increased since the introduction of resistant clones (Carson and Young 1987; Newcombe *et al.* 2001).

4.2.2 Isolate selection

The results described in Chapter 3 as well as the studies conducted by Krupinsky (1989) and Strobl (1992) indicate that the relative resistance rankings of clones would remain the same irrespective of the isolates used for inoculation. These data, in combination with the potential for reduced cost associated with using a small number of isolates, suggest that forest companies should determine a minimal number of isolates necessary for screening. There are two possible strategies which forest companies may use to achieve this goal. The first would involve an experiment similar to that described in Chapter 3, using clones planned for deployment on the landscape and a random sample of isolates from their region in which they are going to develop a program. Once the results from such an experiment had been collected, it would be possible to examine the correlation over all clones between the average disease severity following inoculation with all isolates and the average disease severity following inoculation with each individual isolate. Then, a forest company or commercial user would choose only the most highly correlated isolates to screen for resistance.

The second option would be for companies to assume that the proportion of correlated and uncorrelated isolates in their planning area is the same as that determined in Chapter 3. Using these proportions and the equation outlined in Chapter 3, it would be possible to calculate the probability of picking only uncorrelated isolates as the number of isolates used in the screening process decreases. Depending on the level of risk a company is willing to take, they could pick a corresponding number of isolates. Finally, there is also the possibility of using some combination of these two strategies to pick the number of isolates to be used in the screening process.

4.2.3 Stability of resistance

One final question remaining to plantation managers is the stability of resistance through time. This is particularly true of diseases like Septoria canker which have a sexual stage in their life-cycle (Bier 1939). This stage allows for recombination and the appearance of new combinations of virulence genes on the landscape. The ability to predict how resistance might change as these new isolates appear is essential to plantation managers

and can be undertaken by analyzing the interaction between the host and pathogen populations.

Theoretically, stability should be dependent upon the degree of specificity between individuals in the host and pathogen populations (Robinson 1976). A stable pathosystem is one where differences in disease severity among clones are similar, irrespective of the isolates used for inoculation (Robinson 1976). Though the average disease severity may increase, the most resistant clone will remain so (Robinson 1976). If, on the other hand, clonal resistance rankings varied with the isolate used for inoculation, the pathosystem would be far less stable (Robinson 1976). In this case, the most resistant clone when inoculated with one isolate might become the most susceptible when inoculated with a different isolate. This situation is inherently unstable since the appearance of new isolates could change the relative resistance of clones.

In this study, the clone (hybrid) x isolate interaction was significant but accounted for only a small amount of the explained model variation. This evidence supports the hypothesis that resistance should be relatively stable through time for this pathosystem. Similar analyses as those described in chapter 3 would allow plantation managers to determine the consequences of their clone selection process.

4.3 Pathosystem management

The analyses described above demonstrate how the results from resistance screening experiments can help plantation managers improve disease resistance in their plantations. Ultimately, it is the combination of data on the clone, isolate, and clone x isolate interactions that will likely be the most powerful tools plantation managers have at their disposal. For example, once resistant clones have been identified, the question still remains as to how many should be planted on the landscape to minimize the risk of plantation failure. This question has, to date, been addressed through various risk assessment models (Roberds and Bishir 1997). However, a simpler approach may be to use variance component estimates generated through large scale screening trials similar to those described in chapter 3. The variance estimates for the clone, isolate, and clone x isolate interaction in combination with the following equation would allow one to

calculate the variance of the means for different combinations of clones, isolates, and their interactions.

$$\sigma^2_{\bar{Y}..} = \frac{\sigma_C^2}{C} + \frac{\sigma_I^2}{I} + \frac{\sigma_{CI}^2}{CI}$$

It would then be possible to calculate the probability of exceeding a threshold level of disease severity when different numbers of clones are deployed. Furthermore, a sensitivity analysis could be conducted to determine how changing the number of isolates would impact the probability of exceeding the threshold level of disease severity. This step could be quite important since the number of isolates that any given clone is exposed to is unknown.

For predictions from this approach to be considered reliable there are two assumptions which need to be met. First, the data must be normally distributed with equal variances. The second assumption deals with the inference space of predictions based on this equation. Until the variation among hybrids has been thoroughly examined, predictions should not be extrapolated to clones of hybrids not used to calculate variance components. For example, using the data from the analysis in chapter 3 it would be reasonable to make predictions for clones of *Populus deltoides* Bartr. ex Marsh x (*Populus laurifolia* Ledeb. x *Populus nigra* L.) or *P. laurifolia* x *P. nigra*; however, extrapolations of these predictions to include hybrids of *P. deltoides* x *Populus balsamifera* L. should not be undertaken. This cautionary note is important given that the distribution of resistance responses of different hybrid types is unknown and needs to be explored.

4.4 Future research

In comparison to many of the other economically important pathosystems, such as white pine blister rust or chestnut blight, very little research has been conducted on the epidemiology of *S. musiva*. A review of the literature has demonstrated many areas where research needs to be conducted. For example, it is unclear how this pathogen penetrates host tissue and causes cankers to form on mature clones. Environmental variation appears

to play a role in disease development; however, it is unclear exactly what that role is. Furthermore, how variation in edaphic and environmental conditions may alter Septoria canker susceptibility and which of these factors are the most important need to be determined. This study has also illuminated several areas where research needs to be conducted. For instance, how is resistance conferred to the progeny of hybrid parents, how is pathogenicity inherited in the pathogen, and how do the population dynamics of this pathogen affect risk of plantation failure? Finally, once some of these questions have been answered, the results can be incorporated into risk assessment models, similar to those described by Roberds and Bishir (1997), to help plantation managers choose the most appropriate number of clones to plant on the landscape.

4.5 Conclusion

In this study, two experiments were conducted to help understand the *S. musiva*–hybrid poplar pathosystem. The first experiment demonstrated that no significant interactions existed between water stress and either clone, isolate, or their interaction, thus indicating that greenhouse results should be reasonably robust under field conditions. The second experiment indicated that clones, rather than hybrid types, should be the focus of a disease management strategy and that a small number of isolates from a single geographic location should be sufficient to screen hybrid poplar clones for *S. musiva* resistance. Finally, the small but significant contribution of the clone x isolate interaction to the pathosystem indicates that resistance is relatively stable and that the most resistant clones should remain so irrespective of which isolates appear on the landscape.

The hope of this researcher is that the general pathosystem management framework developed in this study will be used by plantation managers to focus the disease resistance aspect of their breeding programs. This strategy will incorporate information on the host, the pathogen, and the interaction between the two to chose and deploy an appropriate number of resistant clones on the landscape while developing an understanding of the pathosystem as a whole. It is, in fact, this understanding that is essential to the development of a comprehensive and dynamic disease management strategy whether these trees are used for fiber production, shelterbelts, reclamation or ornamentals.

4.6 Literature cited

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