University of Alberta

Survival of lodgepole pine trees following infection by western gall rust

by

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ABSTRACT

The survival of lodgepole pine [*Pinus contorta* Dougl. ex Loud.] following infection by western gall rust (WGR), *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka was evaluated. An 11-year survival function predicted that galls encircling >80% of the stem circumference increased the risk of mortality relative to non-galled trees; survival was similar for infections occurring on the main stem and those growing into the stem from a nearby branch infection. The application of an earlier model of gall expansion predicted that 38-43% of stem-galled trees would survive until age 80. The hydraulic conductivity (K_ψ; $m^2s^{-1}Pa^{-1}$) through stem galls encircling between 0 and 100% of the stem, the leaf area (A_L), sapwood area (A_S), and foliar nitrogen [N] above galls were measured on 12-year-old trees in the field. The K_ψ was reduced proportionally with gall encirclement. The A_L, A_S and foliar [N] decreased with increasing gall encirclement and decreasing K_ψ, resulting in the leaf-area-to-sapwood area ratio (A_L:A_S) remaining constant across gall encirclement and K_ψ values. Xylem and phloem wounds were applied to 13-year-old galled and non-galled trees in the field on either the galled or non-galled side of trees. Although galls and xylem wounds reduced K_ψ, tree growth was unaffected. Trees inoculated with WGR in the greenhouse had root-to-shoot-ratios (R:S) similar to control trees; however, the coarse root concentration of total non-structural carbohydrates [TNC] was lower in galled than control trees, suggesting that galls also disrupt phloem transport to the roots. The whole-tree transpiration (E) of greenhouse inoculated galled and control trees was constant until the soil water content (WC) reached 11%, after which E decreased linearly with decreasing soil WC. Under well-watered conditions galled trees reduced A_L relative to control trees as a consequence of reductions

in K_ψ (g H_2O cm^{-1} MPa^{-1}). However, the reduced A_L of galled trees did not compensate for the lower K_ψ values, and as a consequence the leaf specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}) was lower in galled than in control trees. The K_ψ, Q_L, and E of galled and control trees were similar under water-limited conditions, indicating that the limitation to water flow was belowground.

DEDICATION

I dedicate this dissertation to my husband Gabriel Wolken. It was Gabriel's support which enabled me to stay on course throughout my academic career, and which also allowed me to get off course to enjoy many adventures together!

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1 CHAPTER ONE

Introduction

1.1 Background

The tree species lodgepole pine [*Pinus contorta*] is comprised of four geographical varieties: *P. contorta* var. *contorta,* the coastal form; *P. contorta* var. *bolanderi,* a Mendocino County White Plains form in California; *P. contorta* var. *murrayana* in the Sierra Nevada; and *P. contorta* var. *latifolia,* the inland form (Lotan and Critchfield 1991). In Canada, lodgepole pine [*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.] extends from the Yukon, through the interior of British Columbia and western Alberta, and southward (Farrar 1995). Lodgepole pine accounts for 15 percent of the mature standing timber in British Columbia and Alberta, and represents 20 and 40 percent of the annual timber harvested in British Columbia and Alberta, respectively (Kennedy 1985).

Lodgepole pine is susceptible to infection by several species of stem rusts: stalactiform blister rust [*Cronartium coleosporioides* Arthur]; comandra blister rust [*Cronartium comandrae* Peck]; sweet fern blister rust [*Cronartium comptoniae* Arthur]; and western gall rust (WGR) [*Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka] (Hiratsuka et al. 1995). In western Canada, WGR is considered the most destructive stem rust of hard pines (Ziller 1974), and is the most common disease in the interior of British Columbia (van der Kamp and Spence 1987). WGR is distributed from Nova Scotia to the Yukon in Canada; it extends southward in the east through New York, Pennsylvania, West Virginia, and Virginia, and in the west to Arizona and northern Mexico (Hiratsuka et al. 1995). The native hosts of WGR in Canada are lodgepole pine, jack pine [*Pinus banksiana* Lamb.], and ponderosa pine [*Pinus ponderosa* Laws.]; WGR has also been

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found on the following introduced pines: mugo pine [*Pinus mugo* Turra var. *mughus* Zenari], Austrian pine [*Pinus nigra* Arnold], Bishop pine [*Pinus muricata* D. Don.], maritime pine [*Pinus pinaster* Ait.], Monterey pine [*Pinus radiata* D. Don.], and Scots pine [*Pinus sylvestris* L.].

1.2   Taxonomy, symptoms and gall development of western gall rust

WGR was previously referred to by its asexual name, *Peridermium harknessii* J.P. Moore), and was renamed *Endocronartium*, as Hiratsuka (1969) proposed that WGR had an endocyclic life cycle. The endocyclic life cycle means that the teliospores morphologically resemble aeciospores (Alexopoulos et al. 1996). In contrast to the other rust fungi in Canada, the autoecious life cycle of WGR enables it to infect pine trees directly without an alternate host (Hiratsuka et al. 1995; Ziller 1974). Aeciospores are produced on the surface of galls from the end of May until July, at which time the spores become airborne (Hiratsuka et al. 1995). Infection occurs through the epidermis of the current year's expanding shoots (True 1938). The frequency of infection varies from year-to-year; however, the majority of infections are concentrated in a few years of high infection referred to as wave years (Peterson 1971).

External symptom development following infection is variable and may or may not occur prior to gall formation (Allen et al. 1990). Red pigmentation of the epidermal cells 14 to 28 days following infection is the first visible response to infection (Allen et al. 1990); infection spots appear in July under field conditions (True 1938). The vascular cambium may be infected as early as 21 days following infection, resulting in the formation of distorted xylem tracheids characteristic of galled tissue (Allen et al. 1990). The cambium and ray initials of WGR infected tissue are stimulated to produce

abnormally large numbers of cells (Peterson 1960); the tracheids of galled tissues are shorter and highly branched in comparison to normal tracheids (Zalasky 1976). Distinct spherical or hemispherical gall development occurs in the second growing season following infection (True 1938) on both the main stem and lateral branches (Hiratsuka and Powell 1976).

1.3 Management implications of western gall rust infected lodgepole pine stands

Site disturbances such as wildfire or logging often result in high density stands of lodgepole pine (Johnstone 1981a, 1981b; 2002). Precommercial thinning increases diameter growth, and reduces mortality loss from inter-tree competition (Johnstone 1981a). WGR is an inefficient natural thinning agent as it kills trees of all sizes and infected trees die slowly (van der Kamp 1988). Delaying thinning has been proposed in WGR-infected stands (Blenis and Duncan 1997), as larger trees are more susceptible than smaller trees to infection (Bella 1985), and the likelihood of infection decreases with tree age (Bella and Navratil 1988; Blenis and Li 2005). Hills et al. (1994) recommended that stocking targets be increased to compensate for future mortality, and that thinning operations should target trees with main stem galls and/or numerous branch galls regardless of tree size.

Branch galls result in the eventual death of the distal portions of branches, whereas stem galls usually result in tree mortality (van der Kamp and Spence 1987; van der Kamp 1988). Stem and branch galls were common in young jack pine stands, while in older stands the frequency of stem galls was low, indicating that stem-galled trees may die as stands age (Gross 1983). However, mortality of trees with small stem galls may not occur as the rate of lateral gall expansion is low (Peterson 1960), and sixty percent of

stem galls were determined to encircle less than 40 percent of the stem (Blenis and Duncan 1997). The only estimate of galled tree survival was based on the assumption that 50 percent of trees with WGR infections on the stem or within 10 cm of the stem would survive (Woods et al. 2000).

1.4   Potential causes of galled tree mortality

Potential mechanisms that could account for the mortality of galled trees relative to non-galled trees include: i) stem breakage at the gall, which is a common occurrence 20 years following infection (Gross 1983; Zagory and Libby 1985; van der Kamp 1988) due to the low mechanical strength of the galled tissue (Peterson 1960); ii) invasion by secondary fungi; WGR-infected tissues were more susceptible to infection by secondary fungi than were non-galled tissues (Byler et al. 1972a). Although secondary pathogenic fungi may reduce rust inoculum by killing galls, *Nectria fuckeliana* Booth was reported to move from dead galled tissues to invade and kill living, non-galled branches in a small number of cases (Byler et al. 1972b). It is unknown if secondary pathogenic fungi associated with galled tissues result in tree mortality; iii) disrupted phloem transport to roots as suggested for galls on slash pine [*Pinus elliottii* Engelm.] and loblolly pine [*Pinus taeda* L.] caused by fusiform rust *Cronartium quercuum* f. sp. *fusiforme* (MacFall et al. 1994); and iv) disrupted water flow through stem galls as was observed in galls caused by *C. quercuum* (MacFall et al. 1994) due in part to the deformed nature of galled tracheids (Zalasky 1976).

1.5 Whole-tree water relations and tree growth

The terminology describing the water movement through trees is variable, as both an Ohm's Law analogy (Tyree and Ewers 1991), and Darcy's law (Whitehead and Jarvis 1981) have been used. To unify the nomenclature, Reid et al. (2005) proposed the following equation, which is consistent with Darcy's law, to describe the hydraulic conductivity (K_ψ) in the xylem of trees:

$$K_\psi = \frac{Q}{A_S}\frac{L}{\Delta\Psi},$$ (1)

where Q is the mean flow rate of water through a stem sample of length L and conducting area of sapwood A_S, under a water potential difference $\Delta\psi$ expressed in pressure units. To describe the functional relationship between the capacity of the stem to transport water and the distal leaf area (A_L), Reid et al. (2005) proposed the term leaf specific hydraulic capacity (Q_L), which is calculated by substituting A_L for A_S in Eq. 1:

$$Q_L = \frac{Q}{A_L}\frac{L}{\Delta\Psi},$$ (2)

The K_ψ and Q_L terminology defined above was adopted to quantify the ability of galled and non-galled stems to transport water, and supply foliage with water, respectively in subsequent chapters.

The pipe model theory of Shinozaki et al. (1964a,b) considers stems and branches to be an assemblage of unit pipes which support a unit amount of foliage. This model has been used to predict A_L; Long et al. (1981) observed a linear relationship between the cross-sectional area of A_S and the amount of foliage above that point. However, the leaf-area-to-sapwood area ratio (A_L:A_S) varies with such factors as tree size, and stand density (Keane and Weetman 1987). Assuming a linear relationship between A_L and A_S of a tree,

the A_L:A_S may be predicted by the hydraulic formula combining the Penman-Monteith

equation describing transpiration, and Darcy's law in Eq. 1 describing water flow through

porous media (Whitehead and Jarvis 1981; Whitehead et al. 1984):

$$\frac{A_L}{A_S} = \frac{k(\Delta\Psi/l)c}{Dg_s},$$ (3)

where A_L and A_S are the leaf area and sapwood area, respectively, k is the sapwood

permeability (which is K_ψ adjusted for the dynamic viscosity of water); therefore, K_ψ as

described above could be substituted for k, $\Delta\psi/l$ is the water potential gradient through

the system, D is the time-averaged vapour pressure deficit of the atmosphere, and g_s is

stomatal conductance. The c coefficient is equal to:

$$c = \rho_w \gamma \lambda / \eta c_p \rho_a,$$ (4)

where ρ_w, γ, λ, η, c_p, and ρ_a are the density of water, the psychrometric constant, the latent

heat of vaporization of water, the dynamic water viscosity, the specific heat of air at

constant pressure, and the density of air, respectively (Whitehead et al. 1984; Mencuccini

and Grace 1995).

Leaf-specific hydraulic conductance and soil-to-leaf hydraulic conductance (K_L)

are commonly used terms in the tree physiology literature (Hubbard et al. 1999; Ewers et

al. 2000; Phillips et al. 2002; Addington et al. 2004; Addington et al. 2006). Although

the use of the term conductance is not incorrect, Reid et al. (2005) proposed that it not be

used when the K_ψ changes along the entire path length. Accordingly, the K_ψ term was

deemed a more appropriate metric to describe the water movement through galled stems,

as presumably the K_ψ is reduced through galls. The soil-to-leaf hydraulic conductance

(K_L) is defined here due to its frequency in the literature:

$$K_L = \frac{E_L}{(\psi_{soil} - \psi_{leaf} - h\rho_w g)}, \tag{5}$$

where E_L represents the whole-tree transpiration per unit leaf area, ψ_{soil} and ψ_{leaf} are the soil and leaf water potentials (MPa), respectively, and $h\rho_w g$ is the gravitational component of the water column of height h, density ρ_w (density of water at 20˚C = 998.23 kg m^{-3}), and g is the acceleration due to gravity. The effect of gravity results in 0.01 MPa m^{-1} of tree height.

An important resistance to water flow not included in the above equations describing the movement of water in xylem occurs at the root-soil interface (Örlander and Due 1986). The volume percentage of water is lower in coarse than in fine textured soils at the same soil matric potential (McColl 1973), and as a result plants growing in sandy soil may become water stressed at relatively high ψ_{soil} (Hacke et al. 2000). Sperry et al. (1998) determined that the limitation to water movement in plants occurs in the rhizosphere for low root-to-leaf area ratios, coarse textured soils, and species resistant to cavitation; roots are more vulnerable to cavitation than stems and branches (Sperry and Ikeda 1997; Hacke et al. 2000; Oliveras et al. 2003; Stout and Sala 2003).

The hydraulic architecture and physiology of trees are interrelated. Mencuccini (2003) noted that the plant hydraulic conductance varies as a result of both short-term physiological regulation and long-term structural acclimation. The gradient in water potential may be minimized when soil water availability decreases by: 1) increasing the resistance to xylem embolism; 2) decreasing the $A_L{:}A_S$ (Eq. 3); and 3) increasing the sensitivity of stomata to drought (Martínez-Vilalta et al. 2004). Pines are more vulnerable to xylem embolism than other species within the Pinaceae; decreases in the $A_L{:}A_S$ were associated with an increase in the vulnerability to xylem embolism

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(Martínez-Vilalta et al. 2004). Adjustments in the hydraulic architecture are species dependent, as the $A_L:A_S$ decreased with increasing D in *Pinus* species, but not in *Abies*, *Pseudotsuga*, *Tsuga* and *Picea* (DeLucia et al. 2000). Long-term environmental conditions may also alter the $A_L:A_S$, as Scots pine trees at a warmer and drier site had lower $A_L:A_S$ than trees at a cooler and wetter site (Mencuccini and Grace 1995). In addition, *P. palustris* trees on a xeric site were shorter and had higher root-to-leaf-area ratios relative to trees on a mesic site, resulting in similar K_L values between the two sites (Addington et al. 2006). The $A_L:A_S$ decreases with increasing tree height for the majority of tree species to compensate for the reduced hydraulic conductance associated with taller trees (McDowell et al. 2002). Although increases in the $A_L:A_S$ with increasing tree height have been reported for *Picea abies* (McDowell et al. 2002; Köstner et al. 2002), and *Abies balsamea* (McDowell et al. 2002), the $A_L:A_S$ of lodgepole pine trees will likely decrease, as early successional, and shade-intolerant species are less likely to compensate for the hydraulic limitations on the supply of water to foliage (Sala 2006).

Stomatal conductance is closely linked to plant hydraulic conductance (Addington et al. 2004; Franks 2004; Hubbard et al. 2001). In the short term, rapid changes in stomatal conductance may occur in response to changes in the water potential gradient from the soil to the leaf (Whitehead 1998). However, the greater vulnerability to xylem embolism of species that grow in dry habitats may be partially compensated for by increased stomatal control over water loss (Piñol and Sala 2000). For example, stomatal closure in response to drought maintained the leaf water potential (Ψ_{leaf}) above -1.5 MPa in *P. sylvestris* (Irvine et al. 1998), and above -2.0 MPa in *P. pinaster* Ait. (Delzon et al. 2004).

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1.6 Plant pathogens, growth and physiological functioning of trees

In addition to factors such as tree age and environmental conditions, the growth and physiological functioning of trees may be altered by plant pathogens. The following are examples of forest pathogens of coniferous tree species: the rust fungus *Cronartium quercuum* f. sp. *fusiforme* results in stem galls (similar to the galls formed as a result of infection by WGR) on slash [*Pinus elliottii* Engelm.] and loblolly pine [*Pinus taeda* L.] that disrupt water movement (MacFall et al. 1991). Infection of seedlings by *C. quercuum* f. sp. *fusiforme* typically results in mortality within only a few years, whereas infection of older trees commonly results in mortality caused by breakage at the gall due to the weakened tissues comprising the gall (Agrios 1997). Dwarf mistletoes (*Arceuthobium* spp.) are caused by parasitic flowering plants (Agrios 1997), and increased the $A_L:A_S$ on Douglas-fir [*Pseudotsuga menziesii* var. *menziesii*] and western larch [*Larix occidentalis* Nutt.] (Sala et al. 2001). In contrast, the $A_L:A_S$ of dwarf mistletoe-infected western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] trees decreased, resulting in reduced whole-tree water use in heavily infected trees (Meinzer et al. 2004). Root diseases of conifers caused by *Armillaria ostoyae* (Romagnesi) Herink, *Phellinus weirii* (Murrill) R.L. Gilbertson, and *Inonotus tomentosus* (Fr.:Fr.) S. Teng. result in reduced growth, foliar discoloration, root and butt rot, and tree mortality (Allen et al. 1996). Mortality of trees infected with root rots such as *Armillaria* is greatest during drought or following defoliation (Agrios 1997). Black stain root disease on conifers is caused by the vascular wilt pathogen *Leptographium wageneri* (Kendrick) M.J. Wingfield (Allen et al. 1996); vascular wilt fungi disrupt water conduction in plants by clogging the water conducting tissues with mycelium and spores (Agrios 1997). Stem

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canker fungi such as *Atropellis piniphila* (Weir) Lohman & Cash on lodgepole pine

reduce tree growth (Allen et al. 1996). Although canker fungi typically do not kill their

host, they increase the likelihood of wind breakage, and infection by wood and root rot

fungi through weakening of the tree (Agrios 1997). Needle casts and blights on conifer

foliage caused by fungi such as *Lophodermella concolor* (Dearn.) Darker, and

Mycosphaerella pini Rost. in Munk result in growth reductions from defoliation;

Elytroderma deformans (Weir) Darker also causes stem and branch deformations due to

the perennial nature of the fungus (Allen et al. 1996). Mortality of trees following

infection by needle casts and blights may occur following repeated defoliations (Agrios

1997).

1.7   Research objectives

The overall objective of this research was to evaluate the responses of lodgepole

pine trees following infection by WGR. Chapters 2, 3 and 4 address the specific

objectives of the research. Each chapter is an independent paper that either has been, or

will be submitted for publication.

Chapter 2, *Predicting survival of lodgepole pine stands infected with western gall

rust* (a version of this paper has been published in the Canadian Journal of Forest

Research, 36:878-885) had the overall objective of evaluating the survival of WGR-

infected trees, but more specifically to: 1) model the 11-year survival of WGR-infected

trees as a function of the degree of gall encirclement of the stem; and 2) evaluate the

effectiveness of scribing [removal of a strip of bark around galled tissues] for the

prevention of gall expansion, and promotion of stem healing. To predict the cumulative

mortality of stem galled trees at a rotation age of 80, an earlier model of gall expansion

(Peterson 1960) was applied to the 11-year survival function. This paper provides the first data based estimate of galled tree survival that closely approximates the previous assumption of 50% survival (Woods et al. 2000).

Chapter 3, *Growth responses of lodgepole pine to the reduced water flow through stem galls resulting from infection by western gall rust*, had the overall objective of determining the impact of the reduced water flow through stem galls on tree growth. The chapter is comprised of three different studies that address the following specific objectives: 1) to test the hypothesis that galls decrease aboveground tree growth by reducing water flow through the stem; 2) to determine the relative importance of xylem and phloem disruptions on the radial and height growth of galled trees; and 3) to test the hypothesis that trees with stem galls allocate more resources to root growth to increase water uptake.

Chapter 4, *Whole-tree water relations of western gall rust infected lodgepole pine trees in response to soil drought*, evaluated the responses of galled and non-galled trees to soil drought. Drought events are regularly experienced by trees growing under natural field conditions. This paper builds on the knowledge gained in the previous two chapters, which determined that galled tree mortality is a function of gall size (i.e. gall encirclement of the stem), and that stem galls result in a hydraulic constriction. Trees respond to the reduced water flow by reducing their aboveground growth; the severity of the impact of galls is related to both tree size and gall size. The following specific objectives were to determine: 1) if the Q_L of galled and non-galled trees differed; 2) given that the K_ψ and perhaps Q_L may be lower in galled trees relative to non-galled trees,

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which of the hydraulic parameters comprising the K_ψ and Q_L metrics changes in response

to soil drought; and 3) the effect of multiple soil drought cycles.

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2 CHAPTER TWO

Predicting survival of western gall rust-infected lodgepole pine stands[1]

2.1 Introduction

In western Canada, the commercially important species lodgepole pine [*Pinus contorta* Dougl. ex Loud.] is susceptible to infection by western gall rust (WGR), caused by the fungus *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka [syn: *Peridermium harknessii* J.P. Moore]. Galls may form on either the main stem or on lateral branches (Hiratsuka and Powell 1976) following infection of expanding shoots (True 1938). The cambium and ray initials of WGR infected tissue produce abnormally large numbers of cells, resulting in pronounced gall development within two years of infection (Peterson 1960).

Lodgepole pine often regenerates at high densities following wildfire or logging (Johnstone 1981a, 1981b). Precommercial thinning of such dense stands can reduce mortality from competition and maximize diameter growth (Johnstone 1981a). However, heavy infection by WGR following thinning could result in understocked stands (Blenis and Duncan 1997). Even if stocking levels were adequate, productivity could be reduced, as WGR tends to infect larger trees (Bella 1985), and such infected trees may withdraw resources from a site before succumbing to the disease. Delaying thinning in stands heavily infected with WGR has been recommended (Johnstone 1981b; Blenis and Duncan 1997), as the risk of infection decreases with tree age (Blenis and Li 2005).

There are essentially no data indicating how long trees with main stem galls are likely to survive. Woods et al. (2000) assumed 50 percent survival of trees with WGR infections on the stem or within 10 cm of the stem, on the basis that half of the WGR infections occurred on the stem. van der Kamp (1988) stated (although no data were presented) that stem galls usually resulted in tree mortality, but not until after 20 years following infection. Both stem and branch galls were fairly common in young jack pine [*Pinus banksiana* Lamb.] stands, whereas trees with stem galls were quite rare in older stands, suggesting that most stem-galled trees died as the stands matured (Gross 1983). However, there is reason to believe that many trees with stem galls may survive. Sixty percent of stem galls encircled less than 40 percent of the stem circumference, while large galls encircling more than 80 percent of the stem comprised only nine percent of stem galls (Blenis and Duncan 1997). Furthermore, gall expansion is very slow; trees occasionally can survive more than 200 years with a stem infection (Peterson 1960). Therefore, it is possible that tree death could be related to gall size, and may be quite low for trees with small galls.

The vertical growth of stem galls is typically less than their lateral growth, which in turn is closely correlated with radial growth of the host stem (Peterson 1960). Thus, it is possible that scribing, a process whereby a strip of bark is removed around the infection, may effectively stop the spread of the pathogen and promote stem healing. Scribing of cankers caused by white pine blister rust [*Cronartium ribicola* J.C. Fisch.] was expected to be successful for trees 3-10 cm and 10-20 cm in diameter with rust covering <50%-70% and <70%-85% of the stem circumference, respectively (Hunt 1988). It is unknown whether this technique would limit the lateral expansion of galls caused by WGR.

The primary objective of this research was to develop a model to predict annual survival of trees with stem galls. The ability to predict tree survival following WGR infection would: 1) help determine the appropriate density targets during precommercial thinning; 2) aid in deciding whether trees with small galls should be considered potential crop trees in juvenile stand surveys; 3) increase the accuracy of growth and yield projections; and 4) improve impact assessments required for decision making with respect to the inclusion of western gall rust resistance in tree improvement programs. A secondary objective was to evaluate the effectiveness of scribing for preventing gall expansion, and promoting stem healing.

2.2 Materials and methods

2.1.1 Field methods

In 1992, two stands, 45 km apart, were located in the Upper Foothills natural subregion (Beckingham et al. 1996), near Hinton, Alberta. Designated 33 and 632, these stands had naturally regenerated following harvest in 1973 and 1972, and had been precommercially thinned in 1987 and 1985, respectively. Forty plots, each containing five trees with at least one stem gall and five control trees with no stem galls, were established 10 to 20 m apart in both stands. In total, 400 galled and 400 non-galled trees were sampled (Table 2-1).

Table 2- 1. Summary of the average tree and stand characteristics in 1992[a]; gall measurements refer to the gall encircling the greatest proportion of the stem circumference.

Average Tree and Stand Characteristics	Stand 33	Stand 632
Stand area (ha)	122	21
Pre-thinning density (stem/ha)	25 000	17 000
Basal area[a] (m^2/ha)	27.9	31.2
Post-thinning density (stems/ha)	2 500	2 200
Stand age at thinning (yrs)	14	13
Height of non-galled trees (m)	6.6	6.4
Height of galled trees (m)	6.7	6.4
Number of stem galls/tree for galled trees[b]	1.8	1.8
Approximate gall age (yrs)	7	7
Height of gall on tree (m)	1.2	0.9
Percent gall encirclement	53.1	61.9

[a] Basal area was measured in 2003.
[b] In calculating the average number of galls/tree, all overlapping galls were counted separately.

The number of galls per tree, gall height (m) and tree height (m) were recorded. Approximate gall age was estimated by counting rings (or counting back growth increments on branches attached to gall-bearing internodes). The stem circumference at each gall location was calculated by averaging the above- and below-gall stem circumferences. The arc length of gall-free circumference at each gall location was divided by the stem circumference at that location and multiplied by 100 to estimate the percent of the circumference that was gall-free. There is considerable year-to-year variation in infection frequency with occasional years of high infection, called wave years, interspersed among numerous years of low infection (Peterson 1971). Since only expanding shoots are infected, the wave year phenomena can result in clusters of galls with overlapping heights. For such galls, vertical lines were projected from the margins of each gall with the distance between them representing the gall-free arc length (Fig. 2-

1). Henceforth, except as noted, the term gall will refer either to single galls or to clusters of overlapping galls of the same age.
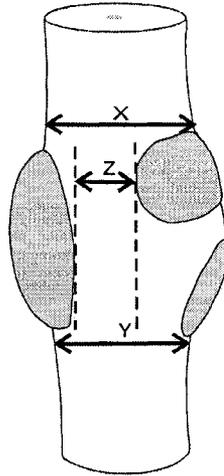
Figure 2- 1. Determination of percent encirclement for three overlapping galls. Vertical lines were projected from the margin of each gall and the distance between them (Z) represented the arc length of gall-free tissue. Stem circumference above (X) and below (Y) the galled area were measured and percent encirclement was calculated as 100-Z/((X+Y)/2)*100.

Branch galls were recorded if they had grown into, or were within 10 cm of the main stem, and were designated as ms_br galls. Thirty galled trees from each stand having only one gall (either a main stem gall or an ms_br gall that had reached the main stem) and having 10 cm or more of gall-free circumference were randomly selected for scribing from a total of 131 trees fitting these criteria. A 1-cm wide channel was scribed around each of the selected galls, exposing the underlying xylem.

In 2003, all trees were re-visited and recorded as living or dead; the basal area of each plot was determined using a wedge prism having a basal area factor of three. Five ms_br and five stem galls located adjacent to the survival plots were dissected and visually examined for differences in gall development. Prior to data analysis, the database was organized so that each tree was represented by a single record with the following elements: 1) whether the tree was alive or dead; 2) number of galls (including

both main stem and ms_br galls). For this variable, all galls in an overlapping cluster were counted; 3) whether or not the tree had ms_br galls; 4) whether or not the tree was scribed; 5) tree height; 6) percent encirclement of the largest gall (gall encircling the greatest proportion of the stem circumference). This variable was calculated by subtracting the percent of circumference that was gall-free from 100 percent (Fig. 2-1); 7) age of the largest gall; and 8) height of the largest gall. The first variable was evaluated in 2003; all other variables were recorded in 1992.

2.2.1 Data analysis

All statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA); significance was assessed using $\alpha = 0.05$. Fisher's exact test (Stokes et al. 1995) was performed to determine if the probability of survival was less for: 1) trees having at least one stem gall than for trees without stem galls; 2) trees with only one ms_br gall and no other stem galls than for trees without stem galls; and 3) unscribed control trees than for scribed trees. The 95 percent confidence intervals (95% C.I.s) for the above differences were calculated based on the binomial probability distribution. For comparisons 1) and 2), above, data were combined over both stands, as log-linear analysis (Kennedy 1992) had indicated that the treatment effect was similar across stands; comparison 3) was performed only for stand 33, as mortality had not occurred in the scribing experiment in stand 632.

Analyses of gall number, tree height in 1992, percent encirclement of the largest gall, age of the largest gall, and height of the largest gall as potential predictors of survival were performed by sorting the individual tree records by the independent variable being analyzed, grouping the data into ten equal sized classes, and regressing the

average percent survival for the 10 classes against the mean of the independent variable for those classes.

Because there appeared to be a strong nonlinear relationship between gall encirclement and survival, nonlinear regression fitting two linear regressions with an unknown knot (Freund and Littell 1991) was employed to evaluate the relationship between percent gall encirclement and tree survival. A dummy variable for stand was included in the model. Initial runs assumed separate parameters for the two stands; however, common parameters were ultimately used if the 95% C.I.s for parameters of the two stands overlapped. As the SAS Proc NLIN procedure uses least squares estimation and the response variable was binary (dead versus alive), iteratively reweighted least squares (IRLS) were used to obtain parameter estimates. Successive models were run, with observations weighted by the variance (estimated from the previous run as $(1/(p_i(1-p_i))$, where p_i is the predicted probability of survival for the ith observation), until the parameter estimates stabilized (Steel et al. 1997).

The probability of infected trees surviving until rotation, as a function of gall encirclement was estimated. First, 100 classes of gall encirclement were created by rounding the percent encirclement of each gall to the nearest one percent. Then, the following assumptions were made. 1) Each year, the infected portion of a trunk would expand to keep pace with the increasing girth of the tree (Peterson 1960). The pathogen would have additional lateral movement at the rate of 1.7 + 1.8r (where r is the rate of radial stem increment in mm [Peterson 1960]) permitting calculation of the increase in gall encirclement over time. 2) Eleven-year survival would be 100 percent for non-galled trees, whereas for galled trees 11-year survival would decrease with percent gall

encirclement as predicted by the nonlinear model described above. This assumption was made to permit estimation of the relative survival of infected and uninfected trees rather than the absolute survival rate of galled trees. 3) The 11-year survival rate could be converted to an annual survival rate by the formula $P_{11}=(P_1)^{11}$, where P_{11} and P_1 are the 11-year and 1-year survival probabilities, respectively. 4) The initial tree radius at the gall location would be the same for each tree, and would equal the average value found in the current study, 43 mm; the average annual radial tree growth would be 1.14 mm year^{-1} (the average annual increment occurring 25 and 45 years after precommercial thinning of lodgepole pine to densities of 2986 [Johnstone 1981a] and 2218 stems/ha [Johnstone 2002], respectively); and stand age at harvest would be 80 years. 5) The probability of a tree with a particular degree of gall encirclement surviving until rotation was the product of the annual survival probabilities. Finally, the overall average probability of stem galled trees reaching rotation age was obtained by multiplying the probability of surviving to rotation for each of the 100 encirclement classes by the proportion of galls in that class.

A sensitivity analysis for stand 33 was performed by changing, by 20%, the values of the 1) knot of the survival function, 2) slope of the survival function after the knot, 3) initial tree radius, 4) radial tree growth, 5) rotation age, and 6) lateral growth rate of the fungus. Each factor was changed while holding the other factors constant at the values used in the initial analysis and was either increased or decreased by 20% to increase the probability of survival to rotation.

2.3 Results

Percent mortality (and 95 % C.I.) of trees with at least one stem gall, trees with only one ms_br gall and control (non-galled) trees were 12% (9%-16%), 10% (5%-19%) and 2% (1%-4%), respectively. The mortality of trees with at least one stem gall and a single ms_br gall were both significantly greater than that for control trees, $p = <0.01$.

Five of the eight dead trees with only one ms_br gall were completely encircled by the gall. Main stem tissue associated with ms_br galls was deformed and discoloured (Fig. 2-2A); however, this deformation and discolouration did not reach the stem centre, as was the case with main stem galls (Fig. 2-2B). Ms_br galls were included with stem galls in all analyses, and were referred to as stem galls except as noted, as they were similar to galls arising from direct infection of the main stem.
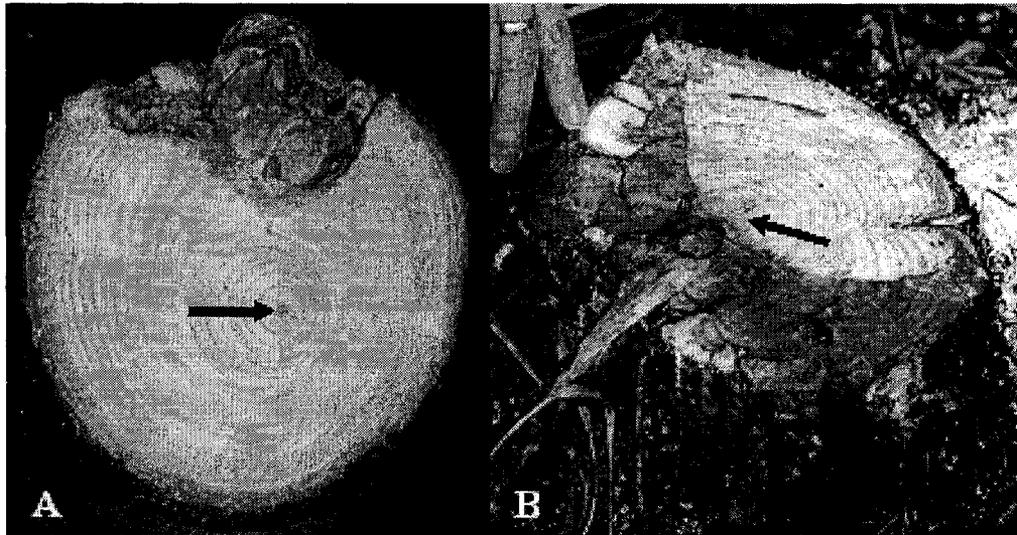
Figure 2- 2. Discoloration and deformation of main stems associated with ms_br (A) and main stem (B) galls. Note that discoloration and deformation extend to the tree centre (indicated by an arrow) only for the main stem gall.

Although galls likely were not aged exactly, it appears that a wave year(s) occurred at or slightly before the time of precommercial thinning, since 87% and 93% of the

largest galls (galls encircling the greatest proportion of the stem circumference) resulted

from infections occurring between 1983 and 1987 for stands 33 and 632, respectively.

As a consequence, there were a large number of trees with main stem galls that were not

removed during the thinning operation. A wide range of gall encirclements occurred in

both stands; 8.5% and 13% of the galls in stands 33 and 632, respectively, encircled the

entire stem. In stand 33 the distribution of encirclements was approximately normal,

whereas in stand 632 there was a nearly uniform distribution of galls having

encirclements of between 15 to 99% (Fig. 2-3).
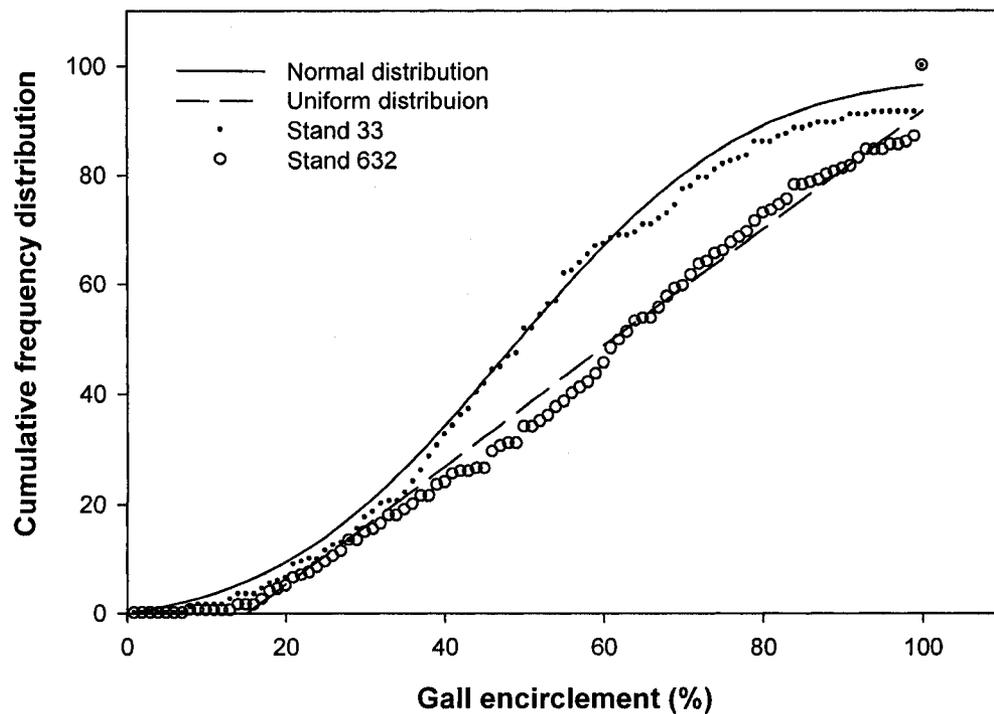
Figure 2- 3. Cumulative frequency distribution of galls encircling different percentages
of the main stem in stands 33 and 632. For purposes of comparison, a normal
distribution with the same mean and variance as stand 33 and a uniform
distribution between 15% and 99% encirclement are included.

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On many galls, rodent feeding had exposed the xylem and resulted in resinosis. However, the feeding typically did not encompass the entire galled surface, and as a result a portion of the gall remained alive.

Percent mortality (and 95 % C.I.) of scribed trees was 7% (1%-23%), and was not significantly different (p=0.64) from unscribed controls, 6% (1%-18%). In most cases the scribed channel had healed over, and there was no evidence that scribing had led to gall inactivation.

There was no association between stem gall number, tree height in 1992, age of the largest gall or height of the largest gall and tree survival. Mortality was best predicted by percent gall encirclement. Nonlinear regression indicated survival was constant at approximately 95 percent survival until the percent gall encirclement reached 79 and 91 percent for stands 33 and 632, respectively (Fig. 2-4), at which point survival decreased rapidly with increasing gall encirclement (Fig. 2-4, Table 2-2).
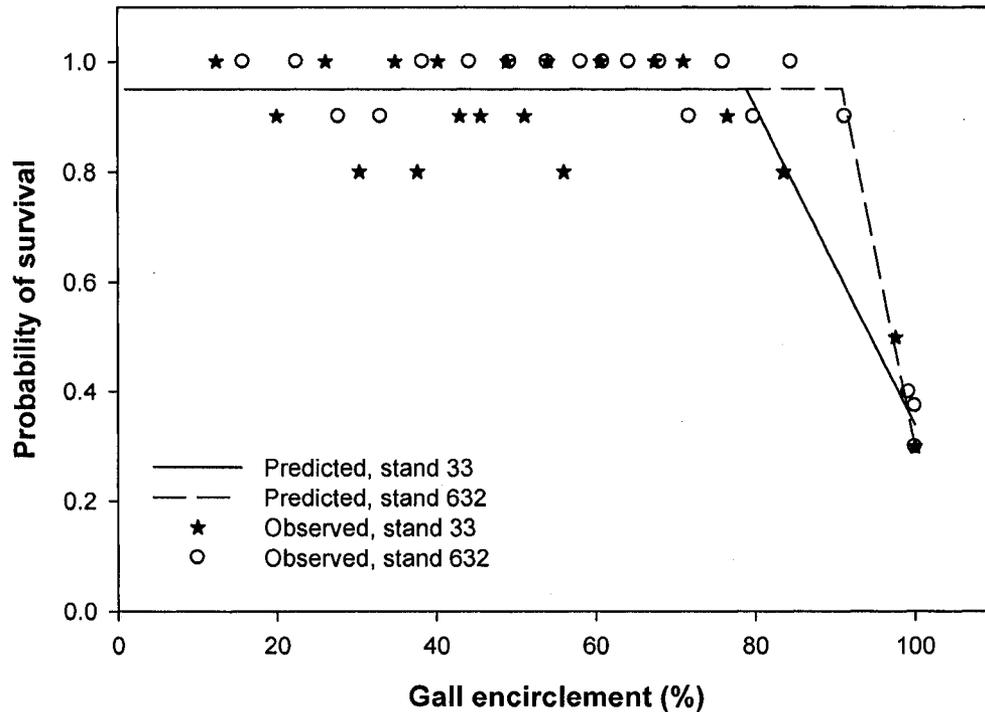
Figure 2- 4. Plot of the mean observed values of survival in 20 equal sized classes (n=10 per class) for stands 33 and 632, and the predicted values from the nonlinear regression.

Table 2- 2. Parameter estimates and the corresponding 95% confidence intervals (C.I.s) of the nonlinear regression model predicting probability of survival as a function of percent gall encirclement 11 years previously.

Parameter	Estimate	Lower C.I.	Upper C.I.
Common intercept[a]	0.95	0.93	0.98
Threshold (knot)[b], stand 33	79	68	91
Slope after threshold, stand 33	-0.029	-0.050	-0.008
Threshold (knot)[b], stand 632	91	88	95
Slope after threshold, stand 632	-0.072	-0.108	-0.035

[a] In initial model runs, confidence intervals for the two stands overlapped, and thus a common intercept was used. Because the slope of the line prior to the threshold was slightly positive and not significantly different from 0, it was set to 0.

[b] For gall encirclements greater than the threshold, survival probability equaled the intercept; for larger gall encirclements, survival decreased with increasing encirclement.

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For both stands, the curves representing survival at rotation as a function of gall encirclement at the time of survey were sigmoidal, reflecting high survival of trees with small galls, low survival of trees with large galls, and a rapid decline in the probability of survival with increasing encirclement for intermediate-sized galls (Fig. 2-5).
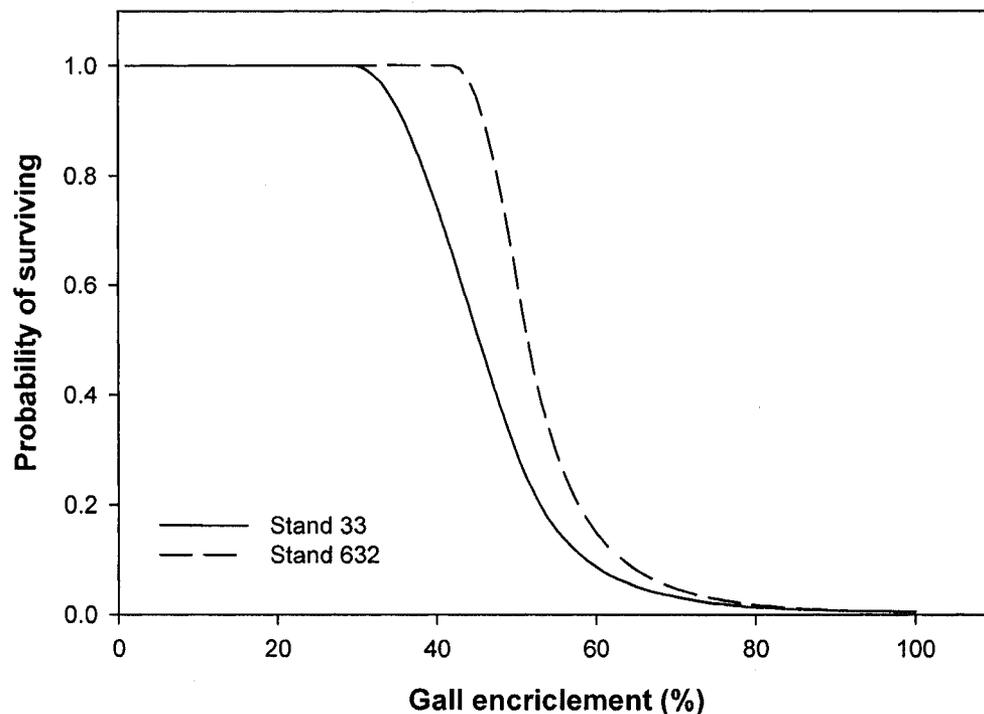
Figure 2- 5. Predicted probability of survival at approximately 31-years of age to rotation of trees with stem galls in stands 33 and 632 as a function of percent gall encirclement in 1992. Curves are based on the assumptions of an 80 year harvest age, 11-year survival probabilities as shown in Fig 2-4, and Peterson's (1960) model for gall enlargement.

Over 80% of the trees with <38% or <47% encirclement in stands 33 and 632, respectively, were predicted to survive to harvest age. In contrast, estimated survival was less than 25% for galls with >52% and >56% encirclement in stands 33 and 632, respectively. Application of the relationship between gall encirclement and survival to

the number of galls in each encirclement class produced overall average probabilities of

survival to rotation of 43% and 38% for stands 33 and 632, respectively.

Sensitivity analysis indicated that survival to rotation was most affected by the

knot, followed by the rotation age, and then the lateral growth rate of the fungus (Fig. 2-

6). Twenty percent changes in these values increased the overall estimated survival in

stand 33 from 43% to 83%, 60%, and 57%, respectively.
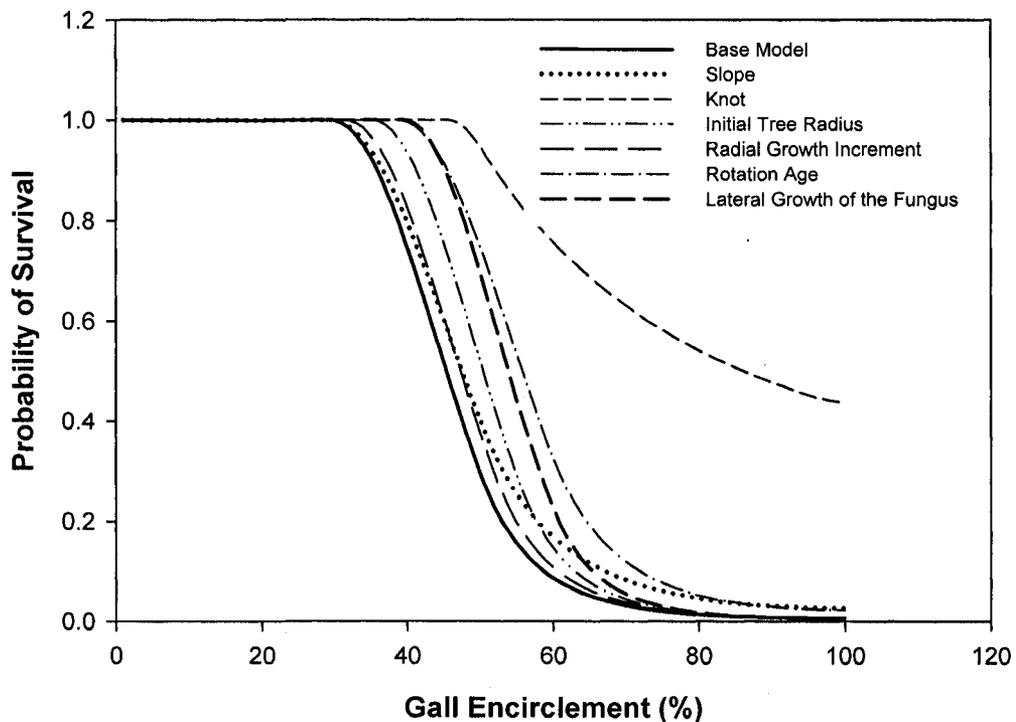
Figure 2- 6. Stand 33 sensitivity analysis of the application of Peterson's (1960) model
of gall expansion. The base model assumed an initial tree radius of 43 mm, radial
growth increment of 1.14 mm, rotation age of 80, slope of 0.029, and a knot of 79
percent; the initial slope and knot were predicted from the nonlinear survival
function. All values were changed by 20% while holding the other factors
constant; values were either increased or decreased to increase the probability of
survival to rotation.

2.4 Discussion

The results of this study provide insights into gall rust induced mortality in the unfortunate situation where heavy infection near the time of precommercial thinning results in moderately stocked stands with substantial gall rust. In the 11 years following the original survey, trees with galls encircling a small percentage of the stem circumference had similar mortality rates as non-galled trees. However, galls encircling more than 79 and 91 percent of the stem in stands 33 and 632, respectively, increased the risk of tree death, with the risk increasing steeply with the degree of gall encirclement.

It is uncertain why the 11-year survival function differed between the two stands. Differences in basal area (27.9 vs. 31.2 m^2/ha) and density (2500 vs. 2200 stems/ha) were likely not large enough to account for the different survival functions. Those differences presumably were due to factor(s) not measured, such as the risk of wind breakage, hydrological characteristics, or rodent feeding. Although the frequency of dead trees broken at the gall was not recorded, many dead trees were unbroken. Nevertheless, stem breakage has been described as a significant cause of mortality (van der Kamp 1988) and might have differed between the stands. Water movement is restricted through galls, such that hydraulic conductivity through fully encircled stem sections was reduced by 59% - 99%, relative to non-galled (above-and below-gall) sections of stem (Wolken and Blenis unpublished). Although both stands were in the Upper Foothills natural subregion, they may have differed in hydrological characteristics, which in turn influenced survival. Most galls in both stands had at least some gnawing by rodents, potentially increasing the risk of stem breakage (van der Kamp and Spence 1987) or

interfering with water flow. Thus, differences in rodent feeding may also have contributed to differences in the relationship between gall encirclement and mortality.

The mortality of trees with ms_br galls was significantly greater than that for non-galled control trees, which might appear to contradict earlier work showing negligible impact of branch galls (Gross 1983). However, five of the eight dead trees with ms_br galls were entirely encircled by the gall. Thus, it appears that the effect of ms_br galls was similar to that of galls arising from direct infection of the main stem: they were likely to be lethal if they encircled the majority of the stem circumference, but were likely to be relatively benign if they did not. Visual examination confirmed the similarity between ms_br galls and galls arising from direct stem infection in that they caused comparable discolouration and deformation of stem tissues, although the impact of ms_br galls did not extend to the centre of affected trees (Fig. 2-2).

Trees selected for scribing had 10 cm or more of gall free tissue, which corresponded to gall encirclements of 20% - 60% of the stem circumference. It is not surprising that scribing did not decrease mortality resulting from WGR, as this range of gall encirclements on unscribed trees did not lead to mortality in either stand. It does not appear that scribing by itself has great potential for WGR management. First, the 1-cm channels used in this study had all healed over. A similar phenomenon occurred for scribed white pine blister rust cankers, leading to the suggestion that channel widths should be 2.5-3.0 cm (Hunt 1988). Since WGR can persist for a long time in the gall, even this channel width may not be adequate unless some additional treatment, such as removing gall tissue (Peterson 1961), was applied. Secondly, scribing must be limited to trees with small stem galls, as it would result in girdling of trees with large galls.

Although scribing small cankers may make sense for an aggressive pathogen such as *C. ribicola*, it would be less feasible for WGR, as small galls do not appear to be lethal.

Application of Peterson's (1960) model to the data from this study enabled the prediction of the cumulative mortality of WGR-infected trees, relative to uninfected trees, at a rotation age of 80 years. This process involved extrapolation in that it assumed that the relationship between gall size and the risk of tree death determined over the 11-year period of this study would hold until rotation. On the other hand, it does provide some data-based quantification for the survival of infected trees, something that has been lacking to date. Although the distribution of gall sizes, and the threshold and slope parameters for the survival functions differed between the two stands, both stands had numerous smaller galls that may not be lethal. The average predicted relative survival values were 43% and 38% for stands 33 and 632, respectively, suggesting that the assumption of 50% survival (Woods 2000) was a reasonable estimate. The sensitivity analysis indicated that the severity of the infection (knot) had the greatest impact on the probability of survival; a 20% increase in the knot of stand 33 resulted in an overall estimated survival of 83%. It is unlikely that many more main stem galls will develop in the two stands, as it has been demonstrated that the susceptibility to infection decreases with tree age (Blenis and Li 2005). However, the absence of obvious main stem galls on trees > 60 years old that we, like Gross (1983), have observed implies that mortality rates could increase as these stands approach rotation age. To some extent the discrepancy between our survival predictions and those observations made by Gross (1983) may be attributed to the fact that the two stands in the current study were approximately 20 years old when first surveyed and thus numerous infected trees may have either died prior to

1992, or were removed at the time of precommercial thinning. In any event, continued monitoring of the survival plots through to rotation would allow for more accurate survival predictions.

In summary, there was a wide range of gall sizes following precommercial thinning of two dense lodgepole pine stands. The 11-year pattern of survival of trees with galls was similar regardless of whether the infection had occurred on the main stem or had reached the stem from a nearby branch infection. In both cases, survival was similar to that of non-galled trees if the galls encircled less than approximately 80% and 90% of the stem for stands 33 and 632, respectively; for larger galls, survival decreased sharply with increased percentage of gall encirclement. Application of Peterson's (1960) equation for gall expansion to the survival function predicted that over 80% of the trees with <38% and <47% encirclement, in stands 33 and 632, respectively would survive to rotation, whereas less than 25% of the galls encircling >52% and >56% encirclement would live to be harvested in stands 33 and 632, respectively. Overall survival of galled trees, relative to non-galled trees, was estimated to be 38% - 43%, which closely approximates the previous estimate of 50% (Woods et al. 2000). Future surveying of the survival plots is essential to determine whether or not these estimates of survival at rotation are overly optimistic. Scribing does not appear to be feasible since large channels cannot be cut into large galls without girdling the tree, and the scribing of small galls appears to be unnecessary.

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3   CHAPTER THREE

Growth responses of lodgepole pine to the reduced water flow through stem galls resulting from infection by western gall rust

3.1   Introduction

Western gall rust (WGR) is an important forest pathogen of lodgepole pine [*Pinus contorta* Dougl. ex Loud.] caused by the fungus *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka (syn: *Peridermium harknessii* J.P. Moore). The spores of WGR infect only the current year's shoots (True 1938); infection is followed by stimulation of the cambium to produce more than five times the normal percentage of ray parenchyma (Peterson 1960). Galls form on the main stem or lateral branches (Hiratsuka and Powell 1976), becoming distinctly spherical or hemispherical 2 years following infection (True 1938).

Branch galls result in the death of the distal portions of branches, while stem galls may result in tree mortality (van der Kamp 1988). Twenty-year-old trees with stem galls were more likely to die (within 11 years) than non-galled trees if the gall encircled greater than 80% of the stem circumference (Wolken et al. 2006). Although the majority of stem galls encircled less than 40% of the stem (Blenis and Duncan 1997), the application of an earlier model of gall expansion (Peterson 1960) predicted that approximately 40% of trees with stem galls would survive until a rotation age of 80 years (Wolken et al. 2006), which was similar to the previous assumption of 50% survival of stem galled trees (Woods et al. 2000).

Plant pathogens alter the growth and physiological functioning of their hosts. For example, water movement was disrupted by galls, similar to those resulting from

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infection by WGR, caused by *Cronartium quercuum* f. sp. *fusiforme* on slash [*Pinus elliottii* Engelm.] and loblolly pine [*Pinus taeda* L.] (MacFall et al. 1991). Dwarf mistletoe (*Arceuthobium* spp.) infections resulted in increased leaf-area-to-sapwood area (A_L:A_S) ratios in Douglas-fir [*Pseudotsuga menziesii* var. *menziesii*] and western larch [*Larix occidentalis* Nutt.] (Sala et al. 2001). The higher A_L:A_S in infected Douglas-fir trees was the result of the combined decrease in A_S and increase in A_L, whereas in infected western larch the higher A_L:A_S was attributed to a decrease in A_S (Sala et al. 2001). The increased A_L:A_S of infected Douglas-fir trees were accompanied by increases in the sap flow per unit A_S, resulting in the whole-tree water use of infected and uninfected trees being similar for Douglas-fir; increases in the sap flow per unit A_S were not observed in western larch, and as a result the whole-tree water use was greater in infected than uninfected trees (Sala et al. 2001). In contrast, Meinzer et al. (2004) reported lower A_L:A_S in dwarf mistletoe infected western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] trees due to a decrease in A_L, resulting in decreased whole-tree water use in infected trees relative to uninfected trees.

The A_L:A_S may be predicted by the following hydraulic formula (Whitehead and Jarvis 1981; Whitehead et al. 1984), which combines the Penman-Monteith equation describing transpiration, and Darcy's law describing water flow through porous media, such as the xylem of trees (Mencuccini and Grace 1995):

$$\frac{A_L}{A_S} = \frac{k(\Delta\Psi / l)c}{D g_s}, \tag{1}$$

where A_L and A_S are the leaf area and sapwood area, respectively, k is the sapwood permeability, $\Delta\psi/l$ is the water potential gradient through the system, D is the time-

averaged vapour pressure deficit of the atmosphere, and g_s is stomatal conductance. The c coefficient is equal to:

$$c = \rho_w \gamma \lambda / \eta c_p \rho_a, \tag{2}$$

where ρ_w, γ, λ, η, c_p, and ρ_a are the density of water, the psychrometric constant, the latent heat of vaporization of water, the dynamic water viscosity, the specific heat of air at constant pressure, and the density of air, respectively (Whitehead et al. 1984; Mencuccini and Grace 1995). In an attempt to unify the nomenclature for water movement through trees, Reid et al. (2005) proposed the following equation, which is consistent with Darcy's law describing the hydraulic conductivity (K_ψ; $m^2 s^{-1} Pa^{-1}$) in the xylem of trees:

$$K_\Psi = \frac{Q}{A_S} \frac{L}{\Delta \Psi}, \tag{3}$$

where Q is the mean flow rate ($m^3 s^{-1}$) of water through a stem sample of length L (m) and conducting area of sapwood A_S (m^2), under a water potential difference $\Delta \psi$ expressed in pressure units (Pa).

Whitehead et al. (1984) determined that differences between the A_L:A_S of *Picea sitchensis* (Bong.) Carr. and *P. contorta* were explained by differences in the sapwood permeability (k in Eq. (1)). Conduction experiments with eosin dye revealed that mature branch galls have a greater amount of non-conducting tissue than non-galled branches (True 1938), and the tracheids of galled tissues are unusually short and branched (Zalasky 1976). In addition, galls fully encircling the stem restricted water flow, resulting in a reduction in the K_ψ by 59% - 99%, relative to non-galled stem sections (Wolken and Blenis, unpublished). However, the impact of the reduction in the K_ψ through stem galls resulting from infection by WGR on the growth of lodgepole pine is currently unknown.

The root-soil interface is an important resistance to water flow (Örlander and Due 1986) that is not included in either equation 1 or 3. Soils and roots shrink as they dry, creating vapour gaps between the roots and soil, which increases the resistance to water flow (Örlander and Due 1986). Water transport is limited by low root-to-leaf area ratios (Sperry et al. 1998); the root-to-leaf area ratio was 42 percent higher on a xeric than on a mesic site, enabling xeric site trees to function similarly to mesic site trees (Addington et al. 2006). Root growth was determined to be negatively related to soil water availability (Gower et al. 1992); the root-to-shoot ratio (R:S) of loblolly pine seedlings increased in response to drought (Bongarten and Teskey 1987). Presumably an increase in the belowground growth relative to the aboveground growth (R:S) might allow galled trees to compensate for the reduced flow of water through the gall.

MacFall et al. (1994) suggested that the galls resulting from infection by *C. quercuum* f. sp. *fusiforme* may also disrupt the phloem. Galled tissue contains a greater proportion of parenchymatous cells than normal tissues (True 1938; Peterson 1960). In galls, these parenchyma cells become filled with large amounts of starches, indicating that WGR causes its host to provide it with additional resources (True 1938). True (1938) attributed the larger stem diameter above galls than below galls on 9-to-30-year-old Scots pine [*Pinus sylvestris* L.] to the excess phloem parenchyma of galls disrupting the normal downward movement of materials.

The goal of this research was to determine the growth responses of lodgepole pine to infection by WGR. More specifically the objectives were: 1) to test the hypothesis that galls decrease tree growth by reducing water flow through the stem; 2) to determine the relative importance of xylem and phloem disruptions on the radial and height growth

of galled trees; and 3) to test the hypothesis that trees with stem galls allocate more resources to root growth to increase water uptake.

3.2   Methods

3.2.1   Field experiment 1. Evaluating the role of reduced water flow in reducing the growth of galled trees

3.2.1.1   Site and sample tree selection

Twelve WGR infected lodgepole pine trees, approximately 12-years-old, were located within a 120 ha cutblock located in the Upper Foothills natural subregion (Beckingham et al. 1996) near Hinton, Alberta. A young planted area that had not yet achieved crown closure was selected to eliminate the confounding influence of inter-tree competition, and reduce environmental and soil moisture variability. Annual precipitation was approximately 470 mm between 2000 and 2005, of which approximately 60% fell between May and September. The mean growing season temperature between May and September during these years was 13°C; the mean maximum temperature was 21°C (*Hinton Valley weather station data, Environment Canada*).

The twelve sample trees were spaced between 10 and 20 m apart, were at least 0.5 m away from potentially competing pines or shrubs and had one stem gall per tree, located within 1-m of the tree base. The trees were carefully selected to represent the full range of gall encirclements (0 to 100 percent of the stem circumference) (Table 3-1).

Table 3- 1. Summary of the characteristics of 12 open grown lodgepole pine trees.

Tree Characteristic	Tree Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Percent gall encirclement	0.0	17.9	21.6	36.8	40.6	58.7	65.3	72.6	83.5	87.8	100	100
Dbh (cm)	4.9	7.3	5.7	5.1	5.3	3.8	4.1	5.8	3.3	7.6	3.9	5.3
Gall height (cm)	.	72	76	44	65	64	45	52	47	49	59	42
Tree height (cm)	353	413	336	305	378	301	308	390	260	381	251	287
Tree age	12	12	12	11	12	12	12	12	12	12	12	12
Gall age[a]	.	9	10	10	10	10	10	11	11	11	10	11

Note: Dbh refers to the diameter at breast height (bh=1.3m).
[a]Gall age was estimated from a disc removed from the stem within 10 cm of the base of the gall.

3.2.1.2 Field sampling

Trees were harvested over two days in August 2005. The percent gall encirclement of each tree was calculated by first averaging the above (X) and below (Y) gall stem circumferences, and then measuring the arc length of gall-free circumference (Z) for each gall. The percent gall encirclement was calculated as $100-Z/((X+Y)/2)*100$ (Wolken et al. 2006). The diameter at breast height (dbh; cm at 1.3 m), and gall height above ground (cm; measured to the base of the gall) were measured prior to felling the trees. The tree age and total tree height (cm) were measured once the trees were felled at ground level (Table 3-1).

The live crown was divided into three sections: 1) below the top of the gall; 2) between the top of the gall and a point 30 cm above the gall top (henceforth referred to as the reference point); and 3) above the reference point. As needle expansion was not fully completed at the time of tree harvest, sub-samples of 30 fascicles per crown section were collected for determination of the specific leaf area (SLA; cm^2 g^{-1}) from each of two cohorts of needles (current vs. all previous years) to increase the accuracy of the

measurement (Whitehead et al. 1984; Coyea et al. 1992). The sub-samples of needles were placed in polyethylene bags, and transported to the laboratory on ice in an insulated box.

A 1.5-m long stem bolt, centred on the gall was cut from the tree for measurement of K_ψ. The stem sections with the branches still attached were placed in a double layer of moistened plastic tubing. The stem sections were covered with ice in an insulated box, transported to the laboratory, and frozen at $-20°C$ within 8 h of harvest (Reid et al. 2003). The branches not associated with this stem bolt were separated into the three crown sections, placed in large bags, and transported to the laboratory.

3.2.1.3 Leaf area:sapwood area ratio (A_L:A_S)

The sub-samples of 30 fascicles per crown section-needle cohort combination were scanned, and the one sided projected surface area was calculated using Sigma Scan-Pro® image analysis software. These needles were then oven-dried at 70°C for 24 h, and the dry weights measured to determine the SLA.

The current year shoots were removed from the branches in the laboratory, and placed in paper bags according to the associated crown section. The branches and current year shoots were then placed in large drying ovens at approximately 60°C until the dry weights stabilized. Once dried, the old and new needles were stripped from the branches and current year shoots, respectively, and sorted into paper bags according to the crown section-needle cohort combination. The total dry weight of needles was determined for each crown section-needle cohort combination by weighing the bags to the nearest 0.1 g, and subtracting the weight of the paper bag. The leaf area (A_L; m^2) was calculated by multiplying the SLA of the sub-samples by the total dry weight of each crown section-

needle cohort combination. The total A_L for each crown section was determined by summing the A_L for the new and old needles.

A 1 cm thick disc was removed from the stem bolt at the reference point, scanned, and the surface area calculated using Sigma Scan-Pro® image analysis software. The sapwood area (A_S; cm^2) was determined by subtracting the centre pith area from the total stem cross-sectional area inside the bark. The A_S was equivalent to the stem cross-sectional area, and therefore was a measure of tree growth, as the trees had not yet formed heartwood and only the pith area was removed. The $A_L:A_S$ ($m^2 cm^{-2}$) was calculated for the total A_L located above the reference point.

3.2.1.4   Hydraulic conductivity (K_ψ; $m^2 s^{-1} Pa^{-1}$)

The K_ψ (Reid et al. 2005) through the gall of each tree was measured according to procedures outlined in detail in Reid et al. (2003). Briefly, 20-to 30-cm long samples (sample lengths varied as a result of gall encirclement, and the location of branch whorls) were cut from the frozen stem sections, scrubbed clean, and thawed overnight in a 10 mmol solution of oxalic acid. The following day, 2 cm from each end of the sample was trimmed with a band saw. The ends of the samples were then planed smooth with a 20° block plane. The samples were then attached to the K_ψ apparatus used by Reid et al. (2003); the hanging water column generated a 16.3 kPa pressure head across the sample; samples were oriented in the natural direction of flow in the standing tree. The flow rate of degassed water at room temperature through the sample was measured once the flow of water stabilized after 5 min; outflow measurements were measured with an electronic balance and recorded with a computer every 30 s for 20 min (Reid et al. 2003). After the outflow measurements had been taken, non-binding 0.1% (w/v) acid fuchsin dye was run

through the samples to delineate the flow path of water through the galls. Samples were then frozen to preserve the staining pattern (Reid et al. 2003). A thin cross section was then removed from the bottom of the sample to expose the underlying dye flow pattern; the exposed cross section was scanned, and the total conducting surface area of the galled stem sections were calculated using Sigma Scan-Pro® image analysis software. However, as the intent of this study was to capture the impact of the gall on the K_ψ, any area obviously disabled by the gall was included in the measurement of the conducting surface area.

The K_ψ ($m^2s^{-1}Pa^{-1}$) was calculated for each galled stem section according to the nomenclature proposed by Reid et al. (2005), and defined in Eq (3).

3.2.1.5 Total foliar nitrogen [N] and phosphorus [P] concentration

A small sub-sample removed from the older cohort of needles (all previous years) above the reference point for all trees was ground with a Wiley mill to pass a 40-mesh screen; ground samples were stored in air tight containers at room temperature in the dark, until the samples were analyzed. The total foliar nitrogen [N] and phosphorus [P] concentrations were determined at the Natural Resources Analytical Laboratory at the University of Alberta using a digestion technique similar to the Kjeldahl method (Richards 1993).

3.2.1.6 Statistical analysis

All statistical analyses were performed using SAS® (SAS Institute Inc., Cary, N.C.). Statistical significance was assessed using $\alpha=0.10$ in order to reduce the Type II error rate, as the sample size was small (n=12). Simple linear regression was used to

determine the relationship between K_ψ and percent gall encirclement of the stem, as well as to regress: 1) foliar [N] and [P], 2) A_L above the reference point, 3) A_S at the reference point, and 4) A_L:A_S ratio against both percent gall encirclement and K_ψ. Multiple regression was used to relate both A_L and A_S as dependent variables to K_ψ and gall encirclement, as independent variables.

3.2.2 Field experiment 2. Impact of galls and xylem and phloem disruptions on tree growth

3.2.2.1 Site and sample tree selection

Thirteen-year-old galled and non-galled trees spaced approximately 20-m apart were selected along seven transects of varying length within a naturally regenerated and unplanted portion of the cutblock described above. All galled trees had a minimum gall encirclement of 40% of the stem circumference. In early May 2004 prior to shoot elongation, the following treatments were applied to 20 trees each:

1) Non-galled unwounded control tree (NGC);
2) Galled unwounded control tree (GC);
3) Non-galled tree with xylem wound (NGX);
4) Galled tree with xylem wound directly above the gall (GSX);
5) Galled tree with xylem wound on opposite side of stem from the gall (GFX);
6) Non-galled tree with phloem wound (NGP);
7) Galled tree with phloem wound directly above the gall (GSP); and
8) Galled tree with phloem wound on opposite side of stem from the gall (GFP);

Both xylem and phloem wounds encircled 50% of the stem circumference and were 2-cm high. Xylem wounds were cut 1 cm into the xylem using a portable drill with a 2-cm diameter drill bit (Fig. 3-1A). Phloem wounds removed the phloem and vascular cambium by scoring the bark with a utility knife, and exposing the underlying xylem

(Fig. 3-1B). Both xylem and phloem treatments applied to non-galled trees were located at breast height (1.3 m).
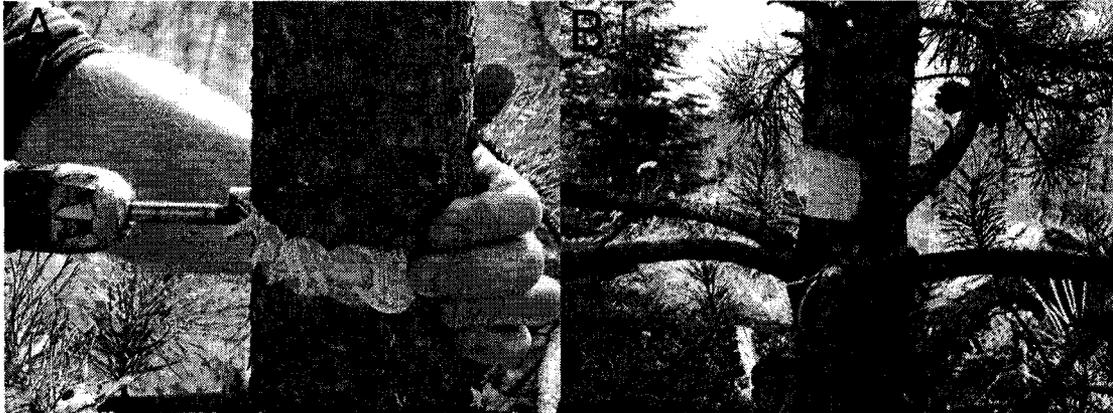
Figure 3- 1. Xylem wounds created using a portable drill with a 2-cm diameter drill bit (A), and phloem wounds created by scoring the bark with a utility knife (B); wounds encircled 50% of the stem circumference.

3.2.2.2 Field sampling

Gall encirclement was calculated as described above. Other tree measurements recorded at the time of wounding included gall height (height to the base of the gall; cm); and dbh (cm). There were only two instances where the dbh could not be measured as a result of the gall location.

In September 2005, two growing seasons after treatment, four trees per treatment were harvested for K_ψ measurement; all had a dbh $\leq$10 cm to minimize the variation in tree size. In October 2005, all remaining sample trees were harvested and returned to the laboratory for analysis. The tree status (living versus dead), tree height (cm), and crown length (CL; cm) were measured on all trees at the time of harvest. Cross-sections located 20-cm above the galled/treated areas were cut for radial growth measurements. The current year's leader was sealed in a polyethylene bag, packed in ice for transport, and

frozen at -20°C until prepared for nutrient analysis. The last four main stem internodes were removed for the measurement of height growth increments.

3.2.2.3 Laboratory measurements

The K_ψ was measured through the galled/treated areas on four replicates of each treatment (n=32) following the methods outlined previously; however, the pressure head generated by the hanging water column across the sample was 15.8 kPa. The gall age for each K_ψ sample was determined by counting the number of growth rings at the inflow side of the sample. A small sub-sample of approximately 20 fascicles from the current year's leader of these trees was processed for foliar [N] and [P] as described above.

The cross-sections located 20-cm above the galled/treated areas were first thawed before being glued to a board for sanding, first with a coarse grit sand paper, followed by a fine grit sand paper. The cross-sections were then scanned; the image analysis software WinDENDRO™ was used to measure the radial growth increment of four radii for the last six years of growth on the scanned cross-sections; the ring width was determined by averaging the four radii for each of the years of growth. The mean radial growth following treatment, and the mean radial growth 4-years prior to treatment were measured. The internode lengths of the last four years of growth on the main stem were measured to the nearest 1 cm; the growth rings at the base of each internode were counted to confirm the year of growth. The mean height growth following treatment, and the mean height growth 2-years prior to treatment were measured; a longer average height growth prior to treatment could not consistently be determined for all trees.

3.2.2.4 Statistical analysis

Average tree characteristics across treatments were compared by an analysis of variance (ANOVA) to determine if differences existed prior to the treatment application (Table 3-2). Tukey's multiple comparison tests following the one way ANOVA were used if differences were detected between treatments prior to the treatment application. Simple linear regression was used to determine the relationship between: i) the mean radial and height growth following treatment and K_ψ (n=32); the mean growth prior to treatment (4-years and 2-years for the radial and height growth, respectively) was used as a covariate; and ii) both foliar [N] and [P] and K_ψ (n=32). An ANOVA was performed to determine: i) if the mean radial and height growth following treatment with the mean growth prior to treatment (4-years and 2-years for the radial and height growth, respectively) as a covariate differed across treatments on all trees (n=157), and was followed by Tukey's multiple comparison tests if treatment differences were detected; and ii) if the foliar [N] and [P] differed across treatments (n=32). Statistical significance was assessed using $\alpha=0.05$.

3.2.3 Greenhouse study

3.2.3.1 Seedling establishment and inoculation

In May 2004, 300 1-year-old lodgepole pine seedlings from Coast-to-Coast Reforestation Inc. (Edmonton, AB, Canada) were planted in 340 mL styroblocks in Pro-Mix® BX growing media. They were placed in a growth chamber at 21°C:18°C (day:night) temperature under a 16 h photoperiod, watered as required, and inoculated 14 days after planting. WGR spores were removed from liquid nitrogen storage, and warmed at 40°C for 5 minutes (Moltzan et al. 2001). Spore germinability on 1.5% water

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agar plates after 96 h (Blenis and Pinnell 1988) was 89 percent. Immediately prior to inoculation, seedlings were well watered and misted with distilled water. Two hundred seedlings were inoculated in the laboratory using the "torn-needle" method in which a single needle fascicle was removed from the lower half of the elongating shoot and dry spores applied directly to the small wound with a fine paint brush (Myrholm and Hiratsuka 1993). The 100 control seedlings were treated by removing a single fascicle. The seedlings were again lightly misted with distilled water, and a wire cage was placed over each styroblock such that it did not contact the seedlings. Wet paper towels were then draped over the wire cages to maintain high humidity, and the styroblock/wire cage units were carefully sealed in large opaque polyethylene bags (Blenis and Pinnell 1988; Moltzan et al. 2001). Both the WGR-inoculated and the control seedlings were incubated in a growth cabinet in the dark for 48 h at 18°C, prior to being returned to the growth chamber.

One week after inoculation, the seedlings were transferred to a greenhouse where they experienced natural light conditions supplemented to provide an 18 h photoperiod. One and 2 weeks after inoculation, the seedlings were fertilized with 10-52-10 (N-P-K) to promote root establishment; every 2 weeks afterwards a 20-20-20 fertilizer was applied. Following 4 weeks in the greenhouse, the seedlings were transferred outside to ensure that they would harden off for the winter. The seedlings were watered as required, and fertilized at 2 week intervals.

After the first snowfall, it was determined that winter conditions might be too harsh for the seedlings, and in November the seedlings were brought inside and removed from

the styroblocks. The root plugs were wrapped in plastic in bundles of five or six and stored in a chest freezer having an average temperature of –3°C.

In April 2005, the seedlings were potted in 2.5 L pots in a 2:1 (sand:peat) mixture. Using a medium feeding rate (5 kg m^{-3}), 12 g of the Nutricote® Total 13-13-13 (Type: 100) controlled release fertilizer with micronutrients was mixed into each pot. Following re-potting, a 10-52-10 (N-P-K) fertilizer was applied to the trees, to aid root establishment. Every 2 weeks afterwards, a 20-20-20 (N-P-K) fertilizer was applied. In June 2005, the pots were placed outside in a cold frame. In November 2005, CloudCover® Outdoor-Indoor plant protector was applied at a dilution rate of 1:10 [CloudClover:Water] to reduce winter water loss. Straw was then packed around the pots to insulate them from the cold temperatures. In February 2006, the trees were returned to the greenhouse and re-potted in 15 L pots in a 2:1 (sand:peat) mixture amended with Nutricote® Total 13-13-13 (Type: 100) controlled release fertilizer with micronutrients.

Two and 9 days after re-potting, the trees were fertilized with the 10-52-10 (N-P-K) fertilizer; every 2 weeks afterwards a 20-20-20 (N-P-K) fertilizer was applied to the trees. The trees were watered twice per week for the remainder of their growth in the greenhouse, and were sprayed with Safers®End All (30 mL L^{-1}) at approximately 2 week intervals to minimize a persistent aphid population in the greenhouse. The trees were grown continuously in the greenhouse for 1 year (February 2006 to January 2007); therefore, the trees did not harden off as they would have under natural conditions.

3.2.3.2 *Harvesting and tissue processing*

In January 2007, 15 galled and 15 control trees were harvested and the tissues processed. At that time only 15 trees with a single stem gall were alive. Fifteen control

trees were randomly selected from among those having a single main stem, and an overall healthy growth form.

The stems of the 15 galled trees were partitioned into three sections: (i) the gall; (ii) above the gall, and (iii) below the gall. As the majority of the foliage of galled trees was located above the gall, the stems of the control trees were partitioned into two sections: (i) above the second major branch whorl, henceforth referred to as the reference point, which represented the majority of the live crown, and (ii) below the second major branch whorl (below the reference point).

All trees were cut at the root collar and the following measurements were made: (i) the root collar diameter (RCD; mm); (ii) the diameter above the gall/reference point; (iii) the diameter below the gall/reference point; (iv) the total tree height; (v) the stem length above the gall/reference point; and (vi) the stem length below the gall/reference point (cm). The stem diameter measurements were made with digital callipers to the nearest 0.01 mm; tree height and length measurements were made with a tape measure to the nearest 0.1 cm.

Approximately 20 needles from the most recent year's leader were removed and dried for 48 h at 68°C. The samples were prepared and processed for total foliar [N] and [P] according to methods described in section *3.2.1.5*. An additional 20 needle fascicles were randomly selected over the entire crown of each tree and frozen in polyethylene bags. They were later scanned and their one sided projected surface area was calculated using Sigma Scan-Pro® image analysis software. They were then oven-dried at 68°C for 48 h, and weighed to within 0.0001 g to determine the SLA. The remaining needles were

placed in paper bags, dried for 72 h at 68°C and weighed. Total tree leaf area (A_L; cm^2) was calculated by multiplying the SLA for each tree by the total needle weight.

For carbohydrate determination, roots were rinsed to remove the growing media, partitioned into coarse ($\geq$ 2 mm diameter) and fine (<2 mm) categories (Samuelson 2000), dried for 72 h at 68°C, and ground to pass a 40-mesh screen. All ground material was stored in air tight containers at room temperature in the dark until the concentration of total non-structural carbohydrates [TNC] were determined (Chow and Landhäusser 2004).

3.2.3.3 Statistical analysis

T-tests were used to compare galled and non-galled trees. Statistical significance was assessed at α=0.05.

3.3 Results

3.3.1 Field experiment 1. Evaluating the role of reduced water flow in reducing the growth of galled trees

The acid fuchsin dye on the galled stem sections for which K_ψ was measured indicated that water flow occurred on the opposite side of the stem from the gall. The K_ψ decreased with increasing gall encirclement (Fig. 3-2); A_L and A_S decreased with increasing gall encirclement (Fig. 3-3A and B) and decreasing K_ψ (Fig. 3-3E and F), resulting in the A_L:A_S ratio remaining constant across the range of gall encirclements (Fig. 3-3C) and K_ψ values (Fig. 3-3G). Foliar [N] decreased with increasing gall encirclement (Fig. 3-3D) and decreasing K_ψ (Fig. 3-3H), whereas foliar [P] remained constant across the range of gall encirclement (Fig. 3-3D) and K_ψ (Fig. 3-3H) values. Multiple regression indicated that both A_L and A_S increased with increasing gall

encirclement following adjustment for K_ψ (Fig. 3-4A and 3-4B for A_L and A_S, respectively), and increased with increasing K_ψ following adjustment for gall encirclement (Fig. 3-4C and 3-4D for A_L and A_S, respectively).
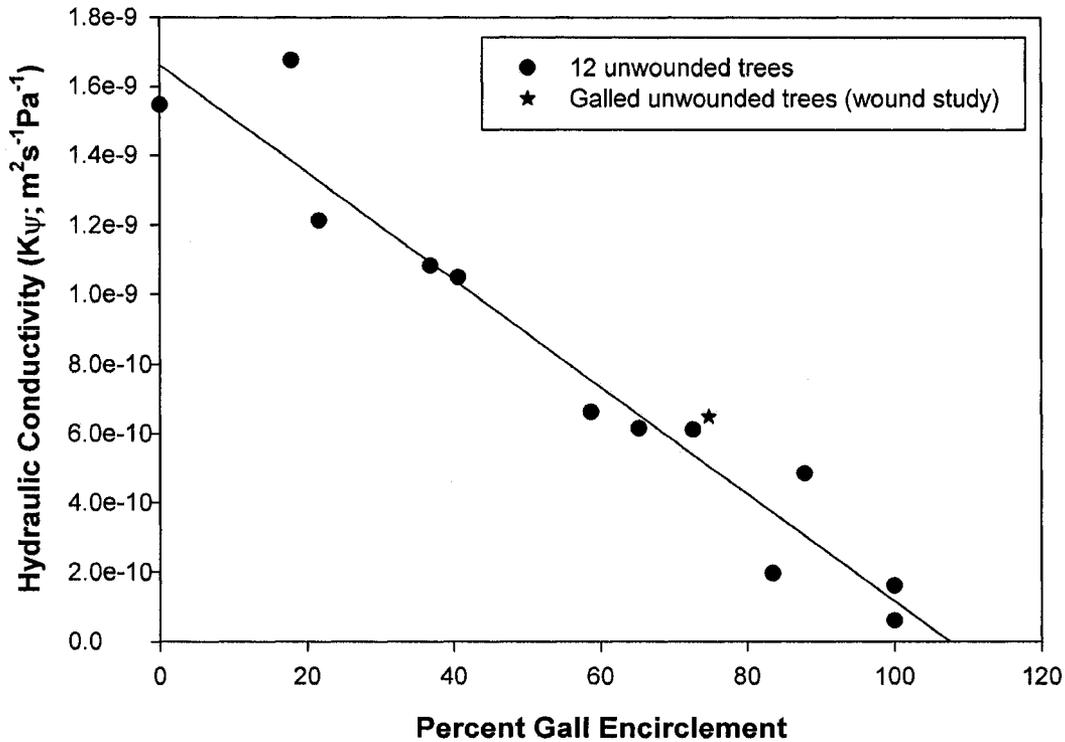
Figure 3- 2. Hydraulic conductivity (K_ψ; $m^2s^{-1}Pa^{-1}$) by percent gall encirclement for 12 open grown trees (p<0.01; R^2=0.94); K_ψ = -1.534E^{-11} * (%Gall Encirclement) + 1.658^{-09}. Closed circles represent data from the first field study. The star represents the average K_ψ of four galled unwounded trees in the second field study, having an average gall encirclement of 75% of the stem circumference.
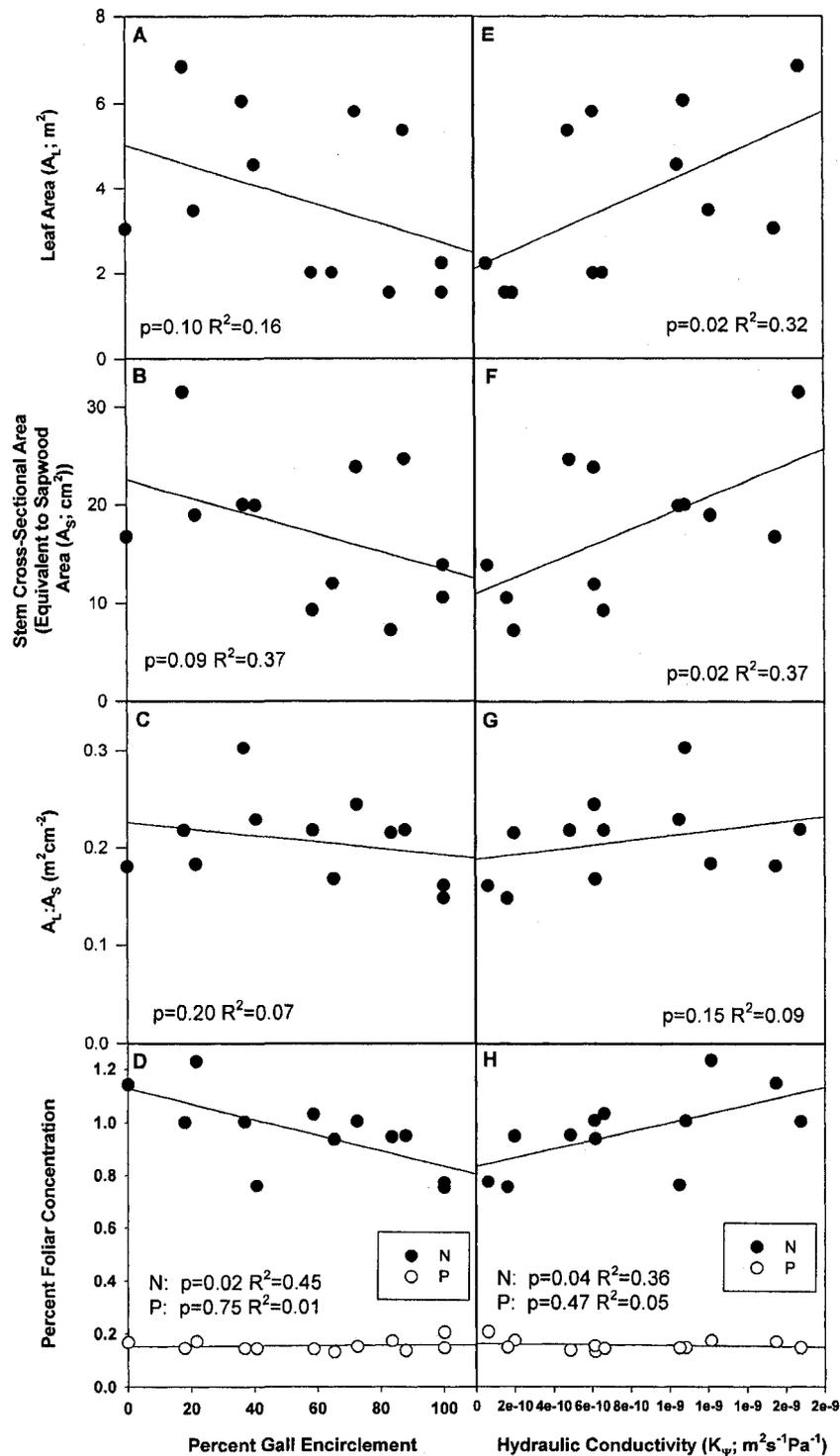
Figure 3- 3. Leaf area (A_L; m^2); stem cross-sectional area (equivalent to sapwood area (A_S; cm^2)); leaf area-to-sapwood area ratio (A_L:A_S; m^2cm^{-2}); and percent foliar nitrogen [N] and phosphorus [P] concentration by percent gall encirclement (Fig. 3-3A, B, C, D) and hydraulic conductivity (K_ψ; $m^2s^{-1}Pa^{-1}$) (Fig. 3-3E, F, G, H) for 12 open grown lodgepole pine trees.
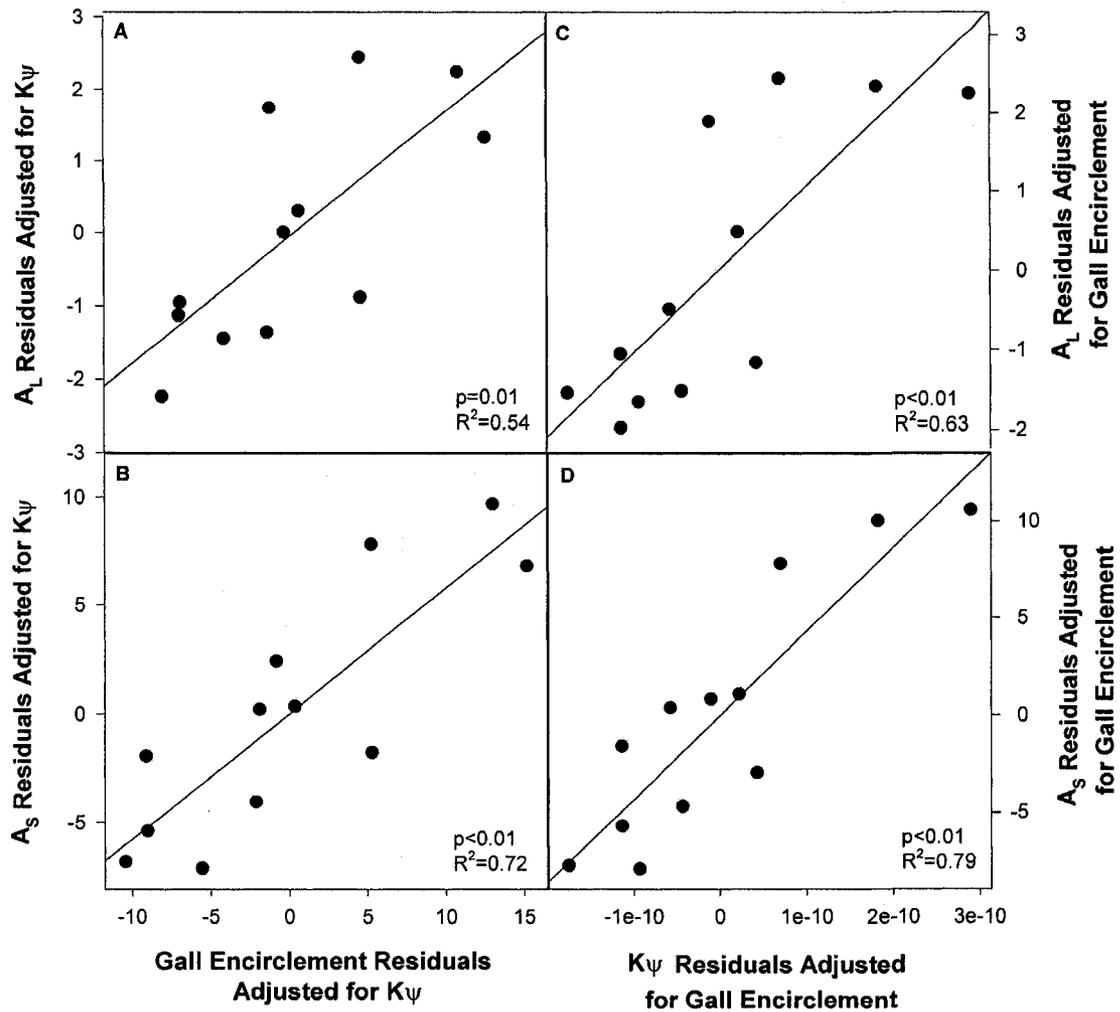
Figure 3- 4. Partial plots of leaf area (A_L; m^2) and sapwood area (As; cm^2) residuals against gall encirclement residuals following adjustment for the effect of hydraulic conductivity (K_ψ; m^{2}s^{-1}Pa^{-1}) (Fig. 3-4A and 3-4B, respectively), and partial plots of A_L and As residuals against K_ψ following adjustment for the effect of gall encirclement residuals (Fig. 3-4C and 3-4D, respectively) for 12 open grown lodgepole pine trees.

3.3.2 Field experiment 2. Impact of galls and xylem and phloem disruptions on tree growth

Throughout the duration of the experiment only three galled trees with xylem

wounds on the opposite side of the stem from the gall died. Prior to treatment

application, the dbh, tree age, percent gall encirclement, gall height, and gall age

measurements were similar (p>0.05) across treatments, whereas crown length differed

between the GFP and NGX treatments (Table 3-2).

Table 3- 2. Summary of the average tree characteristics in May 2003 prior to the
application of the wound treatments (n=157 for all variables except gall age).

Tree Characteristic	Treatment							
	GFP	GFX	GSP	GSX	GC	NGC	NGP	NGX
Dbh (cm)	7.3	7.8	7.3	7.5	7.1	7.7	7.3	8.1
Percent gall encirclement	53.2	60.4	51.8	54.6	61.2	.	.	.
Gall height (cm)	108.3	92.9	123.2	97.2	100.7	.	.	.
Crown length (cm)	407.6[a]	453.9[ab]	468.5[ab]	453.4[ab]	438.5[ab]	462.9[ab]	430.9[ab]	493.2[b]
Gall age*	8	8	9	9	10	.	.	.

Note: Treatment abbreviations refer to the treatments as follows: GFP=galled tree with phloem wound on
opposite side of stem from the gall; GFX=galled tree with xylem wound on opposite side of stem
from the gall; GSP=galled tree with phloem wound directly above the gall; GSX=galled tree with
xylem wound directly above the gall; GC=galled unwounded control tree; NGC=non-galled
unwounded control tree; NGP=non-galled tree with phloem wound; and NGX=non-galled tree with
xylem wound.
[ab]Treatments with different subscripts are significant according to Tukey's multiple comparison test
(p<0.05).
*Average gall age of trees for which K_ψ was measured; estimated from the inflow side of the
galled/wound stem section (n=32).

The acid fuchsin dye patterns of the K_ψ stem sections indicated that water flowed

through stems on the opposite side of the stem from the gall, and that xylem wounds

resulted in dye patterns that were similar to those created by galls (i.e. both galls and

xylem wounds reduced water flow). The eight treatments clustered into three groups

based on their effect on K_ψ (Fig. 3-5): i) xylem wounds on the opposite side of the gall

(GFX), which had the lowest K_ψ; ii) trees without galls with or without phloem wounds,

which had the highest K_ψ; and iii) all other treatments, which had intermediate K_ψ values

(Fig. 3-5). Although the treatments resulted in a range of K_ψ values, there was no

significant relationship between K_ψ and either radial (p=0.86) or height (p=0.41) growth

(Fig. 3-5A and 3-5B, respectively). The radial growth did not differ among the

treatments (p=0.51); however, there was a significant height growth effect (p=0.03) with

Tukey's multiple comparison tests indicating that the height growth was less for trees

with xylem wounds on the opposite side of the stem from the gall than for trees with

xylem or phloem wounds directly above the gall (p=0.01 and 0.04, respectively). There

was no significant relationship between the K_ψ and foliar [N] and [P] (p=0.68 and 0.93,

respectively).
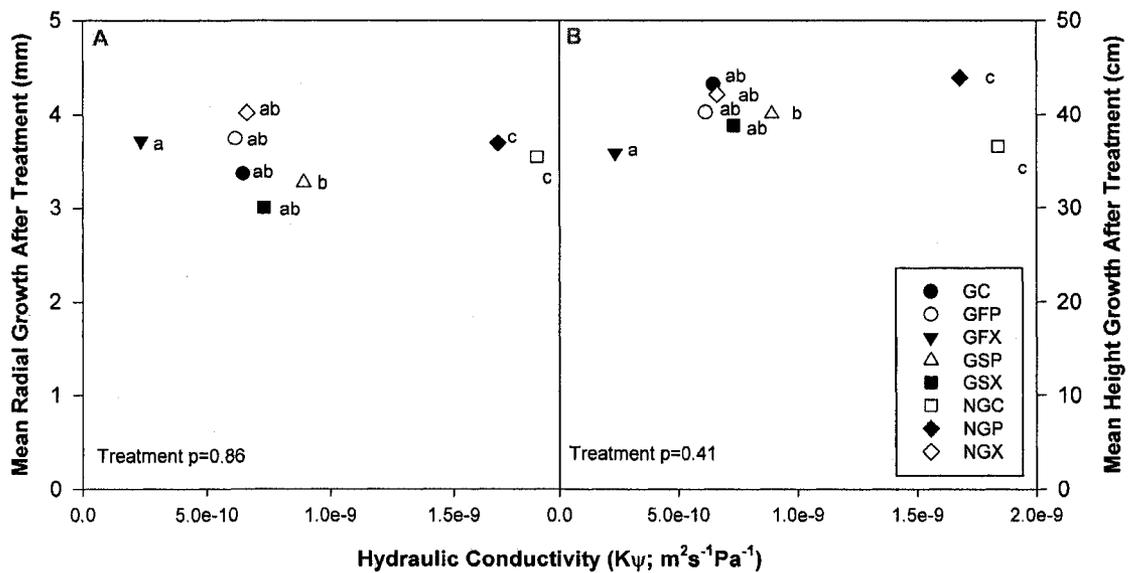
Figure 3- 5. Plots of mean radial and height growth following treatment by the mean
hydraulic conductivity (K_ψ; $m^2s^{-1}Pa^{-1}$) for each treatment (n=32; 3-5A and 3-5B,
respectively), adjusted for the mean radial and height growth 4-years and 2 years-
prior to treatment, respectively. Treatments having different letters have
significantly different K_ψ values according to Tukey's multiple comparison test
(p>0.05). Abbreviations refer to the wound treatments: GFP=galled tree with
phloem wound on opposite side of stem from the gall; GFX=galled tree with
xylem wound on opposite side of stem from the gall; GSP=galled tree with
phloem wound directly above the gall; GSX=galled tree with xylem wound
directly above the gall; GC=galled unwounded control tree; NGC=non-galled
unwounded control tree; NGP=non-galled tree with phloem wound; and
NGX=non-galled tree with xylem wound. P-values for the regression of radial
and height growth versus K_ψ (following adjustment for growth rates prior to
treatment) were 0.86 and 0.41, respectively.

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3.3.3 Greenhouse study

At the time of harvest, 92 and 57 percent of the galled and control trees had died, respectively. The majority of the control tree mortality occurred following transfer outside from the greenhouse in June 2004, and in February 2006 following winter storage. Survival of the control trees was relatively constant during the last year of growth in the greenhouse, whereas the mortality of the galled trees occurred throughout the entire duration of growth. The R:S was similar for galled and control trees (p=0.47). Nevertheless, galled trees were shorter (p<0.01), and had less A_L (p<0.01) than control trees (Table 3-3). The $A_L:A_S$ of galled trees was significantly lower than that of control trees (p<0.01) regardless of whether A_S was measured at the root collar, or above the gall/reference point. Although A_S at the root collar of galled and control trees was similar (p=0.35), A_S at the gall/reference point was 35 percent less in galled than control trees. The [TNC] of coarse roots was lower in galled than control trees (p<0.01).

Table 3- 3. Summary of the average tree characteristics of 15 galled (WGR) and 15 control (CTRL) trees, and t-test results.

Tree Characteristic	Treatment		
	WGR	CTRL	p-value
Height (cm)	40.1	57.6	<0.01
Leaf area (A_L; cm^2)	1781.6	4594.3	<0.01
Sapwood area at root collar ($A_{S(RC)}$; mm^2)	230.9	241.0	0.35
Sapwood area above gall/reference point ($A_{S(Above\ gall/ref\ pt)}$; mm^2)	80.2	127.9	<0.01
Leaf area-to-sapwood area ratio at the root collar ($A_L:A_{S(RC)}$; cm^2; mm^2)	7.7	19.8	<0.01
Leaf area-to-sapwood area ratio at the gall/reference point ($A_L:A_{S(Above\ gall/ref\ pt)}$; $cm^2\ mm^{-2}$)	24.4	36.4	<0.01
Root-to-shoot ratio (R:S; $g\ g^{-1}$)	0.25	0.25	0.47
Foliar nitrogen concentration [N]	1.83	2.07	<0.01
Coarse root concentration of total non-structural carbohydrates [TNC]	4.56	7.55	<0.01

3.4 Discussion

The first field study was conducted to test the general hypothesis that stem galls reduce tree growth by reducing water flow. There were three elements to this general hypothesis: first, reduced K_ψ was expected to reduce tree growth; secondly, larger galls were expected to result in a greater reduction in K_ψ than smaller galls and finally, tree growth was expected to decrease with larger galls as a consequence of the first two elements. As for the first element, reduced K_ψ was clearly associated with a decrease in tree size as reflected by the stem cross-sectional area (Fig. 3-3F), which was equivalent to the A_S, given the absence of heartwood formation. Although A_L generally cannot be used as a measure of tree growth, given that crown closure had not yet occurred for the trees in this study A_L represented an index of tree growth, and decreased with decreasing K_ψ (Fig. 3-3E). Furthermore, following adjustment for gall encirclement there was a strong partial correlation between K_ψ and tree growth, further emphasizing the importance of water transport in determining tree growth. As for the second element, there was strong evidence that K_ψ decreased with increasing gall encirclement (Fig. 3-2). It was somewhat surprising, therefore, that the third element (the relationships between gall encirclement and A_L and A_S) was quite weak (Fig. 3-3A and 3-3B); the partial correlations involving A_L and A_S may provide an explanation for this weakness. Both A_L and A_S increased with increasing gall encirclement (Fig. 3-4A and 3-4B), following adjustment for K_ψ. Furthermore, Peterson (1960) reported that faster growing trees had faster growing galls. It is possible, therefore that faster growing trees lead to larger stem galls, thus weakening the overall negative correlation between WGR encirclement and A_L and A_S.

The wound treatments in the second field study had the expected impact on the K_ψ, as xylem wounds on the opposite side of the gall had the greatest impact on K_ψ, and trees without galls (i.e. with or without phloem wounds) had the least impact on K_ψ. However, the reduction in the K_ψ associated with galls and xylem wounds did not translate into the expected impact on either the radial or the height growth (Fig.3-5A and 3-5B, respectively), even though the range in K_ψ values of the galled control trees in this study was the same as for the 12 unwounded trees in the first field study (Fig. 3-2). As a consequence, these data could not be used to test the relative importance of xylem and phloem disruption in reducing tree growth. However, the results are useful in showing the remarkable resilience of lodgepole pine to short term insults such as wounding. These findings are not unprecedented; Hubbard et al. (1999) attributed the lack of a reduction in the leaf specific hydraulic conductance following the notching of branches to a redundancy (i.e. an excess capacity to transport water) in the sapwood of *Pinus ponderosa* Dougl. ex P. & C. Laws.

There are a number of other possible reasons why decreasing K_ψ was associated with decreased growth in the first, but not the second field study. First, although the field studies were contained within the same cutblock it is possible that the soil and microclimate conditions of the two studies differed. The 12 unwounded trees in the first field study would have experienced less inter-tree competition than the trees in the second field study, due to the absence of neighbouring trees within a 0.5 m radius; water use was reported to increase in thinned (i.e. open) lodgepole pine stands (Reid et al. 2006). Many of the trees in the wounding experiment had neighbours immediately adjacent to them, which would have increased the inter-tree competition for water and nutrients. However,

given that the K_ψ of the galled control trees in the second experiment was similar to that which would have been predicted based on the relationship between K_ψ and gall encirclement in the first experiment (Fig. 3-2), microclimatic differences may not be the best explanation for the discrepancy between the two studies.

Secondly, diameter growth of the 2 years following the imposition of wounds may have been supplemented by stored carbohydrate, as diameter growth only occurs once the resource demands of foliage and root growth have been met (Waring 1987). Root grafts in lodgepole pine trees were determined to support shaded suppressed trees (Fraser et al. 2006); therefore, it is possible that the wounded trees were supported by stored carbohydrate obtained from root grafts with more vigorous trees. The measurement of growth between 2000 and 2005 coincided with the two wettest years, as the average annual precipitation in the area was 418 mm and 581 mm for 2000-2003, and 2004-2005, respectively. Had the wound treatments been imposed during years with limited precipitation, it is possible that growth would have been reduced following the 2 year duration of the wound study, as prolonged drought depletes carbohydrate reserves (Waring 1987).

Thirdly, the wounding treatments may have failed to reduce growth because the length of stem affected by the gall or wound treatment was small relative to the total tree height. To test this possibility, overall tree hydraulic conductivity ($K_{effective}$: $m^2 s^{-1} Pa^{-1}$) was predicted for trees with galls encircling 0 to 100 percent of the stem circumference. The following assumptions were made: 1) Water movement in trees could be described by the following formula (Jury et al. 1991) for water movement through layered soils:

$$\text{Overall Conductivity} = K_{effective} = \sum L_j / \sum (L_j / K_j),\qquad\qquad(5)$$

where L_j is the segment length, and K_j is the K_ψ associated with a particular stem segment. 2) The equation $K_\psi = -1.534E^{-11} * (\%\text{Gall Encirclement}) + 1.658^{-09}$ (Fig. 3-2) could predict K_ψ values for stem segments of length 0.21 m (the average length of stem segment tested in the evaluation of K_ψ for the 12 unwounded open grown trees). 3) Tree height equalled 3.30 m (the average height of the 12 unwounded open grown trees); hence each tree was comprised of 15.71 segments. 4) Of those 15.71 segments, 14.71 had a K_ψ of 2.00^{-09} $m^2s^{-1}Pa^{-1}$ (the predicted K_ψ of non-galled trees), and the galled segment of length 0.21 m had a K_ψ value predicted by the percent gall encirclement of the stem. Plots of overall conductivity of trees with a range of gall encirclements illustrated that stem galls would not have had a large impact on the overall conductivity until the percent gall encirclement was approximately 60% of the stem circumference (Fig. 3-6); at 60% gall encirclement of the stem the overall conductivity would be 93% of a non-galled tree. Acknowledging the limitations associated with the assumptions made to apply the layered soil analogy to the entire tree, it appears evident that small stem galls have a relatively small impact on whole-tree water movement, particularly over a short time period. After many years the disruption in flow could affect tree growth, but over a two year period this effect might not be evident.
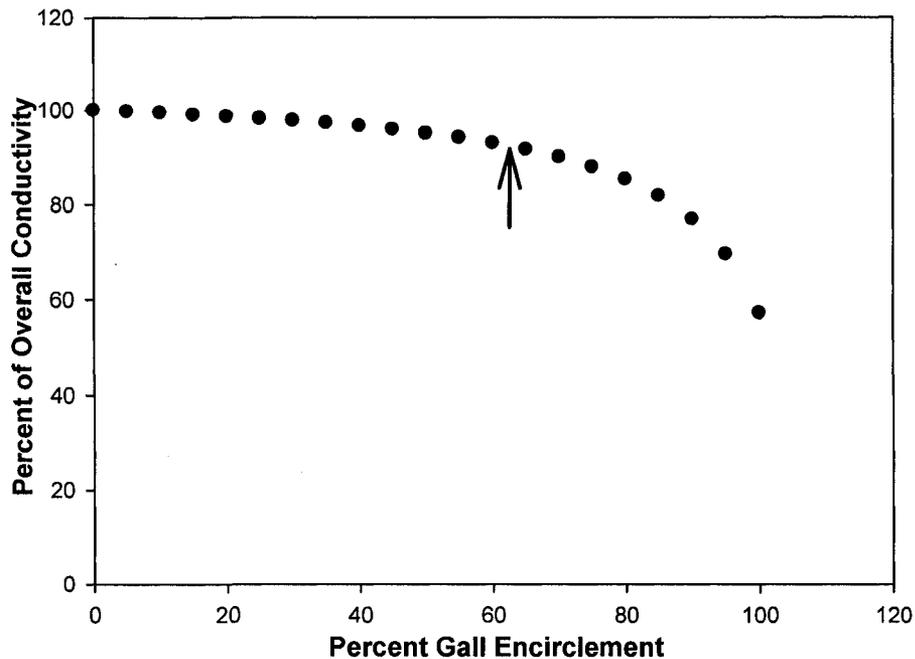
Figure 3- 6. Percent of overall conductivity ($K_{effective}$; $m^2 s^{-1} Pa^{-1}$) by percent gall encirclement of the stem. $K_{effective}$ estimated by applying the layered soil analogy described in Jury et al. (1991) using the average tree characteristics from 12 open grown lodgepole pine trees. Arrow indicates that the $K_{effective}$ is 93% of a non-galled tree at a percent gall encirclement of 60%.

Fourthly, although the trees in the field studies had galls of similar age (Table 3-1 and 3-2), indicating that the majority of the galls in both experiments formed as a result of a wave year of infection (Peterson 1971), the length of time trees grew following the imposition of stress differed between the two studies. The effect of gall encirclement on A_L and A_S of the 12 unwounded trees in the first field experiment reflected the trees' growth response for 9-11 years following infection by WGR (Table 3-1). In contrast, only two years of growth response were captured following wound treatments in the second field experiment. Given the wetness of the two years following imposition of the wounding treatments, the potential for stored carbohydrates to supplement tree growth, and that disruption of water flow over a small portion of the bole may not greatly affect

total stem conductivity (Fig. 3-6), the absence of a treatment effect on tree growth in the second field experiment is not that surprising.

It was hypothesized that galled trees would respond to the reduced water flow by increasing the R:S relative to control trees to increase water uptake, as seedlings grown in dry moisture regimes tend to allocate more resources to root growth (Bongarten and Teskey 1987). Contrary to expectation, the greenhouse study determined that galled trees do not increase root growth relative to total aboveground growth (Table 3-3). However, the [TNC] results (Table 3-3) support the earlier suggestion that galls disrupt phloem transport (True 1938; MacFall et al. 1994), and they may provide a possible explanation for the similar R:S of galled and non-galled trees. Assuming that WGR results in sequestration of carbohydrates at the gall, the resources required for increased root growth may be unavailable to the tree.

Although galls partially encircling the stem do not kill trees immediately, the chronic hydraulic constriction may ultimately result in tree mortality. Because A_L and A_S both decreased with increasing gall encirclement (Fig. 3-3A and 3-3B), the $A_L:A_S$ remained constant (Fig. 3-3C), indicating that trees adapt over time to the reduced capacity of the stem to supply the foliage with water. The decreased A_L and A_S associated with increasing gall size (Fig. 3-3A and 3-3B) and decreasing K_ψ (Fig. 3-3E and 3-3F) suggests that galled trees in the field will be suppressed as stands age, resulting in further reductions in the K_ψ. Presumably the impact of galls on tree growth increases with time as gall encirclement increases (Peterson 1960), and in the long term, WGR-infected trees may die as inter-tree competition for resources increases. Reid et al. (2003) noted that dominant trees contain a greater proportion of earlywood in the outer rings of

the sapwood than suppressed trees, and as a result had greater hydraulic permeability. Shade induced foliage and lower branch mortality may be greater in galled than non-galled trees as the associated reductions in the earlywood, tracheid diameter and branch hydraulic permeability result in reduced stomatal conductance and photosynthesis (Protz et al. 2000). Furthermore, the decrease in the foliar [N] with increasing gall encirclement (Fig. 3-3D) and decreasing K_ψ (Fig. 3-3H) suggests that the reduced water flow through large stem galls reduces water and N uptake, and ultimately the photosynthetic efficiency of foliage above galls, as the majority of foliar N is allocated to photosynthetic proteins (Evans 1989). Although both foliar N and P are typically correlated with photosynthesis, Reich and Schoettle (1988) proposed that only N impacts photosynthesis when P:N ratios are greater than 0.10 to 0.14, while both N and P influence the photosynthetic efficiency when the ratio of P:N is lower; the P:N ratios of the 12 unwounded trees (Fig. 3-3D and 3-3H) were consistently greater than 0.14, suggesting that only N was limiting photosynthesis. Reduced photosynthesis would in turn reduce A_S, leading to further reductions in water and N uptake, and compromised photosynthesis and tree growth.

Although the $A_L:A_S$ was constant across the range of gall encirclements (Fig. 3-3C) and K_ψ (Fig. 3-3G) values, the $A_L:A_S$ of galled trees may decrease relative to non-galled trees with age. Meinzer et al. (2004) suggested that the development of mistletoe infections over several years may result in transient changes in tree allometry; the constant $A_L:A_S$ in the 12 unwounded trees may represent a transient stage in tree growth following infection by WGR. Several species show a decrease in the $A_L:A_S$ with increasing tree height: Douglas-fir (McDowell et al. 2002); ponderosa pine (McDowell et al. 2002); Scots pine (McDowell et al. 2002; Mencuccini and Grace 1996); and

maritime pine [*Pinus pinaster* Ait.] (Delzon et al. 2004). McDowell et al. (2002)

indicated that the majority of studies suggest that the decrease in the $A_L:A_S$ with tree

height occurs to minimize the reductions in hydraulic conductance as trees grow taller.

Although increases in the $A_L:A_S$ have been observed in *Picea abies* (McDowell et al.

2002; Köstner et al. 2002), and *Abies balsamea* (McDowell et al. 2002), the

compensation for hydraulic limitations on water supply to foliage is less likely to occur in

early successional, and shade-intolerant species (Sala 2006), such as lodgepole pine.

Hence, a greater reduction in the $A_L:A_S$ of galled trees relative to non-galled trees with

increasing height is expected.

Mortality of WGR-infected trees may be higher in low density stands, resulting

from planting or precommercial thinning. Although precommercial thinning of dense

lodgepole pine stands reduces mortality resulting from competition, increases diameter

growth (Johnstone 1981), and improves site water relations (Donner and Running 1986),

trees may become infected with WGR following planting or thinning. The remaining

larger, faster growing trees will presumably result in larger galls (Peterson 1960) that

have a lower probability of survival (Wolken et al. 2006). Increased tree sway resulting

from increased wind speed through low density stands may further reduce the K_ψ through

stem galls. The sapwood permeability of slash pine was increased by staking seedlings to

reduce wind sway (Dean 1991); Liu et al. (2003) attributed the reduced specific

conductivity observed in thinned versus unthinned stands to the damage resulting from

the increased bending of stems following thinning. The increased bending of trees in low

density stands may increase the mortality of galled trees resulting from breakage, as stem

breakage at the gall has been reported as a cause of galled tree mortality (Gross 1983; van der Kamp 1988).

Both tree size and gall encirclement determine the severity of the growth response following infection by WGR. It is noteworthy that 92 percent of the galled trees in the greenhouse had succumbed to mortality prior to the time of harvest in contrast to the 57 percent mortality of the control trees, indicating that large galls on small trees are far more detrimental to tree growth and survival than smaller galls on large trees. The high mortality of control trees in the greenhouse study coincided with exposure to natural conditions following transfer from the greenhouse, and winter drying, whereas the mortality of galled trees occurred throughout the duration of the study, and was most likely the result of the hydraulic constriction created by the gall. The response of small trees in the greenhouse study was considerably different than that of large trees with smaller galls in the field studies. The small, young trees in the greenhouse had galls that fully encircled the stem, resulting in a profound affect on tree growth; the majority of the growth characteristics of galled trees were less than those of healthy trees (Table 3-3). The significant reduction in A_L accounted for the lower A_L:A_S in galled than non-galled trees regardless of whether A_S was measured at the root collar (where A_S was similar for galled and non-galled trees) or above the gall/reference point (where A_S was significantly lower in galled than non-galled trees). Although A_S at the root collar was not reduced, the above gall stem diameter was greatly diminished, which contradicts the observation that the stem above galls is larger than that below galls (True 1938). However, direct comparisons of stem diameters may be inappropriate as the trees in True's study were between 9- and 30-years old.

In summary, these results indicate that the reduction in K_ψ through galled stem sections is proportional to the percent of the stem encircled by the gall. Galled trees in the field reduced both A_L and A_S to accommodate the reduced capacity of the stem to supply the foliage with water, resulting in the A_L:A_S remaining constant. The reduction in K_ψ associated with a series of wounding treatments did not translate into the expected impact on either the radial or height growth. The lack of a growth response may be attributed to the fact that growth was measured only two years after the treatments were imposed, which may not have provided adequate time to detect effects, especially since those two years were fairly wet and stored carbohydrate may have supplemented tree growth. Although galled trees did not increase belowground growth relative to non-galled trees to compensate for the reduced K_ψ through galls, phloem disruption combined with the large size of the galls may explain the similar R:S between galled and non-galled trees in the greenhouse; the impact on the growth of small trees in the greenhouse was more severe than that of larger trees with smaller galls in the field. WGR-infected trees are resilient; however, the chronic hydraulic constriction created by galls, and the increase in inter-tree competition and gall size with tree age suggest that the A_L:A_S will decrease with tree height, and that trees with large stem galls will ultimately die. The diminished growth of galled trees associated with reduced water flow has important management implications associated with tree survival and stand density at harvest age. To more accurately model the growth of WGR-infected stands, future studies need to relate above and belowground growth to the whole-tree water relations of galled and non-galled trees.

3.5 References

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4   CHAPTER FOUR

Whole-tree water relations of western gall rust infected lodgepole pine trees in response to soil drought

4.1   Introduction

The fungus causing western gall rust (WGR), *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka (syn: *Peridermium harknessii* J.P. Moore) is an important forest pathogen of lodgepole pine [*Pinus contorta* Dougl. ex Loud.] trees. Tissues of the current year's expanding shoots are susceptible to infection (True 1938). Infection results in gall formation on the main stem and lateral branches (Hiratsuka and Powell 1976). Stem galls result in tree mortality (van der Kamp 1988); the likelihood of mortality increased when galls encircled more than 80% of the stem circumference (Wolken et al. 2006). Stem breakage at the gall accounted for mortality 20 years following infection (Gross 1983; Zagory and Libby 1985; van der Kamp 1988). The tracheids of galled tissues are deformed relative to non-galled tissues (Zalasky 1976), and water flow through stem galls was reduced proportionally with the percent encirclement of the stem, resulting in reduced leaf area (A_L) and sapwood area (A_S) above galls (Chapter 3). The reduced water flow and associated reductions in tree growth suggest that galled trees may be more negatively impacted by drought events than non-galled trees.

Reid et al. (2005) proposed that the term hydraulic conductivity (K_ψ) be used to describe the water conducting properties of sapwood xylem. K_ψ is consistent with Darcy's law, and is defined as:

$$K_\Psi = \frac{Q}{A_S}\frac{L}{\Delta\Psi},$$

(1)

where Q is the mean flow rate of water through a stem sample of length L and conducting area of sapwood A_S, under a water potential difference $\Delta\psi$ expressed in pressure units. The leaf specific hydraulic capacity (Q_L) describes the functional relationship between the capacity of the stem to transport water and the distal A_L supported by the stem, and may be calculated by substituting A_L for A_S in Eq. 1 (Reid et al. 2005):

$$Q_L = \frac{Q}{A_L} \frac{L}{\Delta\Psi},$$ (2)

where A_L represents the leaf area (cm^2), and Q, $\Delta\psi$, and L are as defined for Eq. 1.

The conductance of water through tree stems is commonly referred to as the leaf-specific hydraulic conductance or soil-to-leaf hydraulic conductance (Hubbard et al. 1999; Ewers et al. 2000; Phillips et al. 2002; Addington et al. 2004; Addington et al. 2006), and is defined as:

$$K_L = \frac{E_L}{(\psi_{soil} - \psi_{leaf} - h\rho_w g)},$$ (3)

where E_L represents the whole-tree transpiration per unit leaf area, ψ_{soil} and ψ_{leaf} are the soil and leaf water potentials (MPa), respectively, and $h\rho_w g$ is the gravitational component of the water column of height h, density ρ_w (density of water at 20°C = 998.23 kg m^{-3}), and g is the acceleration due to gravity. The conductance term is used to quantify the movement of water from the soil to the leaves, as the path length that water travels cannot be accurately quantified.

Trees respond to long term environmental conditions by adjusting their hydraulic architecture. The leaf area-to-sapwood area ratio (A_L:A_S) of *Pinus sylvestris* L. trees growing on a warm and dry site was lower than trees growing on a cool and wet site (Mencuccini and Grace 1995). *Pinus palustris* P. Mill. trees growing on a xeric site were

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shorter and allocated more resources to root growth than mesic site trees, resulting in similar K_L values on the two sites (Addington et al. 2006). The $A_L:A_S$ decreased in *Pinus ponderosa* P.& C. Lawson with increasing vapour pressure deficit (DeLucia et al. 2000), and vulnerability to cavitation, resulting in increased water supply to foliage (Piñol and Sala 2000). It is currently unknown if the growth reductions associated with galled trees (Chapter 3) result in similar responses to soil drought as non-galled trees.

The goal of this research was to determine the responses of galled and control (non-galled) trees to soil drought. Assuming based on previous results (Chapter 3), that the K_ψ of galled trees would be lower than that of control trees, the specific objectives were to determine: 1) if the Q_L of galled and control trees differed; 2) given that the K_ψ and perhaps Q_L would be lower in galled trees relative to control trees, which of the hydraulic parameters comprising K_ψ and Q_L would differ between galled and control trees and how would they respond to soil drought; and 3) if multiple soil drought cycles would have a greater effect on galled than control trees.

4.2   Methods

4.2.1   Seedling establishment

In May 2005, 460 1-year-old lodgepole pine seedlings were obtained from Coast-to-Coast Reforestation Inc. The seedlings were planted in Pro-Mix®BX growing media in 450-mL styroblock containers one day following removal from cold storage. The seedlings were grown in a greenhouse with an 18 h photoperiod, and watered as required. Two and 9 days after planting, the seedlings were fertilized with 10-52-10 (N-P-K) fertilizer, to aid root establishment.

4.2.2 Seedling inoculation

Eleven days after planting, 315 seedlings were inoculated with *E. harknessii* spores, and 145 left as controls. Spores were removed from storage in liquid nitrogen and warmed in a 40°C water bath for five minutes (Moltzan et al. 2001). Germinability on 1.5% water agar averaged 85 percent after 96 h.

Immediately prior to inoculation, the soil was saturated and the trees misted with distilled water using a chromatography sprayer. Inoculation was done by the "torn-needle" method (Myrholm and Hiratsuka 1993), in which a single needle fascicle was removed from the lower half of the elongating shoot and dry spores were applied directly to the small wound and around the entire stem circumference near the wound. A single fascicle was also removed from the control seedlings. The seedlings were again lightly misted with distilled water and a wire cage draped in wet paper towels was placed over each styroblock. The styroblocks were sealed in large opaque polyethylene bags (Blenis and Pinnell 1988; Moltzan et al. 2001) and placed in a dark growth cabinet for 48 h at 18°C, after which time the seedlings were returned to the greenhouse.

4.2.3 Seedling growth and fertilization

The seedlings were watered as required and fertilized at two week intervals with 20-20-20 (N-P-K) fertilizer. At the end of June, the seedlings were transferred outside, where the watering and fertilizing schedule was maintained until October.

In mid-November 2005, the seedlings were brought indoors, and CloudCover ® Outdoor-Indoor plant protector was applied at a dilution rate of 1:10 [CloudClover:Water] to reduce water loss during winter storage. The seedlings were removed from the styroblocks, and the root plugs wrapped in plastic in bundles of four

trees which were then placed in storage bins in a freezer at –2°C. Due to freezer malfunction, the bins of trees were placed outside on the roof of the greenhouse while the outside temperature was between 0 and –6°C. When the outside temperatures decreased below –6°C, the bins were moved to a cold room with a temperature fluctuating between 4° and 7°C, where they were stored until February 2006.

4.2.4 Greenhouse establishment 2006

In February 2006, WGR-inoculated and control trees were potted in 2.5 L pots in a 2:1 (sand:peat) mixture. Nutricote® Total 13-13-13 (Type: 100) controlled release fertilizer with micronutrients was mixed into each pot at a medium feeding rate (5 kg m^{-3}). The trees were watered twice per week for approximately 3 months, after which time they were watered every other day until July 2006, when they were watered daily. The trees were fertilized with a 20-20-20 fertilizer at 2 week intervals. Galled trees were sprayed with Safers®End All (30 mL L^{-1}) at approximately 2 week intervals to control aphids attracted to the galls.

4.2.5 Soil water measurements

A soil water retention curve was developed specifically for the 2:1 (sand:peat) mixture to convert soil water content (WC; %H_2O (v/v)) measurements to soil water potential (Ψ_{soil}; MPa) values. The portion of the soil water retention curve between - 0.015 and -1.0 MPa was developed using pressure plates. The wetter portion of the curve was developed using a soil column containing the 2:1 (sand:peat) mixture. Briefly, five time domain reflectometry (TDR) probes were inserted along the vertical length of the soil column at a spacing of 2 cm; a reservoir of water was lowered to generate a range of

soil WC once the TDR readings stabilized. The van Genuchten function (van Genuchten 1980) was fit to the data obtained from both the pressure plates and the soil column with the Levenberg-Marquardt algorithm as implemented in MathCad 13. To improve the accuracy of the TDR measurements, a calibration equation specific to the 2:1 (sand:peat) mixture was developed and employed in the calculation of the soil WC.

4.2.6 Drought stress experiment

4.2.6.1 Greenhouse experiment (GH)

In June 2006, two galled and two control trees were randomly selected from the population of "acceptable" trees. Acceptable galled trees had galls encircling the entire stem circumference, and sufficient stem length above the gall to perform the necessary experimental measurements; acceptable control trees had good growth form and an overall healthy appearance. At approximately 1700 hr, trees were watered until water drained from the bottom of the pots. The pot weight at 900 hr the following day was recorded as the field capacity weight (FC; g). A TDR probe, consisting of two 10.5 cm long metal rods was inserted into each pot, and left in place throughout the duration of the experiment to measure the volumetric soil WC. The experiment was performed three times with each time considered a separate block.

4.2.6.1.1 Stomatal conductance, leaf and soil water potential (ψ_{leaf} and ψ_{soil})

The stomatal conductance (mmol m^{-2} s^{-1}) was measured at 1200 hr each day on the same two fascicles of needles using a steady state porometer (LI-1600; Li-cor, Lincoln, NE) with a conifer cuvette. At the end of the experiment the leaf surface area that had been inserted into the conifer cuvette was determined by scanning the needles and using

Sigma Scan-Pro® image analysis software. The projected leaf surface area was calculated and stomatal conductance values were adjusted for the actual leaf area housed in the conifer cuvette.

Every second day the leaf water potential (ψ_{leaf}; MPa) was measured at 1200 hr using thermocouple psychrometer chambers (C-52 sample chambers; WESCOR Inc.), and a CR7 datalogger (Campbell Scientific, Logan, UT). The psychrometer chambers were calibrated using a range of NaCl solutions of known osmotic potential (Andraski and Scanlon 2004). A single fascicle on each tree was selected at the start of the measurement day (1000 hr); the fascicle was marked with a small rubber band, wiped with a Kimwipe moistened with distilled water to remove any foreign residues, and then blotted dry. The fascicles were then left to equilibrate until the 1200 hr measurement period, when they were removed with a razor blade, immediately sealed in plastic bags, and transported to the laboratory on ice. In the laboratory the needles were cut to length with a razor blade to fill the 9.5 mm diameter by 4.5 mm deep C-52 sample holders. Once filled, the C-52 chambers were placed in an insulated box to reduce the fluctuation of the temperature; the chambers were left to equilibrate for approximately 24 h, at which point the readings were deemed stable. The readings at the end of the equilibration period were converted to ψ_{leaf} values using the individual psychrometer chamber calibration curves and the temperature correction equation supplied by WESCOR Inc.

At 1200 hr each day the soil WC was measured using the TDR probes and a Tektronix 1502B cable tester. Soil WC measurements were converted to ψ_{soil} values using the calibration equation and soil water retention curve.

4.2.6.1.2 Whole-tree transpiration (E), hydraulic conductivity (K_ψ), and leaf-specific hydraulic capacity (Q_L)

At 1000 hr each day, the pots were sealed with a double layer of plastic bags so that any water loss would be from transpiration only. Pot weights were taken at 1000 and 1400 hrs. The 4-hr whole-tree transpiration (E; g H_2O) was determined by the difference in pot weights between 1000 and 1400 hrs. Following the 1400 hr pot weighing, the pots were uncovered to prevent anaerobic conditions. The E measurements were adjusted for the total tree leaf area (A_L; cm^2). The A_L was calculated by determining the specific-leaf area (A_L per dry weight of leaves ($cm^2 g^{-1}$)) of a sub-sample of needles, and multiplying this by the total dry weight of leaves. The 4-hr whole-tree transpiration per unit A_L will be referred to as E_L (g H_2O cm^{-2} A_L).

Although the conductance term in Eq. 3 is typically used when the path length through which water moves from the soil to the leaves is unknown, the K_ψ (Eq. 1) metric was used to quantify the movement of water through galled and control tree stems to incorporate the geometric properties of the flow pathway, specifically the length (L), and the cross-sectional area (A_S) (Whitehead et al. 1984). The K_ψ of individual trees was calculated as Whitehead et al. (1984) demonstrated for the permeability (K_ψ adjusted for the fluid viscosity of water) of individual trees. In using the K_ψ terminology, the L term in Eq. 1 was assumed to be equivalent to the distance over which $\Delta\psi$ was measured. The K_ψ was calculated according to Darcy's law (Eq. 1), where Q in Eq. 1 was measured gravimetrically, and is equivalent to the 4-hr whole-tree unadjusted transpiration (E; g H_2O) between 1000 and 1400 hr through the stem of length L (cm; the length between the root collar and the height of the needles used to measure the ψ_{leaf}) and conducting sapwood area (A_S; cm^2 calculated at the root collar diameter (cm)), under a water

potential difference ($\Delta\psi = \psi_{soil} - \psi_{leaf} - h\rho_w g$, where the ψ_{soil} and ψ_{leaf} are the 1200 hr soil

and leaf water potentials (MPa), respectively, and $h\rho_w g$ is the gravitational potential

(MPa) of a column of water of density ρ_w (998.23 kg m^{-3}) and height (0.01 MPa m^{-1}); g

(9.8 m s^{-2}) is the acceleration due to gravity) expressed in pressure units (MPa). The

resulting units for K_ψ were g H$_2$O cm^{-1} MPa^{-1}.

The leaf-specific hydraulic capacity (Q_L; g H$_2$O cm^{-1} MPa^{-1}) was calculated

according to Eq. 2, where Q was measured gravimetrically and is equivalent to E defined

above. The $\Delta\psi$ and L variables are as defined for K_ψ above. The A_L is the whole-tree

leaf area (cm^2) calculated as described previously.

4.2.6.1.3 Drought cycle

For the first three days of the measurement period, water was added to the pots with

a large syringe prior to sealing the pots at 1000 hr, so that the pot weight was

approximately 95% of the FC pot weight at the start of the measurement period. The pots

were not watered to FC in order to prevent water loss from the pots via drainage; it was

not always necessary to add water to all pots on these three days. Following the three

measurement days in the well-watered condition, water was withheld for 10 days, at

which time the trees were harvested.

4.2.6.2 Growth chamber experiment (GC)

The greenhouse experiment (GH) was repeated for three experimental blocks each,

with two galled and two control trees, in a growth chamber (GC). Light and temperature

were maintained at 18 h:6 h, and 21°C:15°C (day:night), respectively. Trees were

transferred to the growth chamber from the greenhouse on day 1 of the experiment

following the insertion of the TDR probes and the determination of the FC of each tree. The following modifications were made to the drought stress methodology in the GC experiment: 1) stomatal conductance was not measured; 2) the ψ_{leaf} was measured daily; and 3) the trees were monitored through two dry down cycles. In the first dry down cycle (C1) water was withheld until the soil WC was at or below 5.10% for three days, at which time the trees were watered to 95% of the FC following the 1400 hr measurement period. The trees were then monitored through a second dry down cycle (C2) until the 5.10% WC threshold was reached, at which time the trees were harvested. The initial belief was that a ψ_{soil} value of -1.0 MPa would be stressful and that this corresponded to a soil WC of 6.90%. However, following modifications made to the fitting of the van Genuchten function, it was determined that trees were stressed at ψ_{soil} values at or below -0.1 MPa, which corresponded to a soil WC of 5.10%.

4.2.7 Statistical analysis

All statistical analyses were performed using SAS® (SAS Institute Inc., Cary, N.C.). Statistical significance was assessed using $\alpha=0.10$ to reduce the likelihood of a Type II error associated with small sample sizes.

Because of the nonlinear relationship between E and soil WC, piecewise regression (Freund and Littell 1991) was used to model their relationship. The relationship between E and soil WC was determined separately for each tree in the GH and for the first dry down cycle in the GC (there were insufficient data to permit analysis of the effect of water content on E for the second dry down cycle in the GC experiment). Data following three days at the -0.1 MPa ψ_{soil} threshold in the GH were removed because there was little change in E after that time. Two different models were assessed: i) Model 1 allowed the

slope before the knot to vary with b0; and ii) Model 2 assumed that the transpiration before the knot was constant (b2=0). The generalized F-test determined that Model 2 best described the trees in both the GH and the GC C1 experiments.

Piecewise regression was not appropriate for modeling the relationship between $\Delta\psi$, K_Ψ or Q_L and the soil WC. Several alternative models (Sit and Poulin-Costello 1994) were evaluated, but none fit the data well. Multivariate analysis of variance was deemed an inappropriate alternative for the comparison of the $\Delta\psi$, K_Ψ and Q_L of galled and control trees due to the small number of sample trees. As a result, the GH, GC C1 and GC C2 data were partitioned into two sets. The first represented well-watered conditions (soil WC $\geq$ 11%, which corresponded to the critical soil WC predicted from the piecewise linear regression of E by soil WC) beyond which transpiration rapidly decreased. The average ψ_{soil} was -0.004 MPa, and thus $\Delta\psi$ was essentially the same as -1 times the ψ_{leaf}. The second set represented water-limited conditions (soil WC $\leq$ 8%, which corresponded to the clustering of the data at low soil WC). Within these two groups there typically was no change in K_Ψ, Q_L, or $\Delta\psi$ with soil WC. Thus, for each tree, measurements of hydraulic parameters measured on different days were averaged (separately for well-watered and water-limited conditions) prior to subsequent analyses.

Two major analyses were performed. First, to compare galled and control trees through one dry down cycle, data from the GH and GC C1 data were combined to increase power and inference space. Separate analyses were done for the well-watered and water-limited conditions. For each of these conditions, restricted maximum likelihood (REML) analysis was used (Littell et al. 2006). A full statistical model, including factors for experiment (GH and GC) and block was specified; random variables

with estimated negative variances were automatically excluded from the models by the REML method. Analysis of variance (ANOVA) was performed on the natural log transformed (ln) variables comprising K_Ψ (K_Ψ, E, $\Delta\psi$, A_S and L), and Q_L (Q_L, E, E_L, $\Delta\psi$, A_L and L). Thus it was possible to partition lnK_Ψ into:

$$lnK_\Psi = lnE + lnL - lnA_S - ln\Delta\Psi, \tag{4}$$

and lnQ_L into either:

$$lnQ_L = lnE + lnL - lnA_L - ln\Delta\Psi, \tag{5}$$

$$\text{or} \quad lnQ_L = lnE_L + lnL - ln\Delta\Psi, \tag{6}$$

Partial correlations, adjusting for differences in treatment (galled versus control) and experiment (GH versus GC), were performed to examine the relationship between both E and $\Delta\psi$ and water movement (either K_Ψ or Q_L). Where the partial correlations were significant, the slopes of the four groups of trees (corresponding to two treatments and two experiments and henceforth referred to as cohorts) were tested for similarity by determining if the 95% confidence intervals overlapped.

In the second major analysis, the impact of two dry down cycles on galled and control trees was assessed. The process was the same as for the first analysis except that the data were analyzed as a split plot because each tree was measured in both cycles.

4.3 Results and discussion

When the drought stress was imposed, approximately 14 and 16 months after inoculation with WGR in the GH and GC experiments, respectively, the long term response of galled trees to infection was a reduction in tree height, A_L, and A_L:A_S relative to control trees (Table 4-1).

Table 4- 1. Summary of the average tree characteristics of galled (WGR) and control (CTRL) trees following the application of a drought stress approximately 14 and 16 months after inoculation with western gall rust spores in the greenhouse and growth chamber experiments, respectively.

Architectural Variables	Greenhouse			Growth Chamber		
	WGR	CTRL	p-value	WGR	CTRL	p-value
Tree height (cm)	32.9	37.7	0.01*	33.3	35.9	0.12
Gall height (cm)	11.8	.	.	11.8	.	.
Height to mature needles (cm)	29.0	29.0	0.47	27.6	28.2	0.34
Leaf area (A_L; cm^2)	801	1201	<0.01*	944	1294	0.02*
Sapwood area (A_S; cm^2)	0.86	0.89	0.40	0.87	0.95	0.32
Leaf area:sapwood area ratio (A_L:A_S; $cm^2 cm^{-2}$)	961	1385	<0.01*	1065	1498	0.03*
Root:shoot ratio (R:S; g g^{-1})	0.19	0.20	0.21	0.17	0.19	0.95
Root:leaf ratio (R:L; g g^{-1})	0.32	0.28	0.11	0.29	0.26	0.13

Note: Asterisks indicate significant differences between galled and control trees within an experiment at $p<0.10$

Galled trees resembled trees growing on xeric sites as conifers on xeric sites were determined to allocate more biomass to A_S and roots (Callaway et al. 1994). Although the R:S were similar for galled and control trees (Table 4-1), suggesting that galled trees do not increase their belowground growth to overcome the hydraulic constriction, the R:L were marginally greater in galled than in control trees (Table 4-1), suggesting that the galled trees may have been in a transient stage of architectural adjustment. The regular fertilizer applications prior to the imposition of drought may also have masked the increase in the R:L of galled trees relative to control trees, as the root area-to-leaf area ratio (A_R:A_L) of *Pinus taeda* L. was determined to decrease with nutrient addition in nutrient poor sand soils, increasing the susceptibility to dieback during drought events (Ewers et al. 2000); belowground growth decreased with both water and nutrient availability (Gower et al. 1992; Gower et al. 1994). Addington et al. (2006) reported that the A_R:A_L of *P. palustris* trees was higher on a xeric site relative to a mesic site.

However, the increased allocation of biomass to root growth in galled trees may have been inhibited by the disruption of phloem at the gall, as the concentration of total non-structural carbohydrates [TNC] of coarse roots was lower in galled than in control trees (Chapter 3), resulting in limited carbon available for increased root growth in galled trees.

4.3.1 Whole-tree transpiration (E) as a function of soil water content

The piecewise linear regression of E by the soil WC fit both the GH and GC C1 data (Fig. 4-1A and E). In the GH and GC C1, the E of galled and control trees remained constant (Fig. 4-1A and 1E, respectively) as the soil dried until the soil WC was approximately 11 percent (Fig 4-1A and 1E, respectively). Further decreases in the soil WC led to a precipitous drop in E. A similar critical soil WC of 12 percent was reported for the E of 41-yr-old *P. sylvestris*; between 12 and 5 percent soil WC E decreased linearly with decreasing soil WC (Irvine et al. 1998), as was observed in the current study (Fig. 4-1A and E).
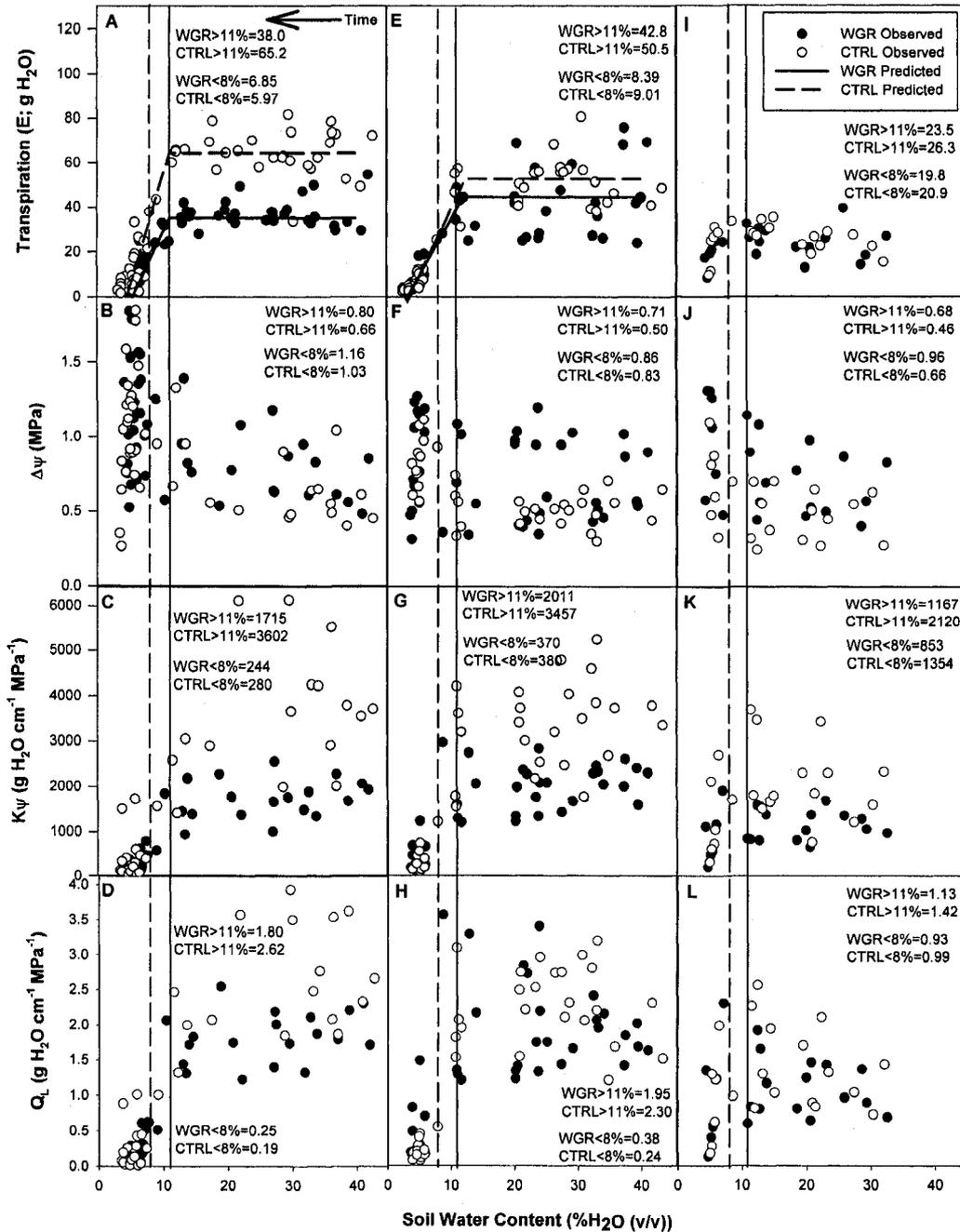
Figure 4- 1. Greenhouse (A-D) and growth chamber cycle 1 (E-H) and cycle 2 (I-L) whole-tree transpiration (E; g H_2O; A, E, and I); water potential difference ($\Delta\Psi$; MPa; B, F and J); hydraulic conductivity (K_Ψ; g H_2O cm^{-1} MPa^{-1}; C, G and K); and leaf specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}; D, H and L) by soil water content (WC; %H_2O (v/v)). Soil WC decreased with time following cessation of watering. The vertical lines represent the soil WC threshold of 11% estimated by the piecewise linear regression of E by soil WC (solid), and the clustering of the data at the dry end below 8% soil WC (dashed); WGR/CTRL>11% and the WGR/CTRL<8% refer to the average values of the hydraulic parameters at soil WC levels >11% and <8%, respectively.

Although the ψ_{soil} threshold of -0.1 MPa at which trees were deemed to be water stressed was relatively high, it was lower than the 11% critical soil WC (Fig. 4-2A), below which the piecewise linear regression model predicted a precipitous linear decrease in E with decreasing soil WC.
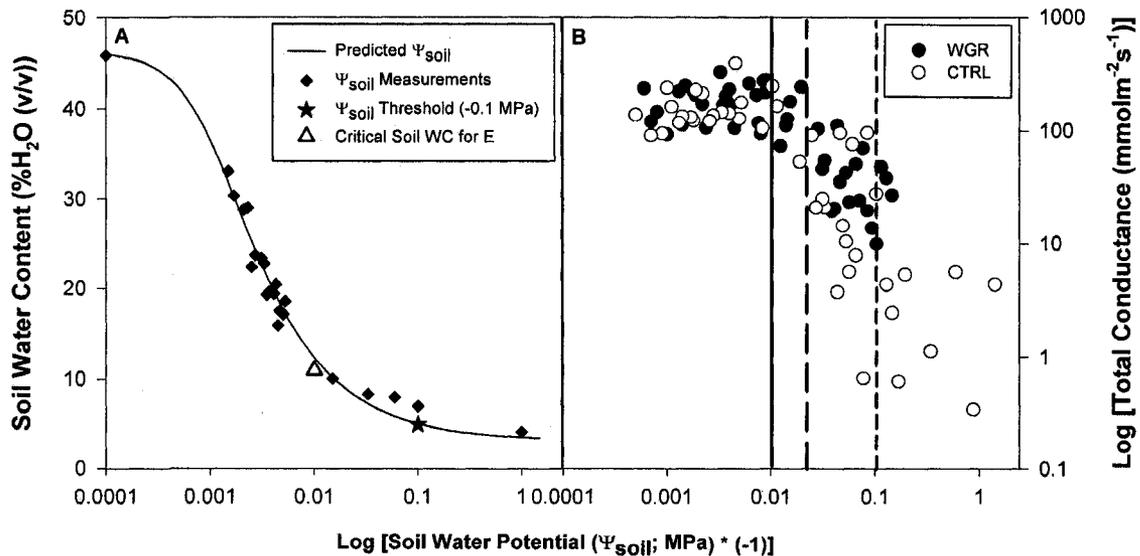
Figure 4- 2. A) Soil water retention curve for the 2:1 (sand:peat) mixture showing the volumetric soil water content (WC; $\%H_2O$ (v/v)) by the Log [Soil Water Potential (ψ_{soil}; MPa)*(-1)]; diamonds represent the ψ_{soil} measurements, the star represents the ψ_{soil} threshold of -0.1 MPa, and the triangle represents the 11% soil WC threshold predicted by piecewise linear regression of the whole-tree transpiration (E; g H_2O) versus soil WC. B) Log [Total conductance ($mmolm^{-2}s^{-1}$)] by the Log [Soil Water Potential (ψ_{soil}; MPa)*(-1)] for galled (WGR) and control (CTRL) trees in the greenhouse experiment; the vertical solid and long dash lines represent the well-watered (soil WC$\geq$11%) and water-limited (soil WC$\leq$8%) partitioning of the data; the vertical short dash lines represents the -0.1 MPa ψ_{soil} threshold.

This ψ_{soil} threshold also corresponded to very low stomatal conductance measurements in the GH experiment (Fig. 4-2B), indicating that the stomates were nearly closed at ψ_{soil} values lower than -0.1 MPa. Reductions in E have been reported for *Pinus radiata* D. Don seedlings at ψ_{soil} between 0 and -0.1 MPa (Squire et al. 1987; Babalola et al. 1968); Lopushinsky and Klock (1974) noted that E began to decline at ψ_{soil} between -0.1 and -

0.2 MPa. Coarse textured soils such as that used in the current study contain lower percent by volume WC than fine textured soils at the same ψ_{soil} (McColl 1973). The weaker capillary forces associated with the large pore spaces in coarse textured soils result in plants exhausting the available water supply at high ψ_{soil} relative to plants in fine textured soils, and as a result plants in sandy soils may become water stressed at relatively high ψ_{soil} (Hacke et al. 2000).

4.3.2 Galled versus control tree comparisons

4.3.2.1 Well-watered condition

The first major analysis combined the results from the GH experiment with those of GC C1 to compare galled and control trees through one dry down cycle. As expected from earlier results (Chapter 3), K_ψ was substantially reduced in galled trees relative to control trees (Table 4-2), indicating that galled trees have a less efficient stem to transport water than control trees. Galled trees responded to this decrease in conductivity in two main ways: reduced E and increased $\Delta\psi$ (Table 4-2). However, these two responses likely reflect different underlying processes occurring on different temporal scales. The reduced E of galled trees likely occurred primarily because of a reduction in A_L relative to control trees. Leaf area of galled trees was only about 70% of control trees (Table 4-1) and showed a high partial correlation with E ($R^2=0.64$; $p<0.01$), following adjustment for cohort (ie. the four combinations of treatment, galled versus control, and experiment, GH versus GC). Thus, because of the reduction in A_L, galled trees showed reduced E even before the process of measuring hydraulic parameters began. However, over the time period in which E and $\Delta\psi$ were measured, E was relatively independent of K_ψ within the cohorts as reflected by the low partial correlation between E and K_ψ ($R^2=0.05$; $p=0.35$,

Fig. 4-3B). In contrast, the partial correlation between $\Delta\psi$ and K_Ψ was quite high

(R^2=0.47; p<0.01, Fig. 4-3A). Furthermore, the slopes of the regressions of $\Delta\psi$ versus

K_Ψ were similar for all four cohorts. Taken together, these results indicate that over the

time frame that E and $\Delta\psi$ were measured, trees under well-watered conditions responded

to differences in K_Ψ primarily through increased $\Delta\psi$ (or equivalently, through decreased

Ψ_{leaf}, since Ψ_{soil} was essentially zero) rather than by decreasing E. This response was

similar for both galled and control trees.

Table 4- 2. Natural logarithm of the means (±standard deviation) of variables comprising the hydraulic conductivity (K_ψ; g H_2O cm^{-1} MPa^{-1}) of galled and control trees through one cycle of drought in the greenhouse and growth chamber experiments; E, A_S, $\Delta\psi$, and L represent the whole-tree transpiration (g H_2O), sapwood area (cm^2), water potential difference between the soil and the leaves (MPa), and the length between the root collar and the height of the mature needles (cm), respectively. The lnA_S and ln$\Delta\psi$ have been multiplied by -1 resulting in lnK_ψ = lnE + lnA_S + ln$\Delta\psi$ + lnL. The difference refers to the difference between control and galled treatments. The value in square parentheses is the 1-tailed p-value for the difference between control and galled treatments, and the value in round parentheses is the percentage of the difference in lnK_ψ between control and galled trees that is accounted for by the lnE, lnA_S, ln$\Delta\psi$, or lnL variables (discrepancies in the addition of the percentage difference are the result of rounding).

Soil WC ≥ 11%	lnK$_\Psi$	lnE	-[lnA$_S$]	-[lnΔΨ]	lnL
Control	8.13±0.33	4.05±0.20	0.12±0.29	0.60±0.27	3.35±0.09
Galled	7.49±0.26	3.67±0.29	0.16±0.19	0.32±0.35	3.34±0.11
Difference	0.64 [<0.01] (100)	0.38 [0.05] (60)	-0.04 [0.36] (-6)	0.28 [0.02] (43)	0.01 [0.38] (2)
Soil WC ≤ 8%					
Control	5.43±0.82	1.78±0.52	0.12±0.29	0.18±0.25	3.35±0.09
Galled	5.47±0.65	1.90±0.32	0.16±0.19	0.07±0.37	3.34±0.11
Difference	-0.04 [0.44] (100)	-0.13 [0.34] (304)	-0.04 [0.36] (85)	0.11 [0.19] (-257)	0.01 [0.38] (-29)
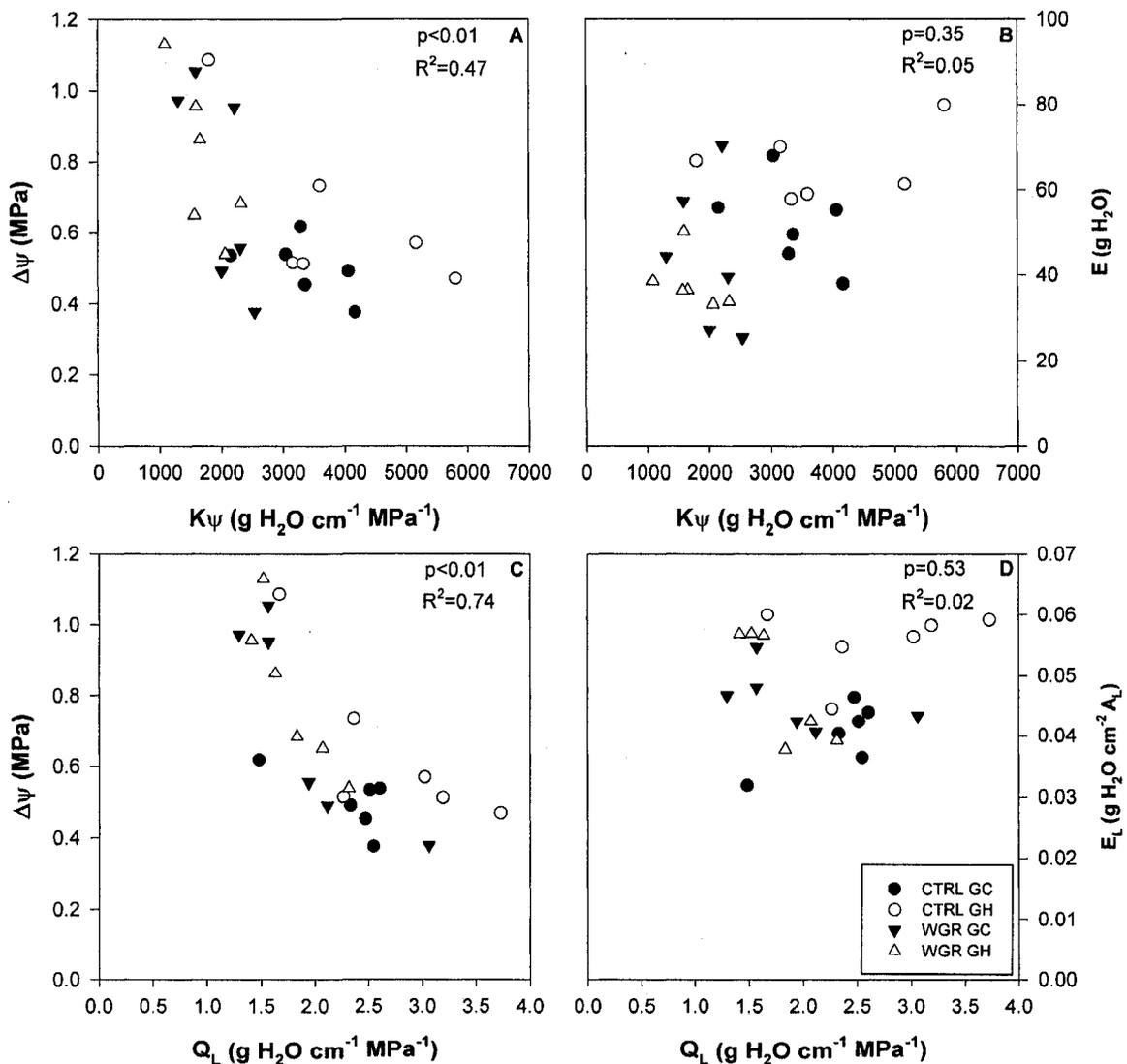
Figure 4- 3. Hydraulic conductivity (K_ψ; g H_2O cm^{-1} MPa^{-1})] by the difference in water
potentials between the soil and the leaves ($\Delta\psi$; MPa) and transpiration (E; g H_2O)
for galled (WGR) and control (CTRL) trees in the greenhouse (GH) and growth
chamber (GC) experiments through one cycle of drought (Fig. 4-3A and B,
respectively), and leaf specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}) by the
$\Delta\psi$, and the whole-tree transpiration adjusted for leaf area (A_L) (E_L; g H_2O cm^{-2}
A_L) (Fig. 4-3C and 4-3D, respectively) for WGR and CTRL trees in the GH and
GC experiments under well-watered conditions through one cycle of drought. P-
values represent partial correlations following adjustment for the treatment (WGR
versus CTRL), and the experiment (GH versus GC). R^2-values refer to partial
correlation plots of the residuals of the variables following adjustment for the
effect of treatment (WGR versus CTRL) and experiment (GH versus GC).

Examination of the components comprising Q_L from the combined GH and GC C1

analysis supports the contention that the host response to galls occurs on two temporal

scales. The explicit partitioning of transpiration per unit leaf area (E_L) into E and A_L

showed that they were both reduced by the same proportion in galled trees relative to

control trees, such that the E_L of galled and control trees was similar. Nevertheless, in

spite of the reduction in A_L, the hydraulic capacity, Q_L was less in galled than control

trees (Table 4-3), likely because the long term reduction in A_L in galled trees relative to

control trees was inadequate to compensate for the reduced ability of galled tree stems to

transport water. Over the shorter time frame in which hydraulic measurements were

taken, trees responded to differences in Q_L through changes in $\Delta\psi$ (R^2=0.74, p<0.01; Fig.

4-3C) rather than through changes in E_L (R^2=0.02; p=0.53; Fig. 4-3D). The similarity of

slopes among the four cohorts indicates that galled and control trees responded similarly

in changing $\Delta\psi$, rather than E_L in response to differences in Q_L. The lower Q_L values of

galled trees were associated with larger $\Delta\psi$ values; *P. sylvestris* trees lowered the leaf

specific hydraulic conductivity (Q_L in the current study) in response to drought, resulting

in higher $\Delta\psi$ (Martínez-Vilalta and Piñol 2002).

Table 4- 3. Natural logarithm of the means (±standard deviation) of variables comprising the leaf specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}) of galled and control trees through one cycle of drought in the greenhouse and growth chamber experiments; E_L, E, A_L, $\Delta\psi$, and L represent the whole-tree transpiration per unit leaf area (g H_2O cm^{-2} A_L MPa^{-1}), whole-tree transpiration (g H_2O), leaf area (cm^2), water potential difference between the soil and the leaves (MPa), and the length between the root collar and the height of the mature needles (cm), respectively. The lnA_L and $ln\Delta\psi$ have been multiplied by -1 resulting in lnQ_L = lnE_L + $ln\Delta\psi$ + lnL, or lnQ_L = lnE + lnA_L + $ln\Delta\psi$ + lnL. The difference refers to the difference between control and galled treatments. The values in square parentheses are the differences between control and galled treatments; the difference is a 1-tailed test for lnE, lnA_L, $ln\Delta\psi$, and lnL, and a 2-tailed test for lnQ_L and lnE_L. The values in round parentheses are the percentage of the difference in lnQ_L between control and galled trees that is accounted for by the lnE_L, lnE, lnA_L, $ln\Delta\psi$, or lnL variables (discrepancies in the addition of the percentage difference are the result of rounding).

Soil WC ≥ 11%	lnQ$_L$	lnE$_L$	lnE	-[lnA$_L$]	-[lnΔΨ]	lnL
Control	0.88±0.25	-3.06±0.21	4.05±0.20	-7.12±0.16	0.60±0.27	3.35±0.09
Galled	0.59±0.24	-3.07±0.15	3.67±0.29	-6.74±0.25	0.32±0.35	3.34±0.11
Difference	0.30 [0.01] (100)	0.01 [0.95] (2)	0.38 [0.05] (129)	-0.38 [<0.01] (-127)	0.28 [0.02] (93)	0.01 [0.38] (4)
Soil WC ≤ 8%						
Control	-1.81±0.68	-5.34±0.55	1.78±0.52	-7.12±0.16	0.18±0.25	3.35±0.09
Galled	-1.43±0.64	-4.84±0.44	1.90±0.32	-6.74±0.25	0.07±0.37	3.34±0.11
Difference	-0.38 [0.13] (100)	-0.51 [0.31] (132)	-0.13 [0.34] (34)	-0.38 [<0.01] (98)	0.11 [0.19] (-29)	0.01 [0.38] (-3)

The second major analysis, which evaluated the effect of two dry down cycles on galled and control trees was simplified by the lack of an interaction between the treatment effect (galled versus control trees) and the cycle effect (cycle 1 versus cycle 2). As a consequence, the effects of treatment and cycle were analyzed separately. The effect of galls on K_Ψ and its components was essentially the same as that found in the combined analysis of GH and GC C1, described above. K_Ψ was reduced in galled trees, which responded by reducing E and increasing $\Delta\Psi$ (Table 4-4).

Table 4- 4. Natural logarithm of the means (±standard deviation) of variables comprising the hydraulic conductivity (K_ψ; g H_2O cm^{-1} MPa^{-1}) of galled and control trees in the growth chamber experiment through two cycles of drought; the absence of a treatment-by-cycle interaction resulted in the pooling of the cycle 1 and cycle 2 data. The E, A_S, $\Delta\psi$, and L abbreviations are the whole-tree transpiration (g H_2O), sapwood area (cm^2), water potential difference between the soil and the leaves (MPa), and the length between the root collar and the height of the mature needles (cm), respectively. The lnA_S and $ln\Delta\psi$ have been multiplied by -1 resulting in $lnK_\psi = lnE + lnA_S + ln\Delta\psi + lnL$. The difference refers to the difference between control and galled treatments. The value in square parentheses is the 1-tailed p-value for the difference between control and galled treatments, and the value in round parentheses is the percentage of the difference in lnK_ψ between control and galled trees that is accounted for by the lnE, lnA_S, $ln\Delta\psi$, or lnL variables (discrepancies in the addition of the percentage difference are the result of rounding).

Soil WC ≥ 11%	lnK_Ψ	lnE	-[lnA_S]	-[$ln\Delta\Psi$]	lnL
Control	7.87±0.30	3.66±0.18	0.11±0.38	0.77±0.25	3.33±0.11
Gall	7.35±0.26	3.50±0.35	0.15±0.18	0.39±0.40	3.31±0.08
Difference	0.52 [<0.01] (100)	0.16 [0.06] (30)	-0.04 [0.41] (-8)	0.38 [<0.01] (73)	0.02 [0.35] (4)
Soil WC ≤ 8%					
Control	6.25±0.79	2.44±0.39	0.11±0.38	0.35±0.31	3.33±0.11
Gall	5.94±0.70	2.31±0.31	0.15±0.18	0.17±0.36	3.31±0.08
Difference	0.31 [0.16] (100)	0.13 [0.33] (44)	-0.04 [0.41] (-13)	0.19 [0.05] (62)	0.02 [0.35] (7)

The reduction in E was likely a long term response as a consequence of reduced A_L ($R^2 = 0.28$; $p<0.01$). Over the short term, $\Delta\Psi$ was more sensitive to differences in K_Ψ ($R^2 = 0.30$; $p<0.01$) than was E ($R^2 = 0.17$; $p=0.13$) with the slopes of $\Delta\Psi$ versus Q_L being similar for galled and control trees. In contrast, the effect of galls on Q_L and its components was slightly different than that found in the combined analysis of GH and GC C1. Although Q_L was numerically greater in control than galled trees, this difference was not significant (Table 4-5).

Table 4- 5. Natural logarithm of the means (±standard deviation) of variables comprising the leaf specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}) of galled and control trees in the growth chamber experiment through two cycles of drought; the absence of a treatment-by-cycle interaction resulted in the pooling of the cycle 1 and cycle 2 data. The E_L, E, A_L, $\Delta\psi$, and L abbreviations are the whole-tree transpiration per unit leaf area (g H_2O cm^{-2} A_L MPa^{-1}), whole-tree transpiration (g H_2O), leaf area (cm^2), water potential difference between the soil and the leaves (MPa), and the length between the root collar and the height of the mature needles (cm), respectively. The lnA_L and $ln\Delta\psi$ have been multiplied by -1 resulting in $lnQ_L = lnE_L + ln\Delta\psi + lnL$, or $lnQ_L = lnE + lnA_L + ln\Delta\psi + lnL$. The difference refers to the difference between control and galled treatments. The values in square parentheses are the differences between control and galled treatments; the difference is a 1-tailed test for lnE, lnA_L, $ln\Delta\psi$, and lnL, and a 2-tailed test for lnQ_L and lnE_L. The values in round parentheses are the percentage of the difference in lnQ_L between control and galled trees that is accounted for by the lnE_L, lnE, lnA_L, $ln\Delta\psi$, or lnL variables (discrepancies in the addition of the percentage difference are the result of rounding).

Soil WC ≥ 11%	lnQ_L	lnE_L	lnE	-[lnA_L]	-[$ln\Delta\Psi$]	lnL
Control	0.61±0.26	-3.50±0.11	3.66±0.18	-7.16±0.16	0.77±0.25	3.33±0.11
Gall	0.40±0.31	-3.30±0.05	3.50±0.35	-6.81±0.33	0.39±0.40	3.31±0.08
Difference	0.21 [0.19] (100)	-0.19 [<0.01] (-93)	0.16 [0.06] (75)	-0.35 [0.02] (-168)	0.38 [<0.01] (182)	0.02 [0.35] (11)
Soil WC ≤ 8%						
Control	-1.02±0.74	-4.71±0.52	2.44±0.39	-7.16±0.16	0.35±0.31	3.33±0.11
Gall	-1.02±0.86	-4.50±0.60	2.31±0.31	-6.81±0.33	0.17±0.36	3.31±0.08
Difference	-0.004 [0.98] (100)	-0.22 [0.27] (4963)	0.13 [0.33] (-3094)	-0.35 [0.02] (8048)	0.19 [0.05] (-4357)	0.02 [0.35] (-506)

More strikingly, E_L was greater in galled than control trees. By rearranging equation 6 to isolate the differences in E_L between control and galled trees as follows:

$$lnE_L = lnQ_L - lnL + ln\Delta\Psi \Leftrightarrow 0.01 = 0.30 - 0.01 - 0.28 \text{ (Table 4-3),} \qquad (7)$$

and

$$lnE_L = lnQ_L - lnL + ln\Delta\Psi \Leftrightarrow -0.19 = 0.21 - 0.02 - 0.38 \text{ (Table 4-5),} \qquad (8)$$

it can be seen that the increased E_L of galled trees relative to control trees in the second analysis was the result of: 1) the superior leaf specific hydraulic capacity of control trees

relative to galled trees being reduced; and 2) the greater $\Delta\Psi$ of the galled trees being increased. Unfortunately, the resolving power of the data are not such to permit further explanation of these results. In spite of this unexplained anomaly, the response of $\Delta\Psi$ and E_L to differences in Q_L was similar to that in the first analysis in that the former ($R^2=0.35$; $p<0.01$), but not the latter ($R^2=0.33$; $p=0.12$), had a significant partial correlation with Q_L.

The lower K_ψ, Q_L, E, and E_L values of galled and control trees in the GC C2 experiment relative to C1 (Table 4-6) may be indicative of some xylem cavitation in the stem.

Table 4- 6. Hydraulic conductivity (K_ψ; g H_2O cm^{-1} MPa^{-1}); leaf specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}); transpiration (E; g H_2O); transpiration per unit leaf area (E_L; g H_2O cm^{-2} A_L), and water potential difference between the soil and the leaves ($\Delta\Psi$; MPa) of galled (WGR) and control (CTRL) trees in the growth chamber experiment cycle 1 and cycle 2.

Parameter	Treatment			
	WGR		CTRL	
	Cycle 1	Cycle 2	Cycle 1	Cycle 2
Hydraulic conductivity (K_ψ; g H_2O cm^{-1} MPa^{-1})	1884	1167	3519	2120
Leaf specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1})	1.88	1.13	2.44	1.42
Transpiration (E; g H_2O)	40.8	23.5	56.9	26.3
Transpiration per unit leaf area (E_L; g H_2O cm^{-2} leaf area)	0.05	0.03	0.05	0.02
Water potential difference ($\Delta\Psi$; MPa)	0.75	0.68	0.57	0.46

However, the reduced values in C2 relative to C1 are more likely the result of root mortality following the first cycle of drought, as roots are more vulnerable to cavitation than stems (Sperry and Ikeda 1997; Hacke et al. 2000; Stout and Sala 2003). Root mortality in dry soils occurs to hydraulically isolate trees from the drying soil (Sperry and

Ikeda 1997), and root vulnerability to cavitation may serve as a sensing mechanism to deliver signals to foliage in response to water stress (Stout and Sala 2003), and may limit gas exchange (Sperry and Ikeda 1997). Also, insufficient time may have elapsed for root regeneration to occur between C1 and C2.

The absence of treatment-by-cycle interactions for K_ψ (p=0.43); Q_L (p=0.86); E (p=0.37); E_L (p=0.41); and $\Delta\Psi$ (p=0.34) in the GC experiment was contrary to the expectation that galled trees would experience greater xylem cavitation during the first dry down cycle than would control trees. The differences in the hydraulic parameters between galled and control trees were expected to have been greater in C2 than in C1 of the GC experiment, as galled trees had reduced K_Ψ. Although the hydraulic parameters were reduced in C2 relative to C1 (Fig. 4-4) it is possible that the dry down stress was not adequate to induce much cavitation. Alternatively, in spite of attempts to keep trees well watered prior to the experiment, it is possible that they may have been subjected to water stress. If so, then any additional cavitation in galled trees relative to control trees may have occurred prior to the experiment. The implication is that in the short term galled trees do not seem any more likely to undergo runaway cavitation (Tyree and Sperry 1988; Tyree and Ewers 1991) in response to drought stress than do control trees.

4.3.2.2 Water-limiting condition

Caution must be taken in comparing galled and control trees under the water-limiting conditions, as the greater amount of A_L in control trees relative to galled trees resulted in the soil drying down more quickly. The faster drying of the soil of control trees resulted in much lower average Ψ_{soil} values in the GH (-0.08 and -0.22 MPa for galled and control trees, respectively), although not in the GC C1 (-0.24 and -0.15 MPa

for galled and control trees, respectively); conditions in the GH were more variable than those in the GC. When soil water was limiting, the K_ψ, Q_L, E_L, E and $\Delta\Psi$ values of galled and control trees were similar (Table 4-2 and 4-3). The extremely low rates of K_ψ (Fig. 4-1C, G and K) at low soil WC likely resulted from below-ground limitations to water flow (Örlander and Due 1986; Sperry et al. 1998), since it is unlikely that such a drastic reduction in K_ψ could result from decreased stem conductivity.

Under water-limiting conditions following multiple drought events galled and control trees had similar K_ψ, Q_L, E, and E_L values (Table 4-4 and 4-5), confirming the combined GH and GC analysis. Significant treatment-by-cycle interactions only occurred with $\Delta\Psi$ (p=0.09), suggesting that the impact of multiple dry down cycles may have been greater for galled than control trees. The $\Delta\Psi$ of galled trees was greater than control trees (p=0.05; Fig. 4-4), suggesting that some xylem cavitation, and/or a greater amount of root mortality may have occurred in galled trees relative to control trees.

4.3.3 Study implications

In retrospect, a finer textured growing medium more representative of soils in the field would have resulted in a slower drying of the soil, and potentially would have enabled the comparison of galled and control trees throughout the entire dry down cycle. The results provide insight into the long term whole-tree responses to stem galls resulting from infection by WGR, as previous research (Chapter 3) on the K_ψ of galled trees applied only to the galled portion of the stem.

The reduced K_ψ (Table 4-2 and 4-4) and Q_L (Table 4-3 and 4-5) values of galled trees relative to control trees under well-watered conditions indicate that the survival of galled trees growing under field conditions may be limited. The high vulnerability of

pines to xylem embolism (Martínez-Vilalta et al. 2004) combined with the: i) expected increase in gall encirclement with tree age (Peterson 1960); ii) greater $\Delta\psi$ of galled trees relative to control trees (Tables 4-2, 4-3, 4-4 and 4-5); and iii) reduction in A_L and A_S with increasing gall encirclement (Chapter 3) indicate that galled trees may be at an increasing competitive disadvantage for the acquisition of water and nutrients relative to control trees as stands age. Assuming that xeric site trees are an analog for the growth of galled trees in the absence of drought, further reductions in the $A_L:A_S$ of galled trees may be expected with the warmer and drier conditions associated with drought. Previous results (Chapter 3) suggest that galls also disrupt phloem transport to the roots, which may limit root regeneration following drought events. Thus, the mortality of galled trees is likely the result of the indirect long term reductions in above- and below-ground growth.

In summary, these results confirm that stem galls reduce K_ψ, and that in the long-term galled trees respond by reducing A_L. Although it is difficult to apply the short term hydraulic measurements of galled and control trees to the long term growth trends, the results suggest that in the short term galled trees are quite resilient. The mortality of galled trees is more likely the result of the indirect long term reductions in tree growth. Large differences in the whole-tree water relations of galled and control trees may only be evident in the transition between well-watered and water-limited soil; however, the nature of the data was not conducive to determining the responses of galled and non-galled trees throughout the entire range of soil WC. The remarkable similarities in the short term responses of galled and control trees to well-watered soil conditions suggests that the disruption of water flow caused by stem galls does not directly result in tree

mortality. The greater likelihood of trees with large galls succumbing to mortality (Wolken et al. 2006) may be attributed to the indirect long term reductions in tree growth associated with the chronic hydraulic constriction imposed by the gall, which are further compounded by environmental stresses such as soil drought. In the long term, trees with large stem galls are presumably out-competed by non-galled trees, as the competition for water and nutrients increases with stand age.

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5 CHAPTER FIVE

Synthesis

5.1 Summary

This research had the overall objective of evaluating the survival of western gall rust (WGR) [*Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka] infected lodgepole pine [*Pinus contorta* Dougl. ex Loud.] trees, but more specifically to: i) determine survival times following infection by WGR; ii) determine the impact of the reduced water flow through stem galls on tree growth; and iii) determine the responses of galled and non-galled trees to soil drought.

Lodgepole pine mortality resulting from infection by WGR was determined not to occur until galls encircled > 80% of the stem circumference (Fig. 2-4), after which the risk of mortality increased with increasing gall encirclement of the stem. Survival of galled trees was similar for infections on the main stem and those that had grown into the main stem from a nearby branch. Application of Peterson's (1960) model of gall expansion to the survival function predicted that the cumulative survival of stem-galled trees was 38-43% at a rotation age of 80 years (Fig. 2-5). Although this estimate closely approximates the previous estimate of 50% survival (Woods et al. 2000), it is the first data-based estimate of galled tree survival.

Although True (1938) determined through conduction experiments with eosin dye that mature branch galls have a larger proportion of non-conducting tissue than non-galled branches, this research is the first to quantify the hydraulic constriction resulting from stem galls following infection by WGR and relate it to reductions in galled tree growth. The reduction in the hydraulic conductivity (K_ψ; $m^2 s^{-1} Pa^{-1}$) through galls was

strongly associated with the percentage of the stem encircled by the gall (Fig. 3-2). There was a weak negative relationship between tree growth (i.e. stem cross-sectional area, which was equivalent to sapwood area (A_S), and leaf area (A_L), which was an index of tree growth, as crown closure had not yet occurred) and gall encirclement (Fig. 3-3), resulting in the leaf area-to-sapwood area ratio ($A_L:A_S$) remaining constant. Xylem and phloem wounds applied to galled and non-galled trees had the expected impact on K_ψ: xylem wounds on the non-galled side of the tree had the greatest impact on K_ψ, and unwound trees without galls had the least impact on K_ψ. Surprisingly, reductions in K_ψ associated with the wound treatments did not translate into the expected reduction in either the radial or height growth. The lack of an expected impact on tree growth may be attributed to the short duration of the treatments, which also coincided with two fairly wet growing seasons. The results emphasize the resiliency of lodgepole pine trees; Hubbard et al. (1999) suggested that *Pinus ponderosa* Dougl. ex P. & C. Laws had an excess capacity to transport water, as the notching of branches failed to reduce the leaf specific hydraulic conductance.

Although the majority of the growth characteristics of galled trees in the greenhouse were lower than those of control trees, galled trees did not increase root growth relative to total aboveground growth (Table 3-3) to overcome the hydraulic constriction created by galls. The concentration of total non-structural carbohydrate [TNC] results support the previous suggestion that galls disrupt phloem transport to the roots (True 1938; MacFall et al. 1994), which could provide an explanation for the similar root-to-shoot ratios of galled and non-galled trees (Table 3-3). The long-term response of galled trees to the reduced water flow through stem galls was a reduction in

A_L (Table 3-3; Table 4-1). In terms of the reduction in A_L, galled trees resembled trees growing on xeric sites; the reduction in biomass allocation to A_L relative to A_S increases the flow of water to leaves (DeLucia et al. 2000; Piñol and Sala 2000).

Although the application of the drought stress experiment results are limited to well-watered soil conditions, the results provide a greater understanding of whole-tree responses to stem galls resulting from infection by WGR. Under well-watered conditions, galled trees had reduced K_ψ (g H_2O cm^{-1} MPa^{-1}) relative to non-galled trees (Table 4-2 and 4-4), confirming that galled trees have a less efficient stem to transport water. Although the whole-tree transpiration (E; g H_2O) of galled trees was less than that of non-galled trees (Table 4-2 and 4-3), both galled and non-galled trees maintained constant rates of E as the soil dried until a critical soil water content (WC; % H_2O (v/v)) of 11% was reached (Fig. 4-1A and E), after which there was a linear decrease in E with decreasing soil WC. A similar critical soil WC of 12% was observed for 41-yr-old *Pinus sylvestris* L. trees (Irvine et al. 1998), suggesting that constant rates of E may be ubiquitous in pines when soil water is not limiting. The lower leaf-specific hydraulic capacity (Q_L; g H_2O cm^{-1} MPa^{-1}) values of galled trees relative to non-galled trees indicate that reductions in A_L were not sufficient to supply the foliage with water (Table 4-3 and 4-5). Irrespective of whether or not the trees were galled or non-galled, under well-watered conditions individual trees responded to reductions in K_ψ by increasing the difference in water potential ($\Delta\psi$) between the soil (ψ_{soil}; MPa) and the leaves (ψ_{leaf}; MPa), through a lowering of the ψ_{leaf}. Similarly, both galled and non-galled trees responded to reductions in Q_L by increasing $\Delta\psi$ rather than decreasing the E adjusted for leaf area (E_L; g H_2O cm^{-2} A_L). In contrast, under water-limiting conditions the steep

decline in K_ψ (Fig. 4-1C, G and K) and Q_L (Fig. 4-1D, H and L) from the well-watered to the water-limited condition suggests that the limitation to water flow was belowground (Örlander and Due; Sperry et al. 1998); both poor soil conductivity and/or root mortality could limit water flow under dry soil conditions.

5.2 Management implications

The current research provides a greater understanding of the mechanisms by which galled trees die. Although disrupted water flow through stem galls is not necessary for tree mortality, as galled trees often die as a result of stem breakage at the gall (Gross 1983; Zagory and Libby 1985; van der Kamp 1988), it is sufficient to account for mortality. In the long term, galled trees responded to the reduced K_ψ through galls by decreasing aboveground growth: A_L and A_S decreased with increasing gall encirclement of the stem (Fig. 3-3A and 3-3B) and reduced K_ψ (Fig. 3-3E and 3-3F; Table 4-2 and 4-4). The increase in gall encirclement with time (Peterson 1960) suggests that the hydraulic constriction created by galls may increase as trees age, resulting in further growth reductions and galled trees being suppressed. Suppressed trees tend to have reduced hydraulic permeability as a result of a reduction in the proportion of earlywood relative to dominant trees (Reid et al. 2003). Although galled trees growing in the greenhouse adapted to the reduced K_ψ by decreasing A_L relative to A_S (Table 3-3 and 4-1), water uptake may be further inhibited by reduced root growth resulting from the disruption of phloem transport at the gall, as indicated by the lower [TNC] (Table 3-3) in the coarse roots of galled trees relative to non-galled trees.

The results of this research have advanced the understanding of the long term growth responses of lodgepole pine trees to the hydraulic constriction created by stem

galls. Unfortunately, the varying temporal scales of the different studies are not conducive to improving the overall galled tree survival estimate of 38-43% at a rotation age of 80 years (Wolken et al. 2006). For example, the first field study in Chapter 3 relating A_L and A_S to the reduced K_ψ and increasing gall encirclement of the stem provide a 12 year integration of the growth of trees that had been galled for approximately 10 years; the results do not provide an indication of the magnitude of the growth reductions in galled trees relative to non-galled trees in older aged stands. The whole-tree growth study conducted in the greenhouse in Chapter 3 suggested that galled trees do not increase their belowground growth relative to non-galled trees to overcome the hydraulic constriction created by galls. Although the greenhouse growth study determined that phloem disruption is also a potential mechanism of galled tree mortality, the 3-year integration of growth following artificial inoculation with WGR has limited application to the prediction of whole-tree growth under field conditions at various stages of stand development. The growth reductions of the greenhouse galled trees were more severe than those of the field study trees, as the galls in the greenhouse encircled 100% of the stem, whereas the field study trees were considerably larger and had proportionally much smaller galls.

It is still unknown if the absence of stem galls in mature stands (Gross 1983; J.M. Wolken, personal observation) is evidence that trees with stem galls succumb to mortality prior to rotation age, or if trees with small stem galls outgrow WGR, and ultimately heal over the gall. As a result, the cumulative galled tree survival estimate may be either an overestimate, or an underestimate, and may be dependent on tree density at various stages of stand development. In natural stands, the higher density of trees and the chronic

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hydraulic constriction created by galls may result in galled trees dying, as the survival

estimates apply to precommercially thinned stands, where many of the galled trees with

lethal stem infections may have been removed at the time of thinning. The observed

reductions in A_L and A_S of galled trees relative to non-galled trees in this research

combined with the increased level of inter-tree competition associated with higher

densities of trees, suggest that galled trees in natural stands have a greater likelihood of

succumbing to mortality. In planted stands, the reduced inter-tree competition for water

and nutrients may result in higher overall survival of stem galled trees. Trees with small

stem galls in managed stands (i.e. planted or thinned) may survive until rotation, as the

majority of trees with galls encircling < 38-47% of the stem circumference were

predicted to survive until 80 years of age (Wolken et al. 2006), and the majority of stem

galls encircled less than 40% of the stem (Blenis and Duncan 1997).

The previous recommendation to delay thinning in combination with taking special

care to eradicate stem galled trees at the time of thinning (Blenis and Duncan 1997) is

probably the best management strategy for WGR-infected stands. Although

precommercial thinning results in increased diameter growth, and reduced mortality from

inter-tree competition (Johnstone 1981), larger trees are more prone to infection by WGR

than smaller trees (Bella 1985). The partial correlation results (Fig. 3-4A and 3-4B)

support the previous observation that larger trees result in larger galls (Peterson 1960);

therefore, delaying thinning has the potential to remove those trees with large (i.e. lethal)

stem galls. The field study trees in Chapter 3 were survivors that were able to tolerate

some degree of infection. Although lodgepole pine trees are resilient to short term insults

such as wounding (Chapter 3), or infections that fail to result in galls, trees between 15

and 20 years old are still at risk of becoming infected and developing large galls (Blenis and Duncan 1997); the incidence of infection was 5% and 20% for jack pine [*Pinus banksiana* Lamb.] stands up to 12 years old and 20 years old, respectively (Bella and Navratil 1988). Even though the number of infections decreases with tree age (Blenis and Li 2005), there was still a potential risk of the non-galled trees in the field studies in Chapter 3 becoming infected in the event of a high infection year, referred to as a wave year (Peterson 1971). Therefore, the recommendation to increase stocking levels to compensate for potential future mortality (Hills et al. 1994) should be considered where the severity of infection is high, as non-galled trees may have only escaped infection, rather than resisted infection.

Growth and survival of galled trees may also be altered by long-term changes in environmental conditions. For example, climate change models predict that the mean annual temperature may increase in some areas by as much as 4°C, resulting in increased evaporation rates (DeLucia et al. 2000), and a global increase in precipitation of $2 \pm 0.5\%$ per 1°C of warming (Coakley et al. 1999). Changes in precipitation will have a greater effect on the development of plant diseases than will temperature (Coakley et al. 1999). Increased precipitation levels could potentially increase the frequency of days conducive to infection by WGR, as WGR spore production occurred under conditions of low vapour pressure deficit (Chang et al. 1987), and high humidity was required for the successful inoculation of lodgepole pine with WGR spores (Blenis and Pinnell 1988). The high vulnerability of pines to xylem embolism (Martínez-Vilalta et al. 2004), and expected increases in the mean annual temperature combined with the lower K_ψ and Q_L, and the

larger $\Delta\psi$ values of galled trees relative to non-galled trees (Tables 4-2, 4-3, 4-4 and 4-5) may further limit galled tree survival.

5.3 Future research

This research has increased our knowledge of the WGR-lodgepole pine pathosystem. However, our understanding of the ability of WGR-infected trees to tolerate infection is still limited, and as a result, our ability to fine-tune the 38-43% overall estimate of galled tree survival is hindered. Future research is required to determine whether trees with small galls should be considered potential crop trees, and to increase the accuracy of growth and yield projections. The role that other mechanisms of galled tree mortality play in either increasing or decreasing mortality also needs to be determined. For example: i) Byler et al. (1972) reported that WGR-infected tissues were more susceptible to infection by secondary fungi than were non-galled tissues; and ii) the gnawing of galled surfaces by rodents could potentially increase the risk of stem breakage (van der Kamp and Spence 1987), or increase the disruption of water flow resulting from stem galls (Wolken et al. 2006).

There are several areas of research which would aid in achieving the overall objective of evaluating the survival of WGR-infected trees that are direct extensions of the current research. First, it is currently unknown if the relationship between gall size and the risk of tree death (Fig. 2-4) will hold until rotation age, and if the rate of gall expansion determined by Peterson (1960) applies to lodgepole pine trees growing in western Canada. This objective could be accomplished by re-evaluating the survival plots at some interval in the future. The cumulative mortality estimates of stem galled

trees could be improved by developing an equation for gall expansion similar to Peterson's (1960) equation that is specific to lodgepole pine growing in western Canada.

Secondly, the K_ψ of large stem galls needs to be quantified. The hanging water column apparatus used in Chapter 3 could not be used to measure the K_ψ of large stem galls because of the deformed nature of older galled tree stems. Although the abnormal tracheid dimensions of galled tissues have been described (Peterson 1960; Zalasky 1976), they have not been related to the reduced K_ψ of galled tissues. Measurements of K_ψ in the thesis research determined that three tissue types comprise galls: i) galled and stained brown, and presumably non-conducting; ii) galled and unstained; and iii) non-galled. The K_ψ associated with the three galled tissue types could be measured according to methods developed for the measurement of K_ψ on excised tissues (Spicer and Gartner 1998). In addition, the water content, average tracheid length and diameter, and conducting A_S at the gall centre could be determined for the representative galled tissue types. Maceration techniques described by Zalasky (1976) could be used to examine the tracheid dimensions of the different galled tissues, which could then be related to the corresponding K_ψ values.

Lastly, it is currently unknown if the leaf area-to-sapwood area ratio (A_L:A_S) of galled trees older than 30 years of age is reduced relative to non-galled trees in response to the reduced K_ψ associated with galls, and whether or not drought years impact the radial growth increment of galled trees more than non-galled trees. To accomplish this objective, trees representing the range of gall encirclements (0 to 100% encirclement of the stem circumference) could be harvested within two different aged stands: i) 20-30-yr-old stand, and ii) 40-yr-old stand or older [Gross (1983) noted an absence of main

stem galls in mature jack pine stands; therefore the age of stands may need to be adjusted]. The $A_L:A_S$, and radial growth increment 30-cm above the galls could be measured. Climate data from nearby weather stations could be used to identify drought years to determine if drought had a greater impact on galled trees relative to non-galled trees. An estimate of the K_ψ of the galls could be made from the excised tissues comprising the galls as previously described.

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