SPORE DISPERSAL AND INFECTION OF LODGEPOLE PINE BY *Dothistroma septosporum* IN NORTHWEST BRITISH COLUMBIA

by

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Abstract

Spore dispersal and infection by *Dothistroma septosporum* which has severely attacked lodgepole pine plantations in northwest British Columbia were studied. Spore abundance was assessed at different distances and heights from single juvenile tree inoculum sources and microclimatic factors were recorded during two consecutive years. One- year-old lodgepole pine seedlings were exposed to natural conditions at the study sites for inoculation. Conidia were assessed from spore traps from June to September in 2009 and were present in the samples whenever rain fell, with a peak in July. It was rare to detect spores more than 2 m away from inoculum sources. The timing and number of conidia dispersed were strongly tied to the climatic variables, particularly rainfall. Infection of the seedlings by the fungus was strongly influenced by the exposure periods, number of spores and high relative humidity. The results suggest increasing the planting distances between susceptible tree species through mixed species plantations and promoting dry conditions within pine plantations may be valuable strategies to reduce the spread of the disease and manage lodgepole pine plantations in the area.

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Chapter 1: Introduction

1.1 Problem statement

Dothistroma needle blight caused by *Dothistroma septosporum* is an important disease affecting pine plantations in most parts of the world (Brown and Webber, 2008). A current epidemic of the disease is causing failure of young lodgepole plantations in northwest British Columbia (BC), Canada, particularly in the Skeena Stikine Forest District (Woods, 2003) (Fig.1). Recently there has been an outbreak of Dothistroma needle blight in northwest BC that has been causing concern due to the extent and severity of the outbreak, and the fact that a native species is being attacked (Woods, *et al.*, 2005). Over 90% of lodgepole pine plantations surveyed have suffered some damage in the current outbreak and the damage in these plantations ranges from low levels of infection to nearly 100% mortality (Woods *et al.*, 2005). According to the aerial overview survey conducted by the British Columbia's Ministry of Forests and Range in 2008 to monitor the forest health conditions in the province, 53,505 ha of the forests were found to be affected by Dothistroma needle blight and damage was greatest in the Skeena Stikine Forest District, where 38,827 ha were affected. The disease was found to be concentrated near the Bulkley, Kispiox and Skeena Rivers (Westfall and Ebata, 2008).

Spore dispersal has an important role in the epidemiology of most diseases; an increase in spore numbers causes greater inoculum pressure for the subsequent infection cycles and generally causes increases in the spread and severity of disease (Runion, 2003). Dispersal has long been recognized as fundamental to the development of plant disease epidemics,

for without dispersal many epidemics would fail to progress (Cooke *et al.*, 2006). Many foliar pathogens including fungi and bacteria encounter atmospheric moisture during dispersal and spread in the form of water droplets. Most fungi that produce spores are dispersed by rain splash (Campbell and Madden, 1990). For foliar pathogens, disease spread is the direct consequence of spore dispersal, although spatial patterns of disease may be quite different from spore dispersal patterns; because spore dispersal is a short term phenomenon compared to most other stages of disease development (Cooke *et al.*, 2006), and because of many environmental factors that affect infection rate. The epidemiology of Dothistroma needle blight has never before been studied in northern BC or for lodgepole pine. Epidemiological information has been obtained from other parts of the world such as New Zealand and other pine species, mainly P. radiata. According to Bulman *et al.* (2004), severity of infection by *D. septosporum* depends on temperature, leaf wetness period, and inoculum density (number of infective unit in a given area). Infection and development of symptoms can occur between 5°C and 26 °C, with infection at lower temperatures thought to be dependent on extended periods of humidity (Gilmour and Crocket, 1972, cited in Bradshaw, 2004). Most infection takes place from late-spring to late-summer (Gilmour, 1981).

To understand epidemics of plant diseases it is important to know how far the inoculum is dispersed from infected plants and in what concentrations it constitutes a risk (Fitt *et al.*, 1989). Measurements of inoculum available in sources within crops may provide an indication of the risk that severe epidemics will occur if weather is subsequently favourable (Fitt *et al.*, 1989). Knowledge of the mode of spore release and dispersal, and

conditions affecting both processes, is just as essential to a full understanding of the epidemiology of a fungus-incited plant disease as information about spore germination, penetration of the host, etc. It is vital to know whether spores are released by wind or water and whether they become freely airborne or are dispersed by water (Meredith, 1973). As suggested by Cooke *et al.* (2006), knowledge of dispersal of spores and infection by *D. septosporum* is needed by policy makers in British Columbia, Canada and other countries to devise plant health protection strategies to control Dothistroma needle blight and manage pine plantations.

Several studies on severity of Dothitroma needle blight in northern British Columbia have been conducted covering the period 1963 to present (Parker and Collis, 1966 and Woods, 2003) but there are no field studies on dispersal patterns and distance that spores are carried, and the relationship between these processes and climatic factors. Therefore, the focus of this study was to understand the spatial pattern and range of spore dispersal by *D. septosporum*, and to examine the influence of climatic variables on spore dispersal and infection by *D. septosporum* in northwest British Columbia.

1.2 Research questions

To achieve the purpose of the study, the following questions were asked:

- What is the difference in frequency of dispersal by ascospores (sexual spores) and conidia (asexual spores) of *Dothistroma septosporum* in rain water and air?

- At what distance can the conidia of *D. septosporum* disperse from a point source?

- What is the relationship between climatic variables (rainfall, temperature, relative humidity and leaf wetness) and the timing and number of spores dispersed?

- What is the relationship between climatic variables and the infection success by *D. septosporum*?

1.3 Study objectives

The specific objectives of this study were to:

- Determine the relative frequency of ascospores and conidia, in rain water and air.

- Determine the dispersal gradient of conidia and ascospores if present from a point source in the field under natural conditions.

- Determine the temporal pattern of spore dispersal by *D. septosporum* during the study period.

- Quantify the association of climatic variables (rainfall, temperature, relative humidity and leaf wetness) with the dispersal of spores of *D. septosporum* in relation to timing and number of spores dispersed.

- Quantify the association of climatic variables (leaf wetness, rainfall, temperature and relative humidity) with infection by *D. septosporum.*

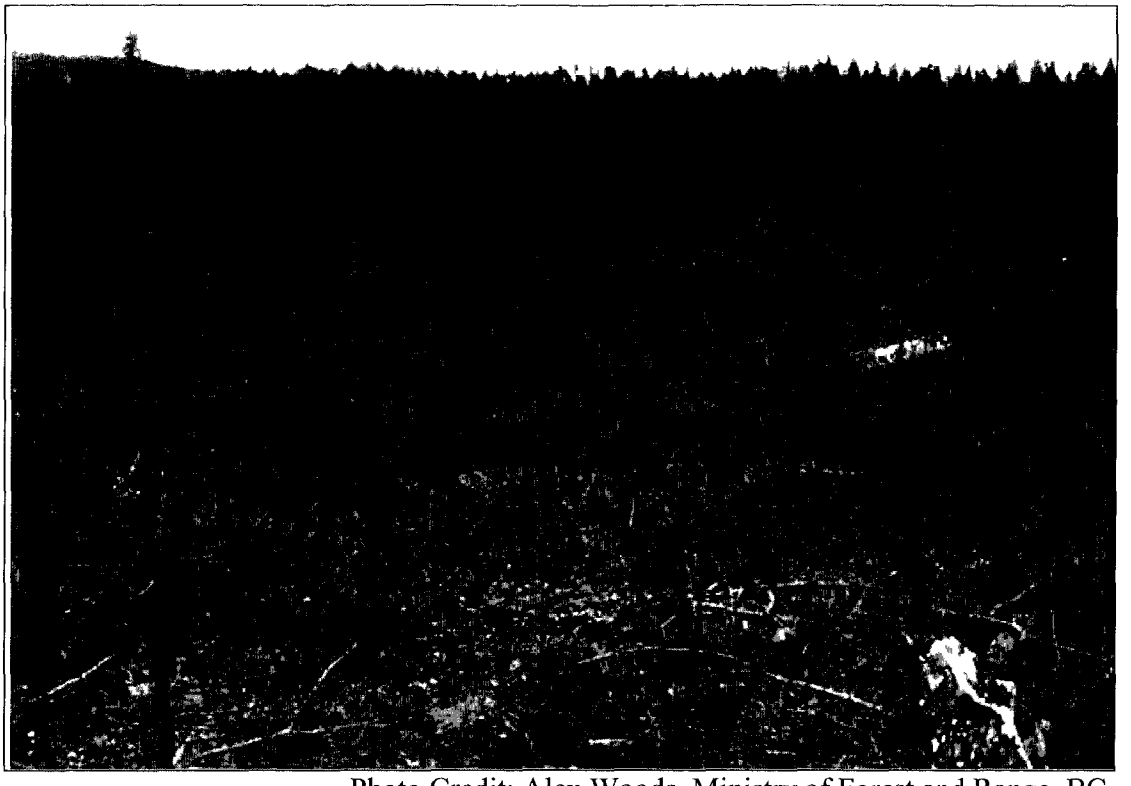


Photo Credit: Alex Woods, Ministry of Forest and Range, BC

Figure 1. Young lodgepole pine plantation affected by Dothistroma needle blight in the Skeena Stikine forest district in northwest BC.

Chapter 2: Literature Review

2.1 Dothistroma needle blight

Dothistroma needle blight, also known as red band needle blight is an economically important disease affecting a number of coniferous trees, in particular pines. In much of the world it is caused by the fungus *Dothistroma septosporum* (Brown and Webber, 2008). In western North America where the fungus is thought to be indigenous, Dothistroma needle blight usually occurs at chronic, endemic levels, but occasionally as sporadic outbreaks that last from one to several years. Severely damaging outbreaks tend to occur only in limited areas (Muir *et al.*, 2004). It has been an important disease in exotic pine plantations in many countries of the Southern Hemisphere since the late 1950s following the widespread planting of exotic *Pinus radiata* D.Don monocultures in large-scale forestry operations (Gibson, 1972). Although the disease can attack both native and exotic pine species, it is generally most damaging in exotic plantations (Gibson, 1974). In New Zealand where the fungus and its main host, *Pinus radiata* were introduced, approximately 50000 ha per year of radiata plantations have been aerially sprayed with copper fungicide to control the diseases over the last 40 years (Bulman *et al.*, 2004). The disease has also been found in countries of the Northern Hemisphere, but past outbreaks have not been as widespread as they have been in the south until recently (Woods, 2003; Bradshaw, 2004). According to Woods (2003) the incidence of Dothistroma needle blight in northern British Columbia (BC) has been documented from 1963, when the disease was first identified (Parker and Collis 1966), to the present. The development of the current disease epidemic has been monitored in northwest BC since

1997 when the first instance of a lodgepole pine plantation failing because of the pathogen was identified (Woods *et al.*, 2005).

A study conducted by Bingzhang *et al.* (1992) in China showed that the disease was most serious in pure stands of susceptible pine and mixed stands with large components of susceptible pine, compared to mixed stands of unsusceptible species with small components of susceptible hosts. They also found that the disease was less severe in pruned stands than in unpruned stands. Elevation has been identified as a factor that influences the severity of the disease in a field. The disease severity increases with decreasing elevation and therefore low-lying areas are more conducive for the disease development (Braun, 2009). This finding has been attributed to pooling of cool moist air overnight in low-lying areas and presence of higher average temperature in areas with low elevation (Kimmins, 1997; Marks and Hepworth, 1986).

2.1.1 Symptoms of the disease

Symptoms of Dothitroma needle blight most often start on the older needles of the lower branches. Symptoms of the disease first appear as yellow or brown spots (Hulbary, 1941, Peterson 1967) followed by the appearance of red bands. The ends of the infected needles turn reddish-brown whilst the bases remain green.

The red banding is due to dothistromin, a mycotoxin produced during initial growth stages of the fungus (Shain and Franich, 1981, Bradshaw and Zhang, 2006). Black fruiting bodies (stromata) can be seen in the red band in later stages of the disease

(Bradshaw, 2004). It has been noted that the stromata containing splash-dispersed conidia are formed on the dead tissues while on the tree, and are the chief means of dispersal of the pathogen within the crop (Gibson, 1973). Older infected needles are usually shed in summer or fall, producing thin crowns in infected stands with a lion's tail appearance. Needles of all ages may be infected, although infections are not uniform throughout the crown. Young trees are more commonly attacked than older trees (MOF, 2002).

Depending on the susceptibility of the host and the extent of infection, the amount of defoliation may be extremely severe as needles of all ages can be affected. This leads to a decrease in yield and in some cases mortality (Brown and Webber, 2008). The most serious impact of *Dothistroma* is reduction of growth or wood yields due to defoliation of needles (Bradshaw, 2004 and Van Der Pas, 1981). Dothistroma blight does not usually kill the infected tree but in extreme cases repeated destruction of foliage by the disease can lead to the death of the tree (Gibson, 1974).

2.2 The pathogen

Dothistroma septosporum (Dorog) Morelet, the causal agent of Dothistroma needle blight is an ascomycete. The life cycle of *D. septosporum* is completed in one year at the coast, but requires two years in most parts of North America (Allen *et al.*, 2003). Its life cycle entails both asexual and sexual stages (anamorph and teleomorph stages) and it is most commonly observed in its asexual state (Allen *et al.*, 2003). The teleomorph of *D. septosporum* is *Mycosphaerella pini* Rost. in Munk (Schoch *et al.*, 2006). Results of

various studies to investigate the fungus showed that the teleomorphic stage of the fungus was rarely found and only the anamorphic stage was observed (Peterson, 1982, Bingzhang *et al.*, 1992; Braun, 2009; Markovskaja and Treigienė, 2009). This suggests that the anamorphic stage is responsible for most diseases on needles in most parts of the world. The abundance of conidia may be the result of selection pressure and a reduced need for sexual reproduction (Evans, 1984). Screening of isolates of *D. septosporum* by Groenewald et al. (2007) showed that both mating types of the fungus were present in the collections from British Columbia (BC), Canada. In contrast, Barnes et al. (2004) reported that the teleomorph *Mycosphaerella pini* was not observed in *Dothistroma* samples collected from different areas including BC.

Numerous conidia are produced in the stromata (fruiting bodies) which develop below the epidermis of infected needles (Peterson, 1982). Stromata can remain viable on dry infected foliage for many months and will produce conidia when suitable moist conditions arise (Hildebrand, 2005). From field investigations and inoculations conducted by Bingzhang *et al.* (1992), the pathogen was found to overwinter as mycelium and unmatured acervuli in infected needles. The process-oriented distribution model developed by Watt *et al.* (2009) indicated that, *Dothistroma* can persist in climate ranging from sub-arctic through temperate, Mediterranean, continental, subtropical to dry tropical regions. Based on the results of DNA analyses of 13 isolates of the fungus conducted by Barnes *et al.* (2004), the fungus has two divergent lineages representing distinct phylogenetic species (*D. pini* and *D. septosporum). D. septosporum* has a worldwide distribution while *D.pini* is restricted to North-Central U.S.A.

The thin-walled conidia are hyaline, smooth, 1-5 septate, short clavate to long filiform, 10-32 x 1.8-3 μm and have a rounded apex and truncated base. Ascospores are hyaline, fusiform, and 11-14 x 2.5-3.5 μm (Cordell *et al.*, 1989). Funk (1985) gave similar description of *D. septosporum* but mentioned that the conidia are 1 to 5 but usually 3-septate and 16-64 x 3.5 μm while the ascospores are 1-septate and 13-16 x 3-4 μm. A recent study by Barnes *et al.* (2004) showed extreme variation in conidial length, ranging from 12-50 μm and even spores from different conidiomata from the same tree differed in average measurement.

D. septosprum produces a toxin known as dothistromin (Bradshaw et al., 1997). This is produced once the needles have become infected. It was believed that dothistromin was required for pathogenicity, but according to Schwelm *et al.* (2008), dothistromin is not a pathogenicity factor for infection by *D. septosporum.* Therefore, Schwelm et al. (2008) proposed that dothistromin has a role in competition against other microorganisms. The toxin also imparts the red coloration to infected needles (Shain and Franich, 1981). Tests have shown that dothistromin can cause chromosome damage in human cells (Bradshaw *et al.*, 2000), leading to concerns in New Zealand in the 1980s about the health risks to forest workers exposed to trees heavily infected with red band needle blight (Brown *et al.*, 2003). Recent studies have shown that dothistromin can only cause large health risk when ingested in large doses because it is a weak mutagen and clastogen (material that can cause breaks in chromosomes), and therefore the need for forest workers to wash their hands well before eating when working in Dothistroma infected stands (Vadim *et al.*, 2000; Bradshaw, 2004; McCulloch and Woods, 2009).

2.3 Fungal spore dispersal

The spores of many plant pathogens can be dispersed only by water because they are contained in mucilage that prevents dispersal by wind (Gregory, 1973; Fitt *et al.*, 1989). Spores that are formed in mucilage are held firmly to the plant surface when dry but are readily released when wetted and become suspended in a film of water on the host surface (Meredith, 1973). The mucilage surrounding splash-borne spores protects them from desiccation and loss of viability during dry weather and this confines dispersal of the spores to periods of rainfall when conditions favour disease spread because free water is available for germination on host surface (Fournet, 1969 cited in Fitt *et al.*, 1989). Water splash directly removes spores from leaf surfaces by incorporating them into splash droplets and such droplets can travel more than a metre from the point of impact but most travel only a few centimetres (Fitt *et al.*, 1989; Madden, 1992, 1997).

Characteristically splash-dispersed pathogens comprise those pathogens that are dispersed from the host in splash droplets because the mucilage surrounding them prevents dispersal by wind. Consequently, dispersal gradients for splash-dispersed spores are generally much shorter than those for wind-dispersed spores (Fitt *et al.*, 1989). It is thought that rain splash may be most significant for local dispersal and may enable some long-distance dispersal in the rebounded small spore-bearing drops after fragmentation when the drops may be small enough to be carried in the wind (Cooke *et al.*, 2006). Splash plays an important role in vertical disease spread because it can transport inoculum vertically (Shaw and Royle, 1993).

Long distance dispersal is limited in spores that are rain-splashed because in still air few inoculum-carrying droplets travel beyond 1m from the source (Fitt *et al.*, 1989). Splash-dispersed spores usually have smooth, thin hyaline walls and elongate shape while dry-dispersed spores often have rough surfaces, thick coloured walls and more or less round shape (Fitt and McCartney, 1986). The rough, thick coloured walls of dry-dispersed spores are thought to protect them against harsh environmental conditions which are not needed by splash-dispersed spores because they are protected by mucilage and water surrounding them. The elongate shape of splash-dispersed spores is thought to aid adhesion to host surfaces by reducing surface mobility (Fitt *et al.*, 1989).

Spore numbers can be estimated with artificial samplers but the choice of sampler and timing of sampling depend on the size of the spores, their mode of dispersal and concentration and the objective of the investigation (Fitt and McCartney, 1986; McCartney *et al.* 1997). Most foliar fungal spores are collected by artificial samplers (slides, funnels) only during rainfall and very few are dispersed to more than 1 m distance or 50 cm above the sources of inoculum (Fitt *et al.*, 1989).

Measurement of disease or spore gradients can be extremely important for identifying sources of disease, for identifying inoculum dispersal mechanisms, for assessing the effectiveness of some disease control strategies and for interpreting the results of field experiments (Cooke *et al.*, 2006). It is important to understand how climate affects disturbances and how forests respond to them (Dale *et al.*, 2001). All diseases are a result of the relationships among host availability, environmental factors, and the pathogen's

ecological requirements, which make up the so-called disease triangle (Gäumann 1950). According to Guo and Fernando (2005), an understanding of climatic factors, especially temperature, relative humidity and rainfall that play important roles in affecting spore dispersal will be useful in modeling and forecasting epidemics of plant diseases.

2.3.1 Spore dispersal by *D. septosporum*

D. septosporum spreads mainly via the conidia, which are produced in an asexual fruiting body or stroma (Gibson, 1972). Conidia are released during moist conditions and spread by splash dispersal (Peterson, 1967, Gibson, 1972). Conidia are dispersed short distances by rain splash and longer distances by wind - dispersed moisture droplets, mist/fog and clouds (EPPO/CABI, 1997; Hildebrand, 2005).

The thin walls of the conidia make them less adapted to exposure and thus less likely to be transported by methods other than rain-splash. However, long distance spread can occur through the movement of infected planting stock, seed mixed with small infected needle pieces and logs which have infected needles lodged in the bark crevices (EPPO/CABI, 1997). According to Jankovský *et al.* (2004), Dothistroma needle blight was first observed in Czech Republic on an imported *Pinus nigra* Arnord in 1999 and it is believed that the disease spread probably with infected planting stock obtained from import at the end of the 1980s and at the beginning of the 1990s. There is also some evidence that clouds may carry conidia over long distances (Gibson *et al.*, 1964, cited in Gibson, 1974). Once the pathogen has been transported to new areas, it is capable of producing spores and thereby spreading to nearby suitable hosts as long as there is

sufficient moisture for spore germination and infection (Hildebrand, 2005). Conidia of *D. septosporum* are readily liberated from stromata into a film of water. They are dispersed by water splash, the dispersal distance normally being quite short (< 2 m) (Bulman *et al.*, 2004).

Peterson (1973) found that the range of dispersal of conidia of *Dothistroma* was limited. High numbers of conidia were collected beneath trees; very few were collected more than 60 cm from infected trees and rarely were any collected over 150 cm. Similarly, the spore-trapping results from Bingzhang *et al.* (1992) study showed the conidia were splashed mainly by rain drops but spread distance was limited. Results of the investigation on effects of spore dispersal on infection by *Dothistroma pini* conducted by Podger (1978) showed that conidia of *Dothistroma* were seldom collected beyond 2 m from the source even during storms with wind speed up to 64 km per hour. However a study conducted by Dale *et al.* (2011) showed compelling genetic evidence for this pathogen's ability to disperse over long distances and suggested that, due to high genetic diversity, splash dispersed conidia are not the only method of dispersal within a stand, or even between trees located 30 cm apart. It is also speculated that ascospores are wind or mist dispersed (Gibson 1974, Bradshaw 2004). Peterson (1973) noticed in his study that more conidia were collected in the months of June, July and August than in September in 1966 and high numbers were trapped in June and July, with considerably fewer trapped in August and September in 1967. He found that though there was significant rainfall, conidia were not collected in October in either year and based on the observed dates suggested the best periods for fungicide application.

2.4 Infection by *D. septosporum*

Infection within a stand is usually only from neighbouring trees and natural transport of spores over long distances is thought to be infrequent. Infected needles attached to the tree crown are the principal source of inoculum; when needles drop to the forest floor, the fruiting bodies are soon overgrown by saprophytes and spore production stops within 2 months (Gadgil, 1970). Under favorable conditions (temperature 18°to 20°C; needle surface moist), most spores that land on the surfaces of needles of susceptible hosts germinate within 3 days, the germ tubes continues to grow on the needle surface, and a very few (about 0.1%) form appressoria over stomatal openings. An infection peg develops between the guard cells and a swollen vesicle forms just below the guard cells (Gadgil, 1967). Further hyphal growth occurs in the mesophyll tissues if the needle surface is wet. Hyphal growth is restricted to necrotic tissue, but the extension of necrosis beyond the region directly infected with the fungus suggests that host cells are killed by the host defense response (Bradshaw, 2004). There is a relatively long incubation period before the appearance of the first symptoms after infection by *D. septosporum* (Gibson, 1974). Peterson (1973) found in his work with *P. nigra* and *P. ponderosa* that the incubation period ranged between 72 and 114 days. In Serbia, Karadžić (1989) stated that the length of the incubation in natural conditions varies from year to year depending on the climatic condition, but on the average it lasts from 4 to 6 months.

According to Bulman *et al.*, (2004), severity of infection depends on temperature, leaf wetness period, and inoculum density. Infection and development of symptoms can occur

between 5°C and 26 °C, with infection at lower temperatures thought to be dependent on extended periods of humidity (Gilmour and Crocket, 1972, cited in Bradshaw, 2004). Most infection takes place from late-spring to late-summer (Gilmour, 1981). Environmental conditions may be favorable, but without high inoculum density, infection may fail (Bulman *et al.*, 2004). The greater the number of pathogen propagules (fungal spores) within or near fields of host plants, the more inoculum reaches the hosts and at an earlier time, thereby greatly increasing the chances of an epidemic (Agrios, 2005). Generally, infection of pines by *D. septosporum* depends on the host susceptibility, inoculum availability and favorable environmental conditions (Bulman *et al.*, 2004).

2.5 The hosts

Dothistroma septosporum is currently known to infect over 60 *Pinus* species in 45 countries, and is especially harmful to *P. radiata* D. Don, *P. ponderosa* Dougl., *P.contorta* Dougl., *P. jeffreyi* Grev. & Balf., and *P. nigra* Arnold (Ivory, 1994; Karadzic, 1994; Brown *et al.*, 2003). Dothistroma has been reported on lodgepole (*Pinus contorta* var *latifolia* Engelm.) and ponderosa (*Pinus ponderosa*) pines, both native species to B.C (Allen *et al.*, 2003). Non-native species such as jeffreyi (*Pinus jeffreyi* Balf.), monterey (*Pinus radiata* D. Don), bishop (*Pinus muricata* D.Don), and maritime (*Pinus pinaster* Aiton) pines, as well as some hybrid pines species have been reported as hosts of *D. septosporum* (Allen *et al.*, 2003). According to McCulloch and Woods (2009), all pine species native to British Columbia are susceptible and the fungus is widely distributed wherever host species can be found. The Kispiox Timber Supply Area, formerly the

Kispiox Forest District has been noted as the most severe Dothistroma needle blight infestation area in northern British Columbia (Woods, 2005). The disease is now so prevalent and chronic that entire plantations of lodgepole pine in northwest B.C are failing (Woods, 2003). Brown *et al.* (2003) argued that several factors including the origin and provenance of a species as well as site and climatic conditions likely affect the susceptibility of a host. Some *Pinus* species such as *P. contorta and P. radiata* have been noted to become increasingly resistant to the fungus with age (Gibson, 1972).

Lodgepole pine is one of the most commonly used species in reforestation because of its wide range of ecological tolerance and quick growth (Lotan and Critchfield, 1990). Prior to 1975 lodgepole pine represented <10% of the landbase in northwest B.C. compared to its current prevalence in 40% of plantations (Woods, 2003). This increase in host availability has undoubtedly played a role in triggering the spread of the disease (Woods, 2003). Prevalence of *Dothistroma* has increased especially in northwestern British Columbia where severe damage to managed and natural stands of lodgepole pine have been reported (Woods, 2003). Moreover, it has been noted that extensive plantations of lodgepole pine in northwest B.C along with a marked increase in the frequency of weather events favourable to the disease have resulted in unprecedented levels of *Dothistroma* infection (Woods *et al.*, 2005; McCulloch and Woods, 2009; Welsh *et al.*, 2009).

2.6 Effects of climate factors on spore dispersal by D. septosporum

Foliar disease fungi may be more responsive to climate change than most other forest disease organisms, as their ability to sporulate and infect is strongly tied to changes in temperature and precipitation (Peterson, 1973; Gadgil, 1977; Hoff, 1985).

Rainfall is an important mechanism for liberation and dispersal of propagules of many species of plant –pathogenic fungi (Fitt *et al.*, 1983). Moisture in such forms as splashing rain and running water plays an important role in the distribution and spread of many pathogens on individual plants and on their spread among plants (Agrios, 2005). Dispersal by rain is usually on a smaller scale than wind dispersal (Esker *et al.*, 2007).

Spores may be moved via rain in two ways; the first is due to rain-splash, where a drop breaks into smaller droplets when it hits a sporulating lesion and each of the smaller droplets will take up several spores and carry them away. The second dispersal mechanism is dry-dispersal, which occurs when a rain drop hits a part of the leaf with no sporulating lesions, and the energy transfer to the leaf results in detached spores being liberated while staying dry (Esker *et al.*, 2007). Dispersal of pathogens involves liberation, transit and impaction and all these are influenced by wind and water. The distance to which spores may be disseminated varies with the agent of dissemination (Agrios, 2005).

Water is believed to be essential for spread of the *D. septosporum*, as spores masses disperse in films of water and are disseminated as water droplets fall from the needle surfaces, resulting in infection within and between neighbouring trees (Brown and Webber, 2008). Bingzhang *et al.* (1992) noted that the time and number of dispersed

conidia in their spore - trapping study were closely related to temperature, humidity and rainfall, especially humidity. Moreover, a study conducted by Peterson (1973) to monitor *Dothistroma* conidia dissemination showed that no conidia were tapped on rainless days and large numbers of conidia were disseminated each day there was significant rainfall, even when there had been a heavy dispersal only 1 or 2 days earlier. The results of an investigation conducted by Karadžić (1989) to define the time of production and dispersal of conidia and ascospores in Serbia showed that conidia were intensely dispersed during wet periods. Based on the results of the evaluation of microclimatic factors affecting ascospores release of *Gremmeniella abietina var balsamea* in Quebec, Canada, Laflamme and Archambault (1990) noted that leaf wetness measurements were best correlated with spore dispersal. Conidia can also be dispersed by wind and cloud transport (Bradshaw, 2004). Favourable temperatures may shorten the period between infection and the production of new spores and so influence the number of spore generations occurring during a season (Colhoun, 1973). It is clear that climatic factors play vital roles in the dispersal of *D. septosporum*, directly as a dispersal agent or indirectly through their effect on the removal of the propagules from an infected needle surface and deposition onto a new needle surface.

2.7 Effects of climate factors on infection by D. septosporum

2.7.1 Rainfall and Leafwetness

Moisture may exist as rain or irrigation water on the plant surface or around the roots, as water vapour in the air, and as dew (Agrios, 2005). Moisture influences the initiation and development of infectious plant diseases in many interrelated ways. It is necessary for the

germination of fungal spores, penetration of the host by the germ tube and for the activation of fungal hyphae before they can cause infection (Agrios, 2005). Moisture increases the succulence of host plants and thus their susceptibility to certain pathogens, which affects the extent and severity of disease (Agrios, 2005). The number of infection cycles per season of many fungal diseases is closely correlated with the number of rainfalls per season, particularly of rainfalls that are of sufficient duration to allow establishment of new infections (Agrios, 2005).

Various studies on the relationship between *Dothistroma* and climate have shown that infection by the fungus is greatly affected by moisture and leaf wetness. In the northern hemisphere the amount of rainfall in June to September is a good indicator of the severity of the disease (Peterson, 1973) and in general a long dry period after infection leads to less severe disease and slower development of stromata than during wet weather (Gadgil, 1977). Temperature and needle wetness are key weather variables in the development of *D. septosporum* (Bulman, 1993).

Gadgil (1974) found that, when all combinations of four temperature regimes (day/night temperatures 24°/16°C, 20°/12°C, 16°/8°C, 12°/4°C) and four leaf-wetness periods (8, 24, 48 hours), and continuous moisture (needles kept wet) were tested, the most infection was obtained at 20°/12°C when continuous moisture was provided . When the effect of moisture was further studied at 20°/12°C, it was found that infection occurred even when there was no additional moisture (mist spraying stopped), but when this happened only a few needles became infected. There was an exponential increase in the severity of

infection with increasing length of wetness periods (Gadgil 1977). The length of the wetness period has also been shown to have a marked effect on the severity of infection and the length of the pre-reproduction period (Gadgil 1976).

In British Columbia, Parker (1972) reported that inoculated radiata pine seedlings had to be continuously exposed to moisture (mist spray) for periods of 3 to 8 weeks or longer before symptoms of disease developed. Inoculated seedlings initially exposed to moisture for approximately 2 to 12 days, then held in a dry regime for several weeks, did not develop lesions. However, when these seedlings were re-exposed to moisture, typical lesions developed within a few to several weeks. Muir and Cobb (2004) also found out that lesions developed first and were more numerous per needle on seedlings held in 24 h/day mist spray than those in 16 h/day mist spray. Their results also indicated that a prolonged exposure to moisture might be necessary for the fungus to overcome host resistance. Infection becomes severe when environmental conditions are conducive to disease development, e.g., gully systems where needles remain moist for long periods, or highly stocked unpruned stands (Bulman, 2004). Woods *et al.* (2005) observed a strong spatial correlation between mean summer precipitation and infection by *D. septosporum* within the area of the current outbreak in BC.

2.7.2 Temperature and Relative humidity

Temperature affects the number of spores formed in a unit plant area and the number of spores released in given time period. In reality, moisture and temperature must be favorable and act together in the initiation and development of the vast majority of plant diseases and plant disease epidemics (Agrios, 2005).

Infection by *D. septosporum* depends on the duration of the needle-wetness period; temperature and the amount of spores present (Bulman, 1993). Although infection by *D. septosporum* is largely dependent on wet conditions, optimal temperatures during extended periods of high humidity have the ability to increase disease severity. Favourable temperatures may shorten the period between infection and the production of new spores (Colhoun, 1973). Muir and Cobb (2004) noticed that typical *Dothistroma* lesions developed at 32 to 83 days after inoculation, depending on the temperature regime and moisture treatments, and penetration on inoculated seedlings was found to be significantly greater in a variable than in a constant air temperature regime. Moreover, wet spores germinated and penetrated needles even in dry conditions, but symptom development required high humidity (Gadgil, 1977). The optimum temperature for successful establishment was found to be 12-18 ^{o}C under conditions of high relative humidity (Brown and Webber, 2008), and according to Gilmour and Crockett (1972), temperature was less important than relative humidity, as infection and development of symptoms occurred between 5 and 26 ^{o}C, although infection at lower temperature was dependent on an extended periods of high humidity. Increased frequency of warm rain in the mid-to-late 1990s has been identified to have coincided with sharp increase in the extent and severity of the current epidemic in northwest BC (Woods et al., 2005).

In a 3-year controlled field study in New Zealand involving a 2 week exposure of individual seedlings to infection in the field, no disease symptoms developed in trees exposed to infection below 7 °C or when the leaf wetness period was <10 h (Gilmour 1981), although the threshold values for these parameters varied from year to year. Light intensity has a strong influence on the severity of disease. Although the germination of conidia and early growth is unaffected, the development of symptoms is drastically reduced with a low light intensity (58 W/m^2) during the day (Gadgil and Holden, 1976).

2.8 Management and Control of *D.septosporum*

Silvicultural techniques in forest pest management manipulate the host or the environment so that they are less favourable or unacceptable to the insects or disease agents. Therefore, the goal of forest management is to create a forest stand that is less susceptible and vulnerable to attack by pests and still economically sustainable (Groot *et al.*, 2003). Chemical control, disease resistance and breeding programs, and stand manipulation are some of the practices found to be effective to reduce the prevalence and control of *D.septosporum* (Gadgil, 1984, Villebonne and Maugard, 1999). According to Brown et al. (2003), combinations of these methods have proved very effective at managing red band needle blight. Two fungicide applications, with one prior to needle emergence and one after emergence, is effective against *Dothistroma* infection (Funk, 1985). Peterson (1973) suggested that because previous seasons' needles can become infected before current-year needles have emerged from needle sheaths, complete

protection of needles of all ages would require two applications of fungicide. A study conducted by Carson (1989) has shown that development of a resistant breeding stock of *Pinus radiata* reduced mean *Dothistroma* infection levels by 11-12%.

Late thinning or a dense understorey of shrubs is found to favour the development of the pathogen because it may keep humidity high at the base of the crown (Villebonne and Maugard, 1999). Therefore thinning and pruning trees will improve the airflow within a stand and make the microclimate less favourable to plant disease (Gadgil, 1984). Low initial stocking, a succession of thinnings, and pruning of lower branches lead to an open, well-ventilated stand with few, if any, dead or dying trees and reduced competition for moisture and nutrients (Gadgil and Bain, 1999). It has been noted that thinning increases the distance between tress and this reduces the effectiveness of rain-splashed spores which travel only short distances. Gadgil and Bain argued that repeated thinning allow individual trees particularly susceptible to infection by the local pathogens to be removed, and the pruning of lower branches lessens the amount of infected foliage and hence the inoculum available for reinfection. Leaf pathogens such as *D. septosporum* therefore, tend to be less severe in managed stands (Gadgil and Bain, 1999). Pruning at the right time can delay the necessity for chemical control of *Dothistroma* needle-blight for several years (Kershaw *et al.*, 1988 cited in Gadgil and Bain, 1999).

In establishing plantations, choosing the right species for a site is important if the species vulnerability to natural agencies is to be minimized (Gadgil and Bain, 1999).

Woods et al (2005) suggested that because extensive planting of favoured species can result in severe damage from pathogen, forest managers should diversify managed stands

to mitigate unexpected negative effects of climate change on forest productivity. In countries where chemical control of the disease is not practiced, disease management is mostly concentrated on silvicultural measures which are believed to reduce inoculum loads and the use of less susceptible species (Brown and Webber, 2008, McCulloch and Woods, 2009).

Chapter 3: Materials and Methods

3.1 Study Area

This study was conducted within the Bulkley Timber Supply Area (TSA) of the Skeena Stikine Forest District in northwest British Columbia, between Smithers and Hazelton, BC (Fig. 2). The study area was located within Interior Cedar-Hemlock moist cold two (ICHmc2) biogeoclimatic zone[1] (Banner *et al.*, 1993). It has low- to mid- elevation (400-1500 m) with a climate that is warm and moist in the summer, cool and wet in the fall and cold in the winter (Banner *et al.*, 1993). According to Coates *et al.* (1997), the area is dominated by a mixture of conifer and deciduous tree species. In mature forests, western hemlock (*Tsuga heterophylla* [Raf.] Sarg.) dominates, but is mixed with western redcedar (*Thuja plicata* Donn ex D. Don in Lamb), subalpine fir (*Abies lasiocarpa* [Hook.] Nutt.), lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.), hybrid spruce - the complex of white spruce (*Picea glauca* [Moench] Voss), Sitka spruce (*P. sitchensis* [Bong.] Carr.), and occasionally Engelmann spruce (*P. engelmannii* Parry ex Engelm.), paper birch (*Betula papyrifera* Marsh.), trembling aspen (*Populus tremuloides* Michx.), and black cottonwood (*Populus balsamifera* ssp. *Trichocarpa* Torr. & Gray).

Dothistroma is currently causing extensive defoliation and mortality in plantations of lodgepole pine within the study area (Woods et al., 2005). The disease has continued to be the most significant forest pathogen of lodgepole pines in the area for the past decade (Westfall and Ebata, 2008).

[1] ICHmc2 biogeoclimatic zone is an ecosystem classification scheme use in BC.

3.2 Site selection

Two sites were selected in severely attacked lodgepole pine plantations within the study area. The selected plantations were between 15 and 20 years old and were located approximately four kilometers apart. Plots were located a minimum of forty meters away from the nearest road, on relatively flat areas that contained trees showing moderate to severe dothistroma needle blight. Each plot measured approximately 15 m long and 15 m wide. All plots were between 400-600 m elevation and positions were recorded with a handheld GPS unit (© GARMIN Corp). Three plots were established at each site, each plot a minimum of 100 m apart.

3.3 Passive spore trapping.

A single lodgepole pine tree infected with *Dothistroma* was retained at the centre of each plot and all other pine trees within 10 m radius were cleared by cutting and removing all the cut trees in order to create a point source for spore dispersal. The retained trees were of similar height (range = 4.5-6.1 m), diameter at breast height (range = 6.3-11.1 cm) and percentage functional live crown[2] (range = 20-30%).

To determine how far the spores disseminated, spores were sampled passively with microscope slides covered with a thin coat of Vaseline (Unilever Canada, Toronto, ON) on an exposed sampling surface area of $10cm^2$ (Peterson, 1973 and Bingzhang et al., 1992). Slides were attached to upright supports at an angle of 45^0 toward the tree at two

[2] Functional live crown is a function of average live crown and average live nodes on a tree (MOF, 2005) i e. Functional live crown = percentage live crown x percentage nodes / 100

different heights above the ground. The lower slides were fixed at the height of the lower live crown and the upper slides were 50 cm higher. Four supports were arranged in lines at distances of 0 (beneath sampled tree), 1, 2 and 3 m from outer crown on two opposite sides of each sampled tree (north – south direction), for a total of forty eight slides per site (Fig. 3). North –south orientation was chosen based on the wind direction at the study area.

All exposed slides were collected and replaced with fresh slides every three days from May 30 to September 26 in 2009. The collected slides were observed under a compound microscope at 100x magnification to count the number of spores collected per 10 cm^2. To facilitate spore counting and for accuracy, the exposed sampling area of each slide was divided into four quarters and two randomly-selected quarters were observed. Identification was based on the morphology of *D. septosporum* as described by Funk (1985). Moreover, to aid the identification, conidia of *Dothistroma* were extracted from a fruiting body on an infected needle, prepared in 10% KOH, and observed on a microscope slide under a compound microscope.

Passive spore sampling was repeated in 2010 using the spore trapping method described above with the aim of confirming the commencement of *Dothistroma* spore dispersal at the study area during the 2009 season and to determine how the variability in climatic factors measured in the two seasons influenced the timing and number of spores dispersed. This second year sampling lasted for nine weeks, from April 23 to June 28 on one plot per site.

3.4 Active spore trapping.

Rotorod spore traps were used in an attempt to trap air borne spores within the live crown. These traps were powered by solar energy and consisted of horizontal brass rods pivoted at the centre with two pieces of vertical plastic rods fixed at the end of each brass rod (Fig. 4). The collecting surface areas of the vertical rods (30 mm in length and 1.5 mm wide) were covered with a thin coat of Vaseline to sample conidia and ascospores which are believed to be dispersed by wind (Gibson, 1974). Two traps were located 40m apart at each site. The traps were mounted at the height of the live crown on top of infected trees using a rope and pulley system. After exposure for three days, the plastic rods were removed and replaced with fresh rods. Exposed rods were mounted on a microscope slide for observation under a microscope at 400x magnifications to count the number of spores sampled. Spore numbers per m^{-3} air were calculated by determining the volume of air sampled by rotorod as a function of rotation (Cox and Wathes, 1995). This was calculated using the formula: $V = 2\ (2\pi rwl)\ s$ where V = volume of air sampled (cm^3 min^{-1}), r = radius of arms (cm), w = width of trapping surface (cm), l = length of trapping surface (cm) and s = speed of rotation (rpm).

3.5 Rain collection and spore sampling.

Rain collectors were mounted on stands about 3m above ground and placed just outside each plot to catch rain-dispersed spores. A total of twelve rain collectors were used with two on each plot. The rain water was collected every three days whenever rain fell during the 2009 field season and stored in a fridge at 4°C to inhibit spore germination.

Benomyl 50 systemic fungicide (Later chemical Ltd, Richmond, BC) (3g/1L of water) was added to the collected rain water to prevent the collected spores from germinating during transportation. One hundred ml of each water sample was pre-filtered with a 125μm mesh sieve (Endecotts Ltd, London, England) to remove debris, and then vacuum filtered through a nitrocellulose membrane filter (Millipore Corp., Billerica, MA) with a pore size of 0.8μm to trap spores. The Millipore filter was transferred with smooth-tip forceps to a clean petri dish, and then cleared with non-drying immersion oil (Type B) for microscopy (Gargille Laboratories, Cedar Grove, NJ) and observed under a compound microscope at 400x magnification for spore counting. Five cm^2 surface area of the Millipore filter was observed to facilitate counting of the spores. Despite the low storage temperature and fungicide treatment, the spores still germinated during storage which impaired the accuracy of counting. Therefore, the numbers of spores collected in the rain water per $5cm^2$ were categorized (0 = no spore, 1 = 1-25, 2 = 26-50, 3 = 51-75, 4 = 76-100, 5 = 101-200, 6 = 201-499 and 7 = 500 and above).

3.6 Stand Data

A stand composition plot was located 40m away from the centre of each of the six plots using a randomly selected bearing to quantify the stand characteristics of the two plantations where the study was conducted. All tree species within 200 m^2 plots were counted, and the height and diameter at breast height (dbh) of all the lodgepole pine trees were recorded. The percentage of functional live crown and all other health agents of the lodgepole pine trees within the composition plots were obtained through visual

observation. Percentage functional live crown was calculated by using the function of average live crown and average live node on a tree (MOF, 2005). Relative soil moisture regime at each plot was also determined using plant species present, topography and elevation of the plots (Delong, 2004). Stand data were used to describe the study area and identify other stand factors that might influence spore dispersal and infection of D. *septosporum* at the study area.

3.7 Climatic data

Weather stations mounted on poles approximately 1m off the ground and housed inside protective solar radiation shields were positioned close to the rotorods and rain collector traps on each plot. All six plots were equipped with HOBOs and microclimate loggers (HOBO Pro Series, © Onset Corp) to record the air temperature, relative humidity and leaf wetness at 15-minute intervals throughout the sampling period in each year. A rain gauge was also mounted on each plot to record the rainfall collected every three days throughout the study period.

3.8 Study 2: Infection under natural environmental conditions.

To determine the effect of spore release and environmental conditions (temperature, rainfall, RH and leaf wetness) on infection by *D. septosporum,* seedlings were exposed to infection at each site during the sampling period in 2009. One-year-old lodgepole pine seedlings from the Bulkley Valley seed zone which were raised in copper treated plug

(PCT) 410 containers were planted in a mixture of 20L sand, 107L peat (Premier Tech Horticulture Ltd., Riviere-du Loup, QC), 20L perlite, 20L vermiculite, 0.2L micromax (Scotts-Sierra Horticultural Products Co., Marysville, OH) and 1L slow release 14-14-14 fertilizer (Polyon Agrium Advanced Technologies Inc., Lathrop, CA) in one gallon plastic containers in a green house. The seedlings were used as trap plants and exposed to natural conditions of *Dothistroma* inoculum at each of the plots in three different groups (A, B and C) and batches for different periods. Groups A, B and C were exposed for two, four and six weeks respectively and each batch of each group was collected and replaced after the exposure periods from May 14 to September 26 in 2009 (Table 1). Six seedlings (two per group) were placed under the canopy of infected trees on each plot at a time. A total of hundred and ninety two seedlings were exposed throughout the study period.

The collected seedlings were sent back to the green house and kept under conditions with temperature ranging between 10 and 20°C, 20 hours of light and watered as necessary. The seedlings were monitored for signs and symptoms of Dothistroma disease for a year. All the seedlings were grouped into infected and non-infected based on the symptoms of the disease. For signs of the disease, some of the needles of all the seedlings that displayed symptoms of Dothistroma needle blight were observed under a dissecting microscope for fruiting bodies.

3.9 Data and statistical Analysis

Data collected during the 2009 field season were used for all statistical analyses of this study. Statistical analyses from the 2010 field season were not reported as there was insufficient resolution in the data to detect meaningful ecological trends.

Daily mean temperature, relative humidity, leaf wetness, and rainfall recorded every 3 days throughout the study period were calculated. The mean minimum overnight temperatures at the two study sites were also calculated. Average numbers of conidia collected per $10cm^2$ of two lower and two upper slides on each row at each distance per 3 days were calculated for each site. Average numbers of conidia per $5cm^2$ trapped in 100ml of rain water collected on each of the two sites were calculated. To determine the differences in stand characteristics of the two study sites, the average tree heights, dbh and percentage of functional live crown of lodgepole pine trees on each site were also calculated.

3.9.1 Relative frequency of ascospores and conidia

The number of conidia of *D. septosporum* collected every 3 days on $10cm^2$ from the lowest slide at 1m distance for each row was averaged for each of the sites. Therefore six slides at each site were averaged for the lowest slides at 1m distance. The numbers of conidia collected every 3 days in 100ml of rain water per $5cm^2$ for each site were also averaged. Numbers of spores recorded on the slides and in the rain water were plotted separately for each site using excel and analyzed graphically to determine the relative numbers of conidia dispersed in air and rain water during the 2009 sampling period. The

frequency of ascospores could not be determined because no ascospores were trapped during the study.

3.9.2 Distance and height of spore dispersal from a point source.

Spore counts per $10cm^2$ from the slides at the 1m distance on each row were combined from each plot and averaged separately for the lower and the upper slides. Each site was analyzed independently. To determine whether more spores were collected from the lower or upper slides, the average number of spores observed every 3 days at each of the heights were plotted over time for each of the sites by using excel.

Spore counts per $10cm^2$ from the lower slides at each of the four distances on the two rows were combined and averaged for each distance and each site. To determine how far the spores dispersed and at which distance was the highest number of spores collected, the average number of spores trapped at each distance every 3 days were plotted over time for each of the sites.

Regression with linear mixed effects was performed to model and examine the effect of heights and horizontal distances from inoculum sources on *Dothistroma* spore dispersal. Spore counts, heights and distances were treated as continuous variables while sites, plots and collection dates were treated as factors. Spore counts were used as response variables, heights and distances as fixed effects while sites, plots and collection dates were modeled as random effects. Residuals plots were used to examine the analytical assumptions of the models. Log(x + 1) transformation was used to transform the spore

counts to satisfy assumptions of the models, namely homogeneity of variances and normal distribution of residuals. Means comparisons were performed using protected t-tests to determine whether significant difference existed (using $\alpha = 0.05$) between the numbers of spore collected at the two vertical heights, and the difference between the numbers of spore collected at the two study sites.

3.9.3 Temporal pattern of spore dispersal by *Dothistroma septosporum*.

Average numbers of spores trapped every 3 days on $10cm^2$ from the lower slides at the 1m interval on each row were plotted for each site from June 2 to September 26, 2009. The average numbers of spores per $5cm^2$ surface area of the membrane filter collected in the rain water every 3 days during the entire 2009 sampling period were also plotted. Graphical inspections of the plots were made to determine the commencement and peak of spore release and dissemination. The date at which spore dispersal halted during the sampling season in 2009 was also determined.

3.9.4 Relationship between climatic variables and spores dispersal.

The average number of spores observed every 3 days on $10cm^2$ of the lower slides at 1m interval from inoculum sources on each row, and the mean daily temperature, relative humidity, percentage leaf wetness and rainfall recorded at each site were plotted to determine the trend of *Dothistroma* spore dispersal in relation with the climatic variables.

Multiple regressions with mixed effects were used to explore, measure, and model the effects of the climatic variables on the number of spores dispersed.

Spore count was used as the response variable; all climatic variables were used as fixed effects and fitted as continuous variables while sites, plots, trees and collection dates were used as random effects to contend with the site to site, plot- to- plot and tree- to-tree variations. The correlations between the climatic variables were tested. The effect of mean minimum overnight temperatures on number of spore collected were also determined using the mixed effects models. The models were checked with residuals plots to examine how well the models fit and to meet the model's assumptions. The response variable was transformed with log(x + 1) transformation to satisfy assumptions of the models. Backward elimination procedure was used to selectively eliminate least significant variables in an iterative model fitting procedure until all remaining variables were significant. Concurrently, I examined AIC values and reported models with lowest AIC values (judged to fit the best) and statistically significant explanatory variables using $\alpha = 0.05$.

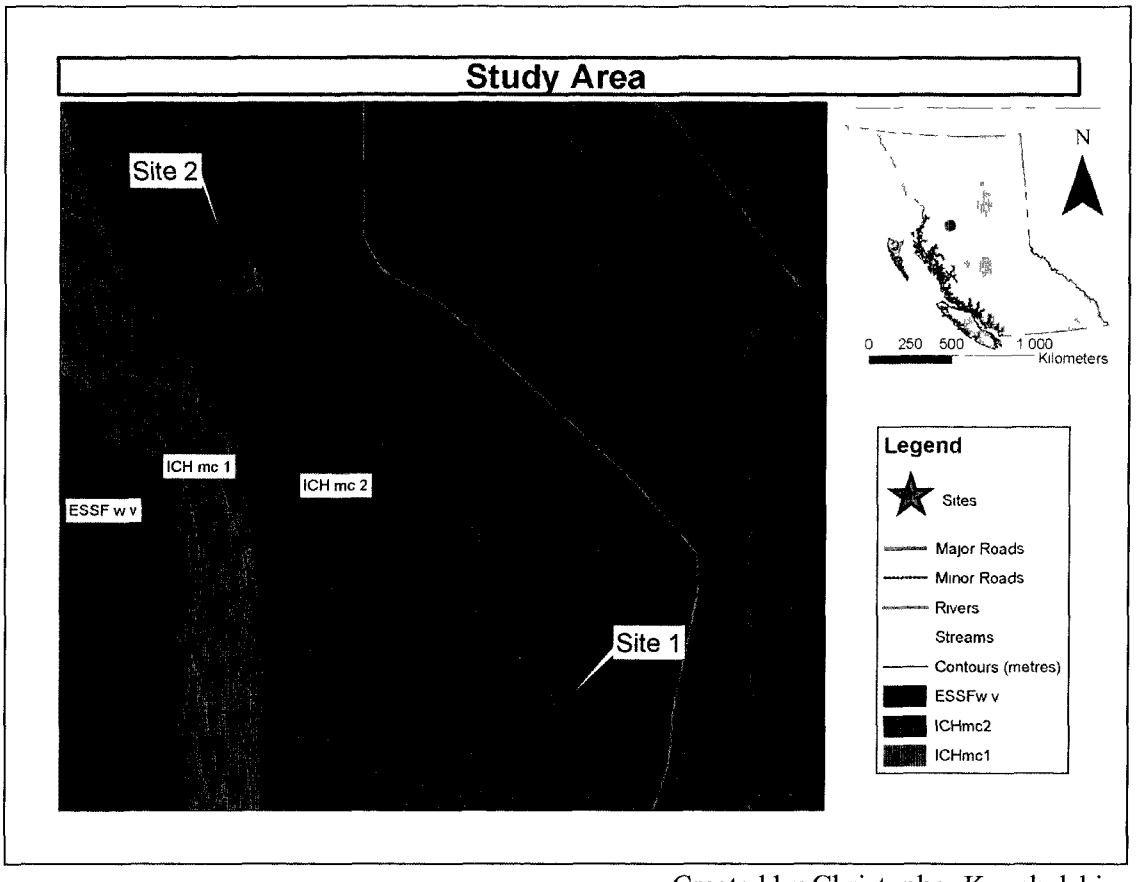
3.9.5 Stand Characteristics

The average tree heights, dbh and percentage of functional live crown of lodgepole pine trees on each site were calculated. Analysis of variance (ANOVA) was used to examine the effects of stand characteristics on number of spores collected. Log (x +1) transformation was used on the response variable to satisfy assumptions of the model, namely homogeneity of variances and normal distribution of residuals. Analytical assumptions were examined by graphical inspection of residual plots. The differences

between the average tree heights, dbh and percentage of functional live crown of lodgepole pine trees at the two study sites were determined by performing mean comparisons using protected t-tests. Statistical significant was determined using $\alpha = 0.05$.

3.9.6 Relationship between climatic variables and infection by *Dothistroma.*

Seedlings infected or not infected based on the symptoms of Dothistroma disease were used as categorical variables with two values. Infected seedlings were assigned the value 1 and the non-infected seedlings were assigned the value 0. Mean daily temperature, relative humidity, leaf wetness and rainfall recorded for each exposure period of the seedlings were calculated. The sum of the average numbers of conidia per $10cm^2$ collected on the microscope slides for every 3 days during each seedlings exposure period were also obtained. Logistic regression with mixed effect models were used to test the significance of all the climatic variables, number of spores and exposure periods of the seedlings on the likelihood of infection of the seedlings. The likelihood of the seedlings to be infected ($y = 1$) or not infected ($y = 0$) were used as the response variable. All the climatic variables, number of spores and exposure periods were used as predictors and fitted as fixed effects. Sites, plots and trees were incorporated in the models as random effects to account for variations in sites, plots and trees. Residuals plots were used to examine how well the models fit and to meet the model's assumptions. I used a backward elimination procedure to selectively eliminate least significant variables in an iterative model fitting procedure until all remaining variables were significant. Concurrently, I examined AIC values and reported models with lowest AIC values (judged to fit the best) and statistically significant explanatory variables using $\alpha = 0.05$.



Created by Christopher Konchalski

Figure 2. Map of the study area showing the location of the two study sites. Inset map of British Columbia showing the location of the study area.

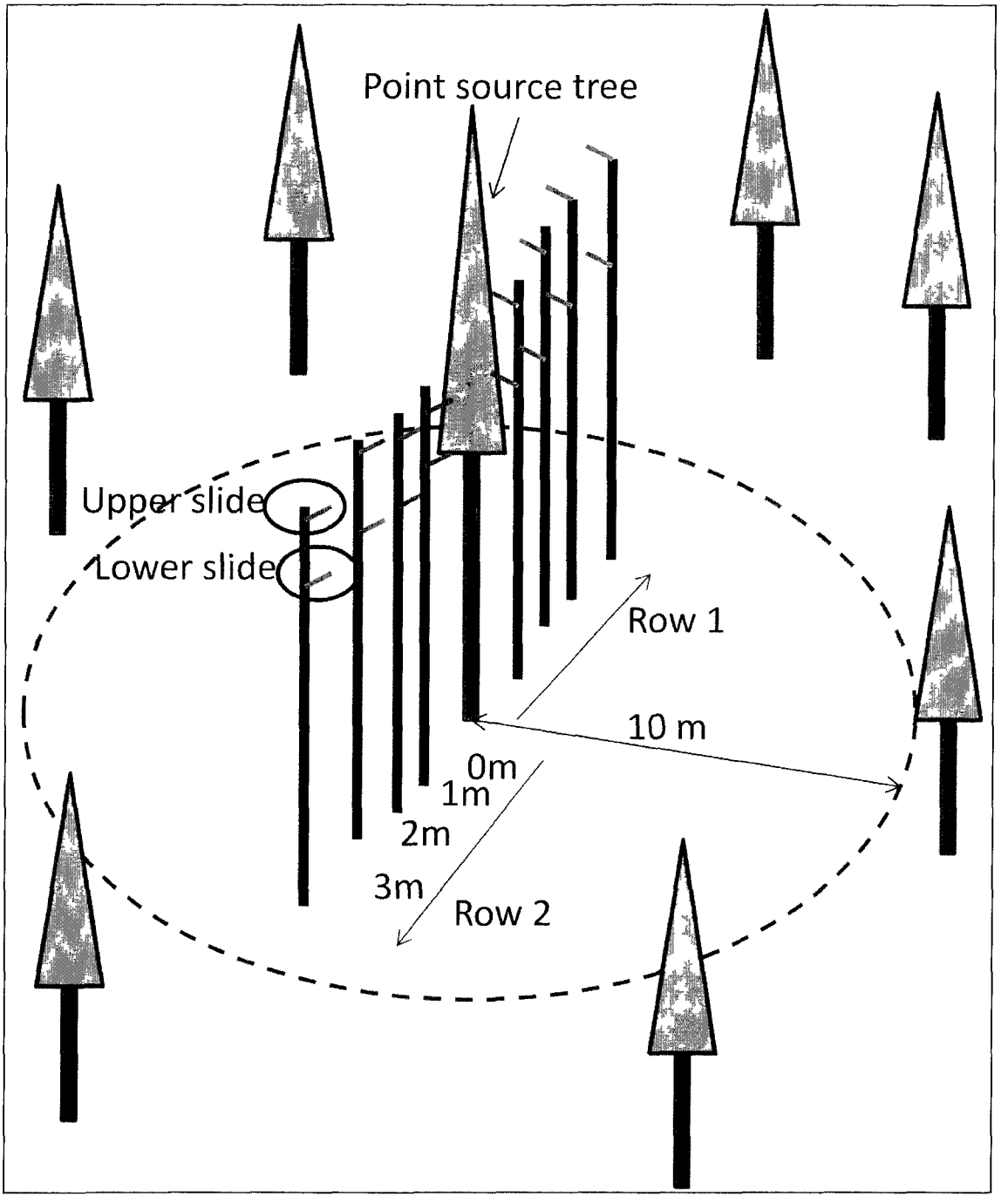


Figure 3 Locations of microscope slides (spore samplers) and retained infected lodgepole pine tree (point source tree) used to determine the dispersal distance of *Dothistroma* spores in the Bulkley TSA in northwest BC, Canada in 2009 and 2010

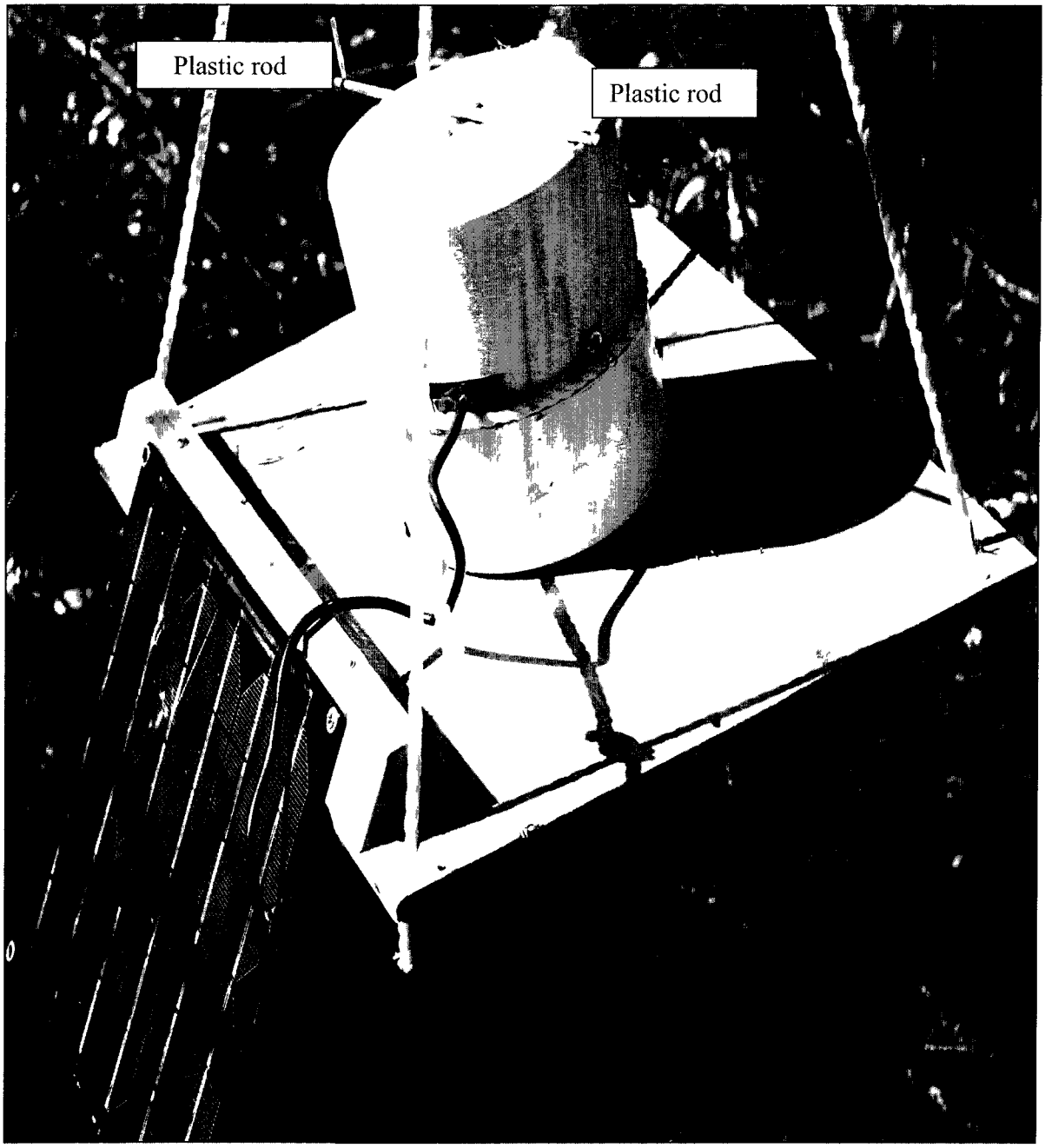


Figure 4. Rotorod spore sampler used for air borne spores sampling in the Bulkley TSA in northwest BC, Canada in 2009.

Table 1. Exposure period and dates of lodgepole pine seedlings to *D. septosporum* spores for field inoculation at the study sites in the Bulkley TSA in northwest BC, Canada during the 2009 field season.

Group	Exposure period	Batch number	Date of exposure
A	2 weeks	1	14 May – 27 May
		2	27 May – 11 June
		3	11 June – 25 June
		4	25 June – 9 July
		5	9 July – 22 July
		6	22 July – 6 August
		7	6 August – 19 August
		8	27 August – 11 September
		9	11 September – 26 September
B	4 weeks	1	14 May – 11 June
		2	11 June – 9 July
		3	9 July – 6 August
		4	6 August – 7 September
C	6 weeks	1	14 May – 25 June
		2	25 July – 6 August
		3	6 August – 20 September

Chapter 4: Results

4.1 Relative frequency of ascospores and conidia

Throughout the study period in both 2009 and 2010, ascospores (sexual spores) of the *D. septosporum* were never observed. Only conidia (asexual spores) were trapped on both the microscope slides and in the rain water. The conidia trapped on the slides were mostly concentrated in water droplets which had collected on the slides; these spores were elongated, straight and hyaline with 1 to 5 septa (Fig. 5). On average, the dimensions of most conidia trapped were 14–40 x 2-3μm. Neither conidia nor ascospores were trapped on the rotorods which were mounted in an attempt to trap spores that might be dispersed by air.

4.2 Temporal pattern of spore dispersal

Spore sampling started on May 30 in 2009 and for almost the first three weeks (May 30 to June 17) of sampling on both sites, no spores were observed by any of the sampling methods used. On June 20th, the first spores were observed in the collected rain water and on the microscope slides on both study sites (Fig. 6). During the 2010 field season, spore sampling started on the 23rd of April and similar to the previous season, no spores were observed until the 10th of June 10, 2010 (Tables 2 and 3). Results from the 2009 sampling season indicated that the numbers of spores collected on the slides increased when it rained after the first observation on June 20, reaching its peak on July 20, and spore counts started decreasing by the second week in September. No spores were collected

between July 22 and August 7 at both sites and spores were absent on the slides and in the rain water on the last day of sampling (September 26) despite the fact that rainfall was recorded (Fig. 6). The nine week sampling period in 2010 also showed increases in the number of spores dispersed after the first observation on both sites (Tables 2 and 3). The peak of spore dispersal and the date dispersal halted at the study area during the 2010 sampling season was not observed since sampling was terminated at the end of June.

Average numbers of conidia per $10cm^2$ collected on the lower slides positioned 1m away from the inoculum sources ranged between 0 and 214 on Site 1, and between 0 and 86 on Site 2 in 2009. In 2010 the average numbers ranged from 0 to 280 on Site 1 and 0 to 96 on Site 2. Comparatively, more spores were counted on Site 1 than Site 2 but the difference was not statistically significant ($t_{198} = -0.0519$, $P = 0.9611$). However, similar dispersal patterns were observed on both sites with most spores collected from June 26 to July 20 and from August 10 to September 20 in 2009 (Fig. 6). The timing and number of spores collected in the rain water were also similar on both sites with most spores trapped on July 20 and August 13 at Site 1, and on July 14 and August 13 at Site 2 (Fig. 7).

4.3 Climatic Data

During the 2009 sampling period, the mean rainfall for every 3 days over a period of time ranged between 0 and 21 mm at Site 1 with an average of 5.9 mm. Similarly, the mean rainfall on Site 2 ranged between 0 and 23.5 mm with an average of 5.3 mm. No rainfall was recorded throughout the first two weeks of sampling (May 30 – June 14) at both sites

in 2009. A prolonged dry period was also experienced on both sites during the 9th and 10th weeks of sampling (July 22 – Aug 7, 2009) as shown in Fig. 8 and 9. The amount of rainfall recorded in 2010 was greater than in 2009 and rainfall was recorded more regularly during the 2010 sampling period without any prolonged dry conditions (Tables 2 and 3).

Daily mean of 24 hour temperature recorded during the 2009 sampling period ranged from 9.8 to 22.7 ^{o}C with an average of 14.3 ^{o}C at Site 1 and between 8.9 and 23.1^{o}C with an average of 14.5^{o}C at Site 2. The temperature was slightly higher on weeks without rainfall and lower on weeks with rainfall. The highest temperature during the sampling in 2009 season was recorded on August 1 at both sites (Figs. 10 and 11). The mean minimum overnight temperatures ranged from -0.7 to 11.4 at Site 1 and -0.9 to 11.7 at Site 2. Temperature was negatively correlated with rainfall (r = -0.46).

The daily mean of 24 hour relative humidity varied from 57.0 to 88.7% with an average of 74.9% and from 53.6 to 91.5% with an average of 76.5 at Site1 and Site 2 respectively. The daily mean relative humidity was high at the end of August through to end of September during the 2009 sampling period at both sites (Figs. 12 and 13).

Generally, the percentage of daily mean of 24 hour leaf wetness recorded in 2009 on both sites was relatively low, with the minimum recorded on June 5 and maximum recorded on September 9. Daily mean of 24 hour leaf wetness at Site 1 ranged from 15.0 to 75.2% with an average of 43.3% and ranged between 0.9 and 83.8% with an average of 44.7% at

Site 2 (Fig. 14 and 15). Leaf wetness and relative humidity were strongly correlated (r = 0.65).

The mean amount of rainfall recorded between (April 23) and (June 28) in 2010 ranged between 0 and 29 mm with an average of 7.2 mm at Site 1, while on Site 2 the range was between 0 and 31 mm with an average of 8.1 mm. Over the entire sampling period, the average daily mean of 24 hour temperature, leaf wetness and relative humidity were 10.0°C, 39.6% and 72.5% respectively (Table 2 and 3)

4.4 Relationship between conidial dispersal and climatic variables

Conidia were only observed on the slides whenever rain fell throughout both sampling seasons after the first conidia were observed. A small amount of rainfall, as low as 0.5 mm triggered release and dispersal of spores. An increase in rainfall amount resulted in greater numbers of conidia disseminated but rainfall frequency was more important than rainfall amount. Most conidia were dispersed when the average amount of rainfall were respectively 17 mm and 15 mm at Site 1 and Site 2 on July 20 in 2009 (Fig. 8 and 9). In 2010 the highest numbers of spores were recorded when the amount of rainfall were 1.8mm and 1.1mm on Site 1 and Site 2 respectively. These peaks in spore dispersal occurred just after a high amount (>20 mm) of rainfall was recorded (Tables 2 and 3). Dispersal of conidia of *D. septosporum* was significantly associated with rainfall (Table 4). No conidia were observed on rainless days irrespective of the temperature and relative humidity.

The number of spores dispersed was negatively associated with temperature. Daily mean temperature within which most spores were released and dispersed ranged between 9.61 and 17.61 °C and a few or no spores were observed when temperature was 18°C and above in 2009 (Figs. 10 and 11). Similarly, in 2010 most spores were observed when temperature ranged between 11.20 and 14.40°C (Tables 2 and 3). From the regression results, daily mean temperature significantly influenced the number of conidia dispersed during the sampling period in 2009 (Table 4). The mean minimum overnight temperature had no significant relationship with the number of spore collected ($t_{197} = -0.725$, $P = 0.4700$).

Most conidia were collected when mean relative humidity during the sampling period in 2009 ranged from 78.70 to 85.42% and percentage leaf wetness ranged from 48.07 to 75.08%. In 2010, most spores were trapped when the daily mean relative humidity and leaf wetness ranged from 72.30 to 78.10%, and 44.20 to 49.50% respectively. The number of conidia collected on the slides was significantly related to relative humidity. The effect of percentage leaf wetness on conidial dispersal was also statistically significant (Table 4). Generally, a few or no conidia were trapped when relative humidity was below 65% (Figs. 12 and 13) and leaf wetness was below 40% on both sites (Figs. 14 and 15).

Average number of conidia trapped increased with increasing rainfall, relative humidity, and leaf wetness but decreased with increasing temperature. From the results of multiple regression models with mixed effects, all the climatic variables significantly correlated

with spore abundance. The relationship between rainfalls, leaf wetness and the number of conidia collected fit the model best, and the relationship was significant (Table 4). Therefore, the best model for the effects of climatic factors on conidia dispersal was the regression equation $Log_{10}\ y = -0.197 + 0.042x_1 + 0.030x_2$, where y is the average number of conidia per 10 cm^2, x_1 is mean amount of rainfall over a 3 day period in mm and x_2 is daily mean leaf wetness in percentage. This relationship holds for a mean 3 days rainfall between 0 – 23.5 mm and daily a mean of 24 hours leaf wetness between 0.9 – 83.8% recorded during this study.

4.4 Relationship between number of conidia dispersed, distance and height from the inoculum source

The greatest numbers of conidia were observed on the slides positioned directly beneath the crowns of the sampled trees (no distance from inoculum sources), followed by those positioned 1m away from the inoculum sources. It was rare to observe spores on the slides positioned 2 and 3 m away from the inoculum sources as shown in Fig. 16A and 16B. On average, the greatest numbers of conidia observed per $10cm^2$ sampling area for every 3 days over the entire sampling period in 2009 were respectively 791, 110, 11 and 4 at 0, 1, 2 and 3 m from inoculum sources at Site 1 (Fig. 16A). From (Fig. 16B), the greatest average numbers of conidia observed for every 3 days over the entire sampling period in 2009 at Site 2 were 228, 84, 4 and 2 at 0, 1, 2, and 3m respectively from inoculum sources. The number of conidia trapped significantly decreased with increasing in distance from the inoculum sources ($t_{611} = -18.187$, $P < 0.0001$). The relationship between distance and spore counts was modeled best by regression equation

Log_{10} y = 1.849 – 0.649x, where y is number of conidia sampled and x is distance from the inoculum source. This relationship holds for numbers of conidia sampled at distances of 0, 1, 2 and 3m from inoculum sources within the study area.

Moreover, the number of spores trapped on the lower slides were slightly higher than the number trapped on the upper slides (Figs. 17A and 17B), but there was no significant difference statistically (t_{401} = -0.502, P = 0.6156). The results showed that more spores were collected on the lower slides than the upper slides but vertical height has no significant effect on number of conidia dispersed, which was modeled by regression equation $Log_{10} y = 1.125 - 0.076x$ where y is number of conidia sampled and x is the vertical distance from the inoculum sources. This relationship holds for vertical distance of 0.5m above inoculum source at the study area.

4.5 Stand Data

The two plantations used for the study were located within the Interior Cedar – Hemlock moist cold two (ICHmc2) biogeoclimatic zone. Site 1 was within site series 06 (ICHmc2/06) whiles Site 2 was within site series 01 (ICHmc2/01). Site 1 had an elevation ranging between 438 and 444 m and Site 2 ranging between 552 and 554 m. At Site 1, heights of lodgepole pine trees ranged between 1.9 and 7.9 m with an average of 5.4 m, the diameter at breast height (dbh) ranged between 4 and 16 cm with an average of 10.2 cm and the percentage functional live crown ranged between 10 and 50% with an average of 27.2%. However at Site 2 the lodgepole pine trees had heights ranging

between 2.1 and 7.3 m with an average of 4.6m, dbh ranged between 3.1 and 11.2 cm with an average of 6.5 cm and the functional live crown ranged between 10 and 45% with an average of 26.2%. (Table 5)

From ANOVA tests, it was noted that the average heights of lodgepole pine trees at Site 1 were significantly different from Site 2 ($F_{1, 89} = 6.468$, $P = 0.013$). Average dbh of lodgepole pine trees at the two sites were also significantly different ($F_{1, 89} = 25.2$, $P < 0.0001$). The functional live crowns of lodgepole pines at both sites were not statistically different ($F_{1, 89} = 0.187$, $P = 0.667$).

The average number of trees within each $200m^2$ composition plot was 25 and 66 for Site 1 and Site 2 respectively. In addition to lodgepole pine, both sites had hybrid white spruce, western hemlock, trembling aspen, black cottonwood, paper birch, sitka alder and willow spp. (Table 6). The only disease found on lodgepole pines apart from Dothistroma needle blight was western gall rust. This disease was found on stems and branches of 5 trees on site 1 and 3 trees on site 2.

4.6 Infection of Seedlings

Lodgepole pine seedlings were placed under the canopy of infested lodgepole pine plantations from mid-May to late September in 2009. Seedlings exposed to this natural inoculum during July, August and September developed symptoms of red band needle blight, whereas those exposed in May and June did not. Symptoms were first observed in

early January 2010 on some of the seedlings and the seedlings were kept and monitored until September 2010 when clear red bands and fruiting bodies were observed on some of the dead needles. Thirty eight of 192 seedlings (19.8%) exposed showed symptoms but only eight (4.17%) produced definite signs of *Dothistroma septosporum*. Of the 108 (56%) seedlings that were exposed for a two weeks period, only ten showed symptoms and no seedling produced fruiting bodies on the needles. Seedlings exposed for a six week period recorded the highest infection (17 seedlings) with fruiting bodies evident on needles of six seedlings. Symptoms of red band needle blight were recorded on 11 of the seedlings exposed for four week periods (Figs. 18 and 19) although fruiting bodies developed on only 2 of the seedlings (both at site 1). The effect of exposure period of the seedlings on the incidence of infection, as determined by the production of symptoms was highly significant (Table 7). Incidence of symptom development was also significantly related to the number of conidia collected at both study sites (Table 7). Development of fruiting bodies occurred on seedlings exposed during the periods when moderate and high numbers of conidia were collected (Figs. 18 and 19).

All the climatic factors apart from temperature were significantly correlated with symptoms especially relative humidity (Table 7). The mean mount of rainfall in each 3 day period which was related to symptoms development during the exposure periods ranged from 5 to 21mm and 5 to 23.5mm at Site 1 and Site 2 respectively (Figs. 20 and 21). The mean daily temperature for symptoms development respectively ranged from 12^0C to 18.1^0C and 12 to 19.2^0C at Site 1 and Site 2 (Figs. 22 and 23). Relative humidity was constantly high during the periods within which infection occurred. The daily mean

relative humidity during exposure periods that resulted in symptom production ranged from 72 to 81.6 % and 72 to 84% at Site 1 and 2 respectively (Figs. 24 and 25). The Symptoms of the disease occurred when the mean daily leaf wetness ranged from 39.2 to 60.3% at Site 1 and 44.4 to 69.2% at Site 2 (Figs. 26 and 27). From the logistic regression with mixed effects, a model with exposure period, number of conidia and relative humidity was best able to predict the incidence of Dothistroma needle blight disease at the study area (equation 1).

[1] $y = -12.978 + 0.569(EP) + 0.120\ (RH) + 0.013(NS)$

EP = exposure period of seedlings to inoculum, RH = daily mean relative humidity during the exposure period and NS = number of spores released and dispersed for infection.

Table 2. Mean numbers of conidia per $10cm^2$ collected on the lower slides positioned 1m away from the inoculum sources in each three day period and the means of daily climatic variables for 24 hours at Site 1 in the Bulkley TSA in northwest BC, Canada in 2010.

Sampling date	Mean number of conidia*	Rainfall(mm)	Temperature(^{O}C)	Leaf wetness (%)	Rh (%)
April 26	0	0.8	6.4	20.6	60.4
29	0	29.0	6.3	67.7	82.7
May 3	0	5.0	4.3	52.7	78.7
6	0	10.0	4.2	62.7	82.1
10	0	0.0	7.5	19.9	61.3
13	0	0.0	9.3	15.0	62.5
17	0	0.6	9.9	39.1	69.2
20	0	24.0	9.2	61.7	84.1
25	0	15.0	8.7	66.9	82.0
28	0	0.0	13.5	12.4	70.6
31	0	0.0	14.1	5.5	60.4
June 3	0	10.0	9.6	52.9	77.0
7	0	0.5	10.8	41.0	73.3
10	4 ± 1	26.0	13.3	44.5	71.8
16	280 ± 23	1.8	11.2	49.5	72.3
21	0	0.0	15.8	11.5	63.3
24	150 ±13	4.0	14.4	44.2	78.1
28	67 ± 6	3.0	11.9	45.5	75.4

* n = 4

Table 3. Mean numbers of conidia per 10cm2 collected on the lower slides positioned 1m away from the inoculum sources in each three day period and the means of daily climatic variables for 24 hours at Site 2 in the Bulkley TSA in northwest BC, Canada in 2010.

Sampling date	Mean number of conidia*	Rainfall(mm)	Temperature(^{O}C)	Leaf wetness (%)	Rh (%)
April 26	0	0.8	6.4	20.6	60.4
29	0	31	6.3	67.7	82.7
May 3	0	14	4.3	52.7	78.7
6	0	11	4.2	62.7	82.1
10	0	0	7.5	19.9	61.3
13	0	0	9.3	15.0	62.5
17	0	0.6	9.9	39.1	69.2
20	0	21	9.2	61.7	84.1
25	0	17	8.7	66.9	82.0
28	0	0	13.5	12.4	70.6
31	0	0	14.1	5.5	60.4
June 3	0	9	9.6	52.9	77.0
7	0	0.5	10.8	41.0	73.3
10	2 ± 1	31	13.3	44.5	71.8
16	96 ± 7	1.1	11.2	49.5	72.3
21	0	0	15.8	11.5	63.3
24	80 ± 5	5	14.4	44.2	78.1
28	45 ± 2	4	11.9	45.5	75.4

* n = 4

Table 4. Table of regressions for the relationship between climatic variables and the mean counts of conidia of *D. septosporum* in the Bulkley TSA in northwest BC, Canada in 2009.

	Variable	Coefficient	SE	t-value	p-value	AIC
A. Univariate regressions				t_{197}		
	Intercept	0.922	0.2340	3.94	0.0001	803
	Rain	0.079	0.0116	6.87	0.001	
	Intercept	-0.443	0.3029	-1.46	0.1451	791
	LW[3]	0.042	0.0052	7.95	0.00001	
	Intercept	-5.975	0.8966	-6.66	0.0001	801
	Rh	0.098	0.0114	8.51	0.0001	
	Intercept	4.844	0.5584	8.67	0.0001	802
	Temp.	-0.239	0.0359	-6.63	0.0001	
B. Multivariate regressions				t_{195}		
	Intercept	0.499	1.0981	0.45	0.6503	793
	Rain	0.044	0.0129	3.45	0.0007	
	Rh	0.031	0.0098	3.18	0.0017	
	Temp.	-0.108	0.0414	-2.61	0.0098	
	Intercept	-1.922	0.6913	-2.78	0.006	793
	Rain	0.054	0.0125	4.29	0.0001	
	Rh	0.043	0.0093	4.59	0.0001	
	Intercept	-0.197	0.3140	-0.63	0.0530	790
	Rain	0.042	0.0135	3.09	0.0023	
	LW	0.030	0.0063	4.82	0.00001	
	Intercept	3.359	0.6333	5.30	0.001	792
	Rain	0.056	0.0126	4.45	0.001	
	Temp.	-0.159	0.0387	-4.12	0.0001	

[3] LW = leave wetness, Rh = relative humidity

Table 5. Stand characteristics of lodgepole pines trees at the study sites (mean dbh, height and percentage functional live crown of lodgepole pine trees) in the Bulkley TSA in northwest BC, Canada in 2009.

Site	Plot	Diameter at breast height(cm)	Tree height(m)	Functional live crown (%)
1	1	11.5 ± 3.0	5.7 ± 0.7	34.1 ± 9.3
	2	7.5 ± 2.2	5.2 ± 1.7	21.7 ± 6.8
	3	11.7 ± 3.5	5.5 ± 1.2	25.8 ± 7.4
Mean		**10.2 ± 2.9**	**5.4 ± 1.2**	**27.2 ± 7.8**
2	1	6.7 ± 1.9	5.4 ± 0.9	28.3 ± 8.9
	2	5.2 ± 1.1	3.7 ± 1.1	21.7 ± 7.2
	3	7.5 ± 1.7	4.7 ± 0.8	28.8 ± 9.5
Mean		**6.5 ± 1.6**	**4.5 ± 0.9**	**26.2 ± 8.5**

Table 6. Tree species composition at the study sites in the Bulkley TSA in northwest BC, Canada in 2009.

Site	*Plot*	*Tree Species*	*No. /7.98m*
1	1	Lodgepole pine	11
		Hybrid white spruce	2
		Trembling aspen	1
		Willow spp.	1
	2	Lodgepole pine	18
		Hybrid white spruce	3
		Paper birch	4
		Willow spp.	6
	3	Lodgepole pine	6
		Hybrid white spruce	1
		Paper birch	4
		Trembling aspen	17
2	1	Lodgepole pine	20
		Black cottonwood	28
		Douglas maple	2
		Paper birch	16
		Western hemlock	8
	2	Lodgepole pine	12
		Black cottonwood	4
		Paper birch	12
		Trembling aspen	1
		Western redcedar	14
	3	Lodgepole pine	24
		Western hemlock	8
		Black cottonwood	32
		Paper birch	13
		Sitka alder	3
		Willow spp.	1

Table 7. Logistic regression with mixed effects models for the relationship between climatic variables, number of conidia, and exposure periods of seedlings and symptoms development of dothistroma blight in the Bulkley TSA in northwest BC, Canada in 2009.

	Variable	Coefficient	SE	Z-value	P-value	AIC
A. Univariate regressions				Z_{190}		
	Intercept	-1.870	0.3139	-5.96	2.57e-09	193
	Rain	0.289	0.1360	2.12	0.0338	
	Intercept	-3.093	0.6113	-5.06	4.18e-07	187
	LW	0.037	0.0117	3.13	0.00174	
	Intercept	-7.901	2.2216	-3.91	9.28e-05	185
	Rh	0.087	0.0262	3.31	0.00093	
	Intercept	-1.865	0.9532	-1.96	0.0505	197
	Temp.	0.035	0.0649	0.53	0.5930	
				Z_{195}		
	Intercept	-1.844	0.2294	-8.04	9.11e-16	179
	Spores	0.013	0.0038	4.15	0.000035	
	Intercept	-3.023	0.4805	-6.29	3.14e-10	181
	Exposure	0.463	0.1138	4.07	0.000047	
				Z_{188}		
B. Multivariate regressions	Intercept	-12.978	3.0730	-4.22	2.41e-05	152
	EP [4]	0.569	0.1351	4.21	0.000025	
	RH	0.120	0.0366	3.28	0.001056	
	NS	0.013	0.0003	3.59	0.000334	

[4] EP = Exposure period, RH = Relative humidity, NS = No. of spores.

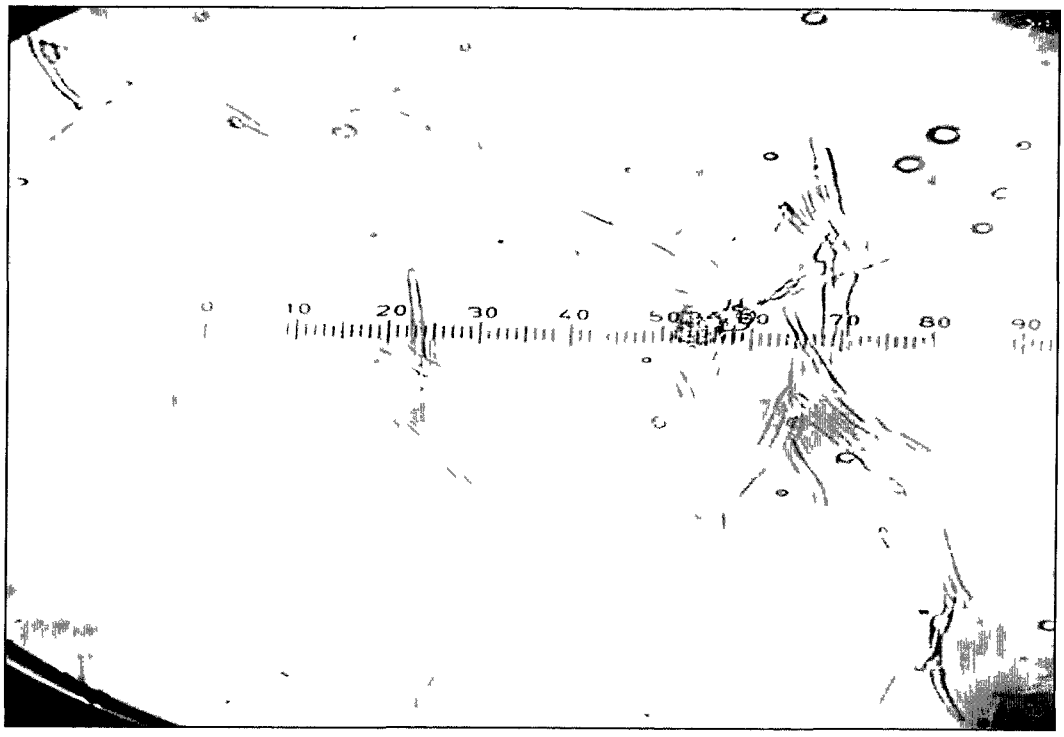


Figure 5. Conidia of *D. septosporum* trapped on microscope slide during 2009 field season in the Bulkley TSA in northwest BC, Canada. (400X)

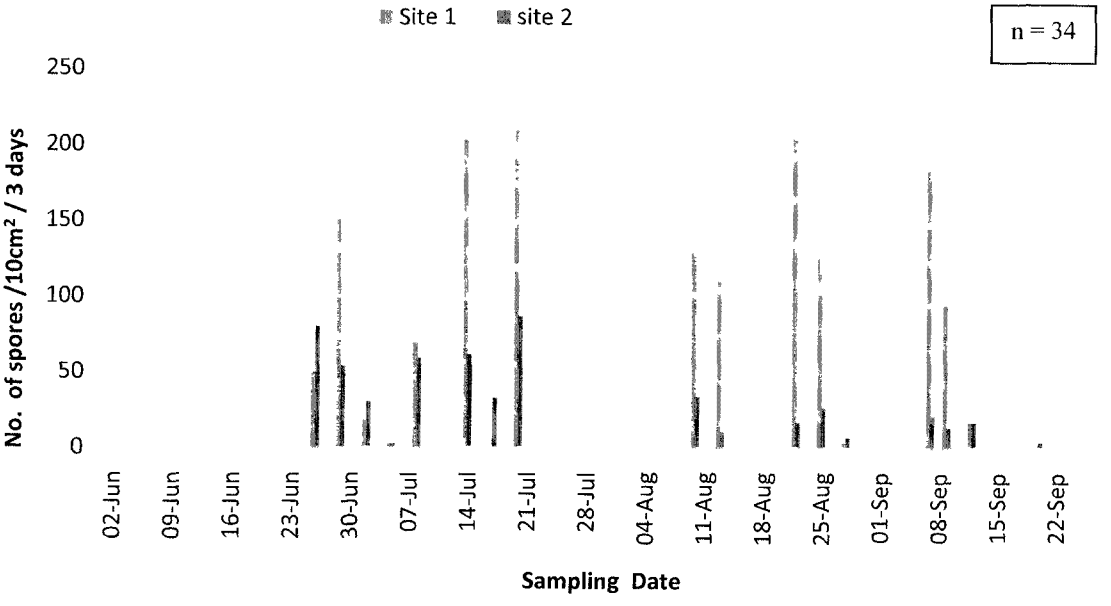


Figure 6. Mean count of conidia collected on the lower microscope slides positioned 1m horizontally from inoculum sources at the two study sites during 2009 sampling period in the Bulkley TSA in northwest BC, Canada. Note: n = sampling size.

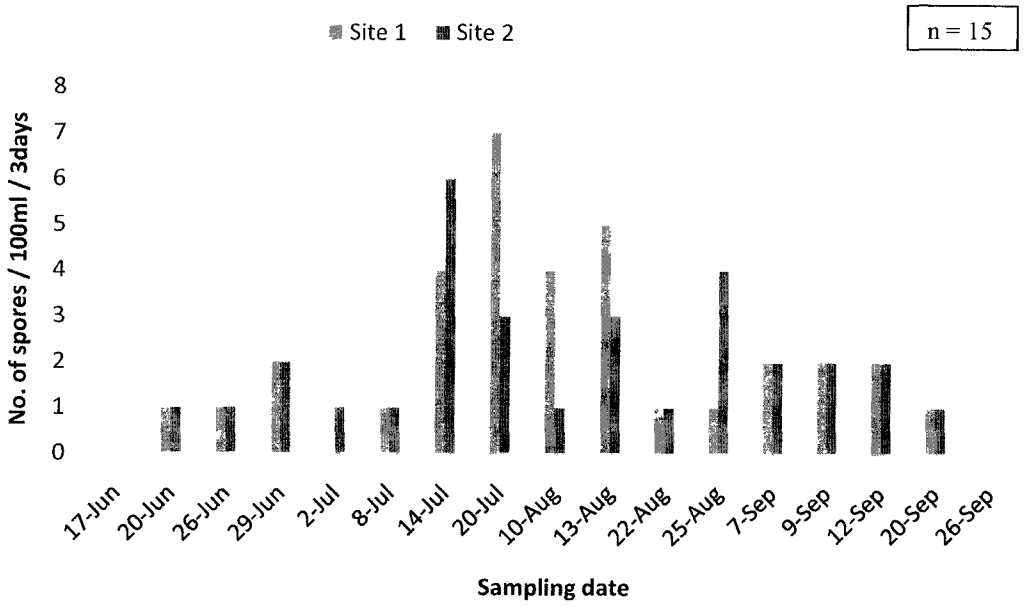


Figure 7. Mean count of conidia trapped in rain water collected at the two study sites during 2009 sampling period in the Bulkley TSA In northwest BC, Canada.

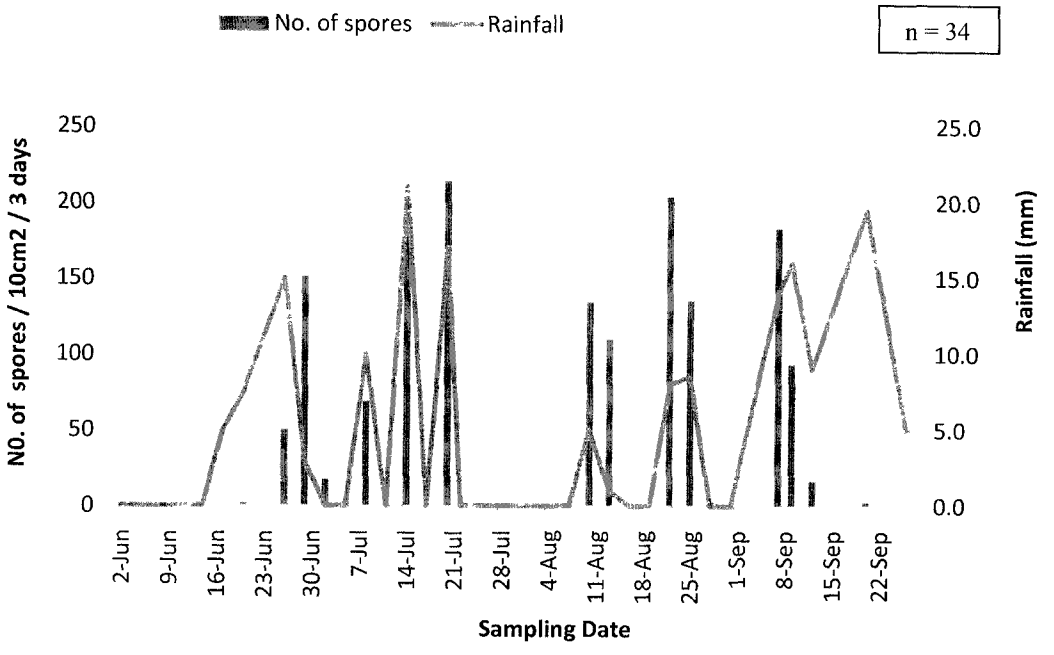


Figure 8. Relationship between mean counts of conidia collected on the lower microscope slides positioned 1m horizontally from inoculum sources and mean amount of rainfall at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

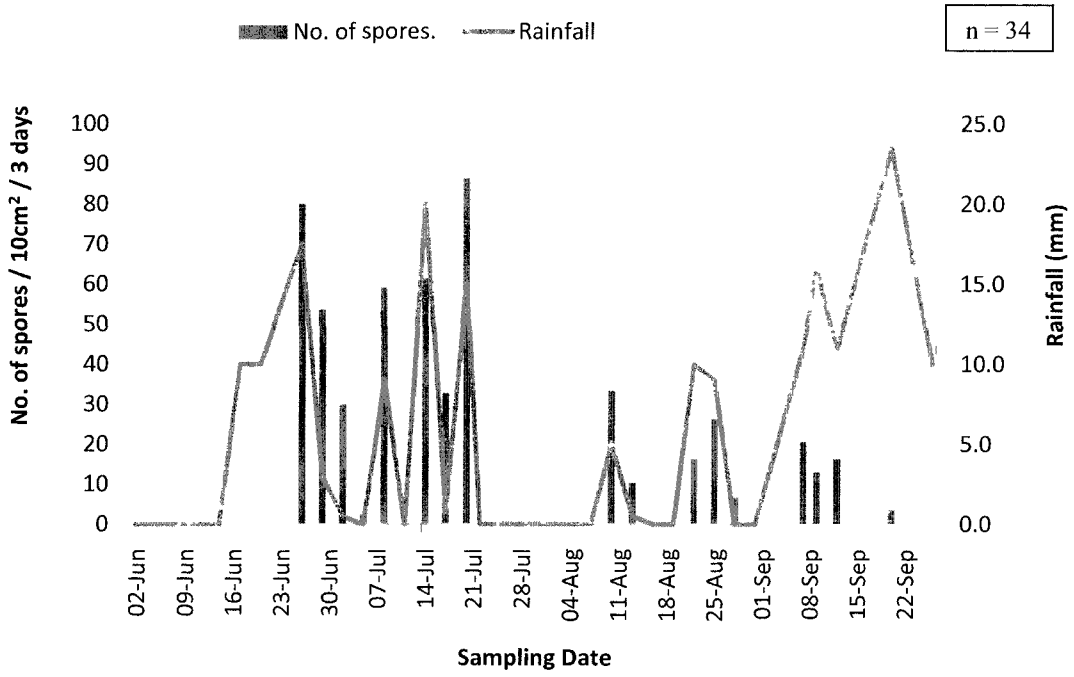


Figure 9. Relationship between mean counts of conidia trapped on the lower microscope slides positioned 1m horizontally from inoculum sources and mean amount of rainfall at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

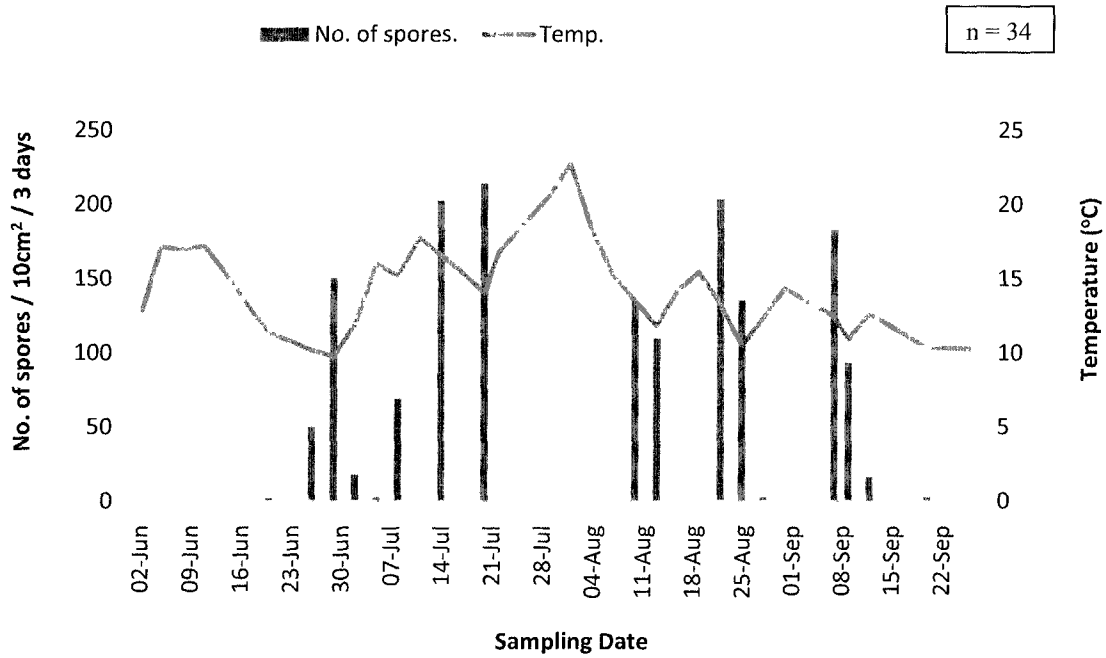


Fig 10. Relationships between mean counts of conidia trapped on the lower microscope slides positioned 1m horizontally from inoculum sources and daily mean temperature at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

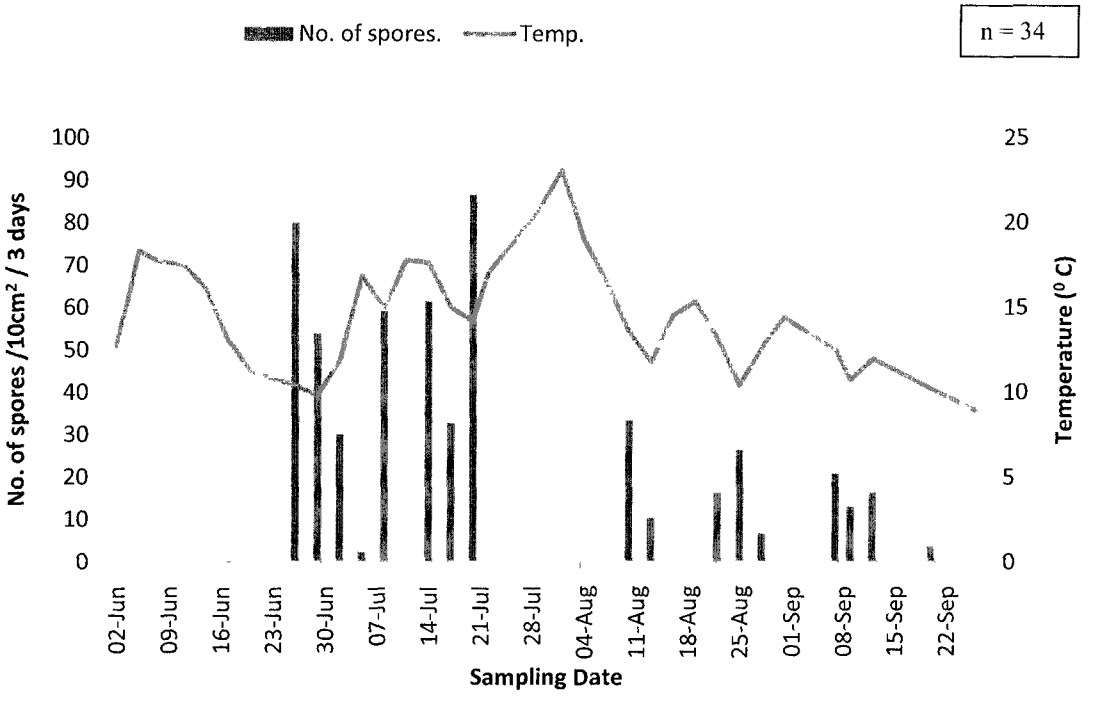


Figure 11. Relationship between mean counts of conidia trapped on the lower microscope slides positioned 1m horizontally from inoculum sources and daily mean temperatures at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

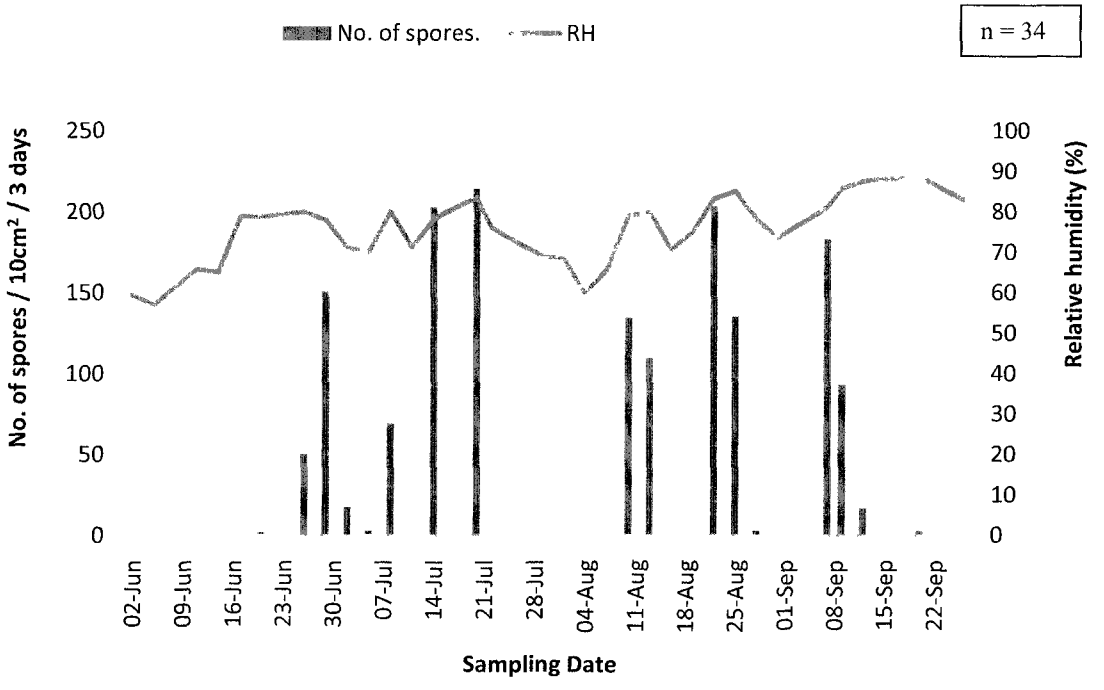


Figure 12. Relationship between mean counts of conidia trapped on the lower microscope slides positioned 1m horizontally from inoculum sources and daily means relative humidity at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

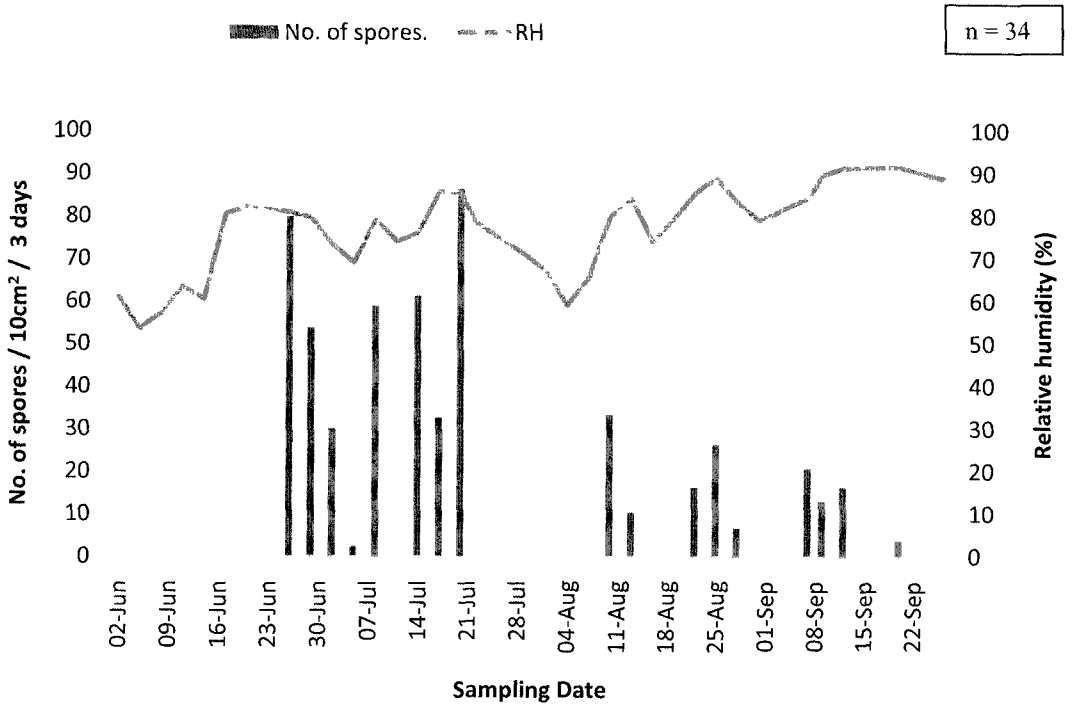


Figure 13. Relationship between mean counts of conidia trapped on the lower microscope slides positioned 1m horizontally from inoculum sources and daily means relative humidity at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

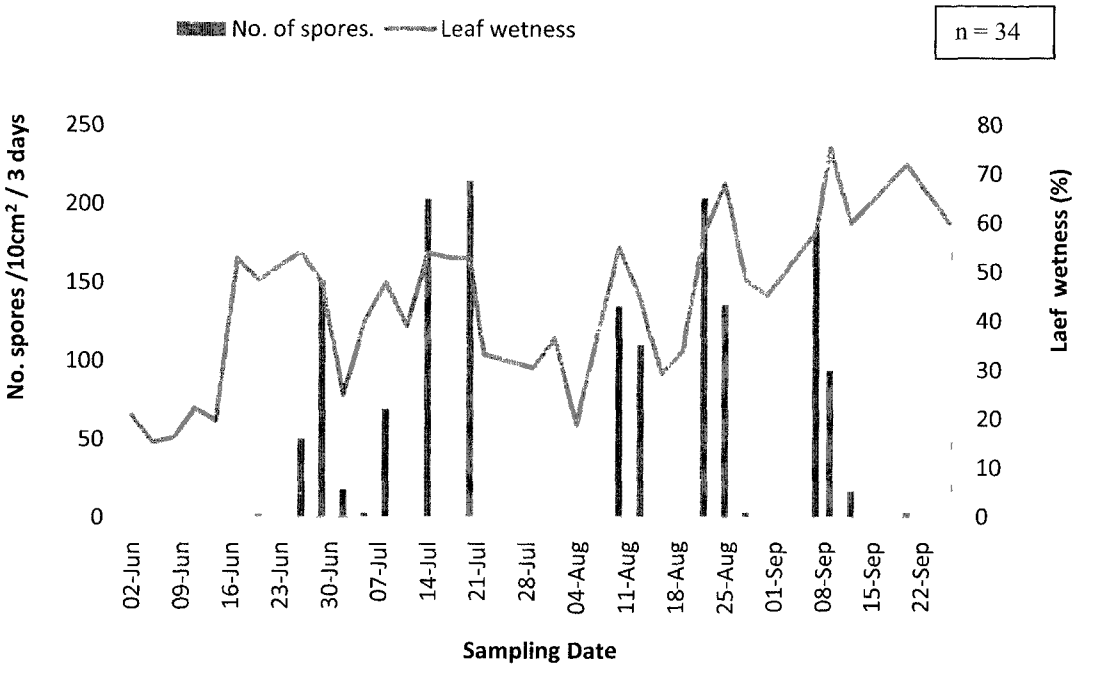


Figure 14. Relationship between mean counts of conidia trapped on the lower microscope slides positioned 1m horizontally from inoculum sources and daily mean leaf wetness at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

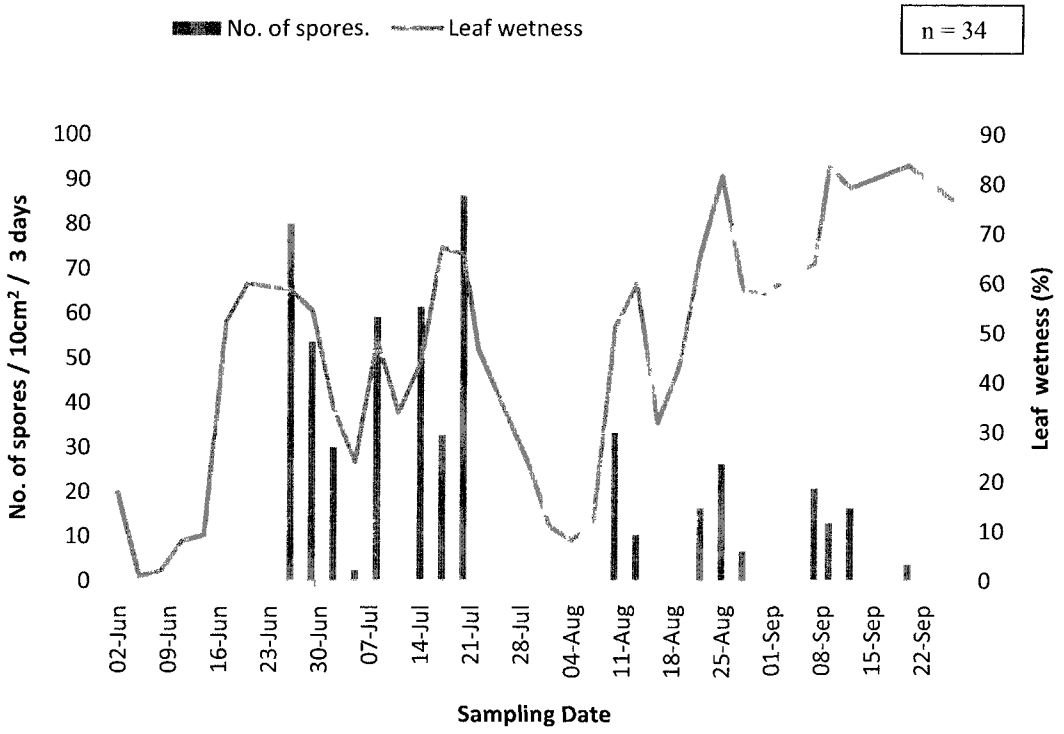


Figure 15. Relationship between mean counts of conidia trapped on the lower microscope slides positioned 1m horizontally from inoculum sources and daily mean of percentage leaf wetness at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

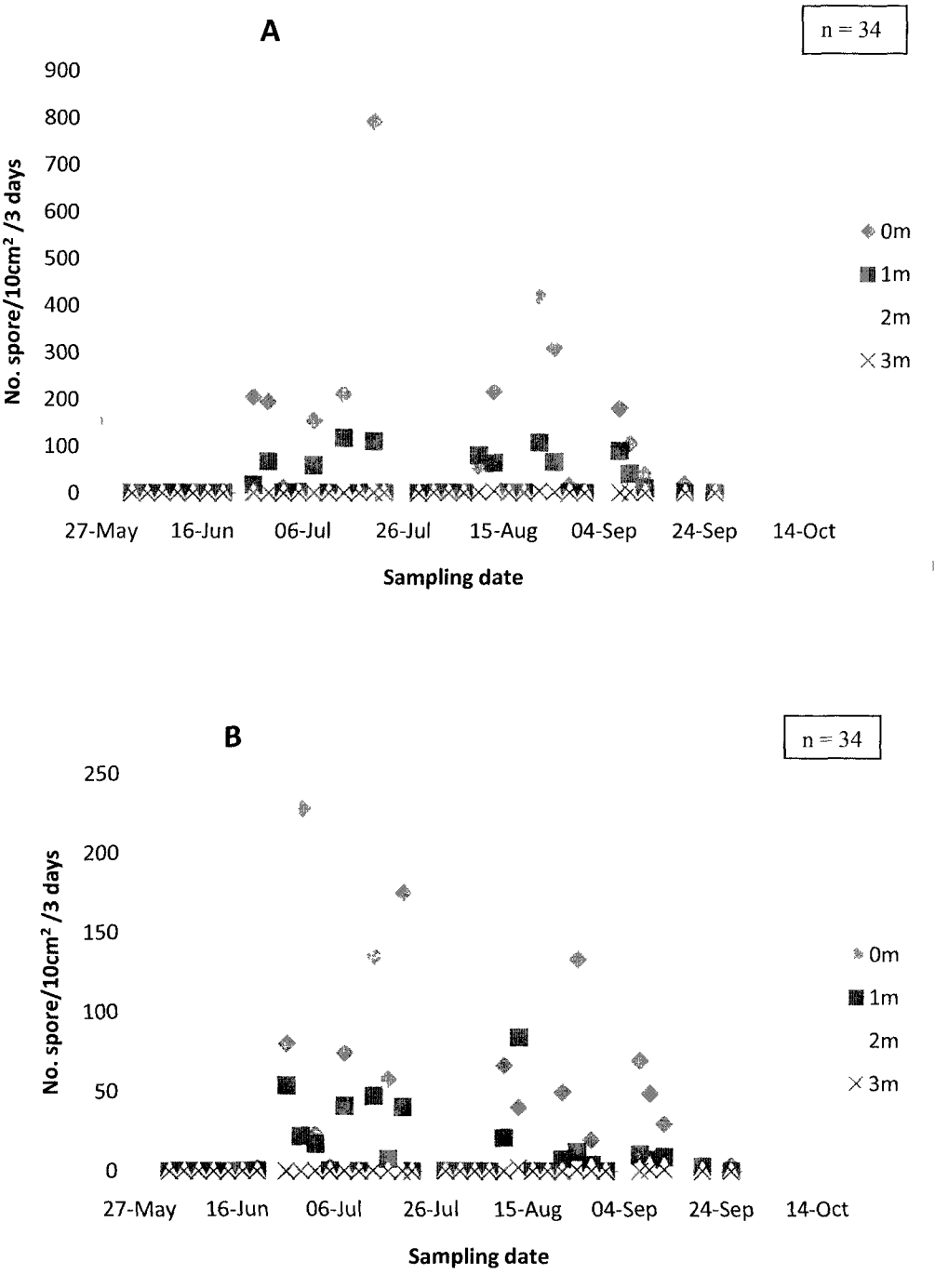


Figure 16. Mean counts of conidia/10cm^2 trapped on the lower slides at different distances from inoculum sources at Site 1(A) and Site 2 (B) in the Bulkley TSA in northwest BC, Canada during 2009 sampling period.

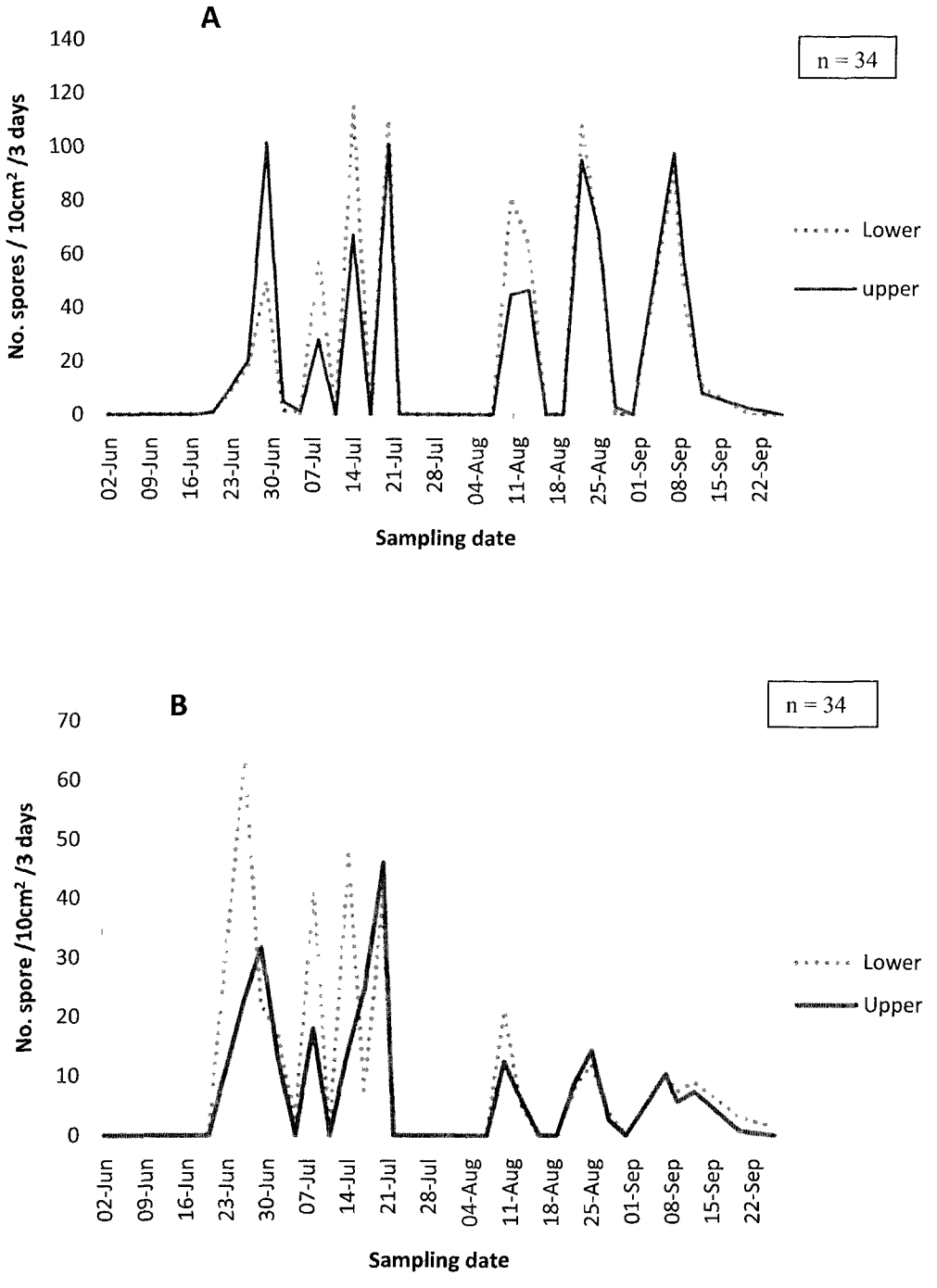


Figure 17. Mean counts of conidia/10cm^2 trapped on the lower and upper slides positioned 1m horizontally from inoculum sources at Site 1(A) and Site 2(B) in the Bulkley TSA in northwest BC, Canada during 2009 sampling period.

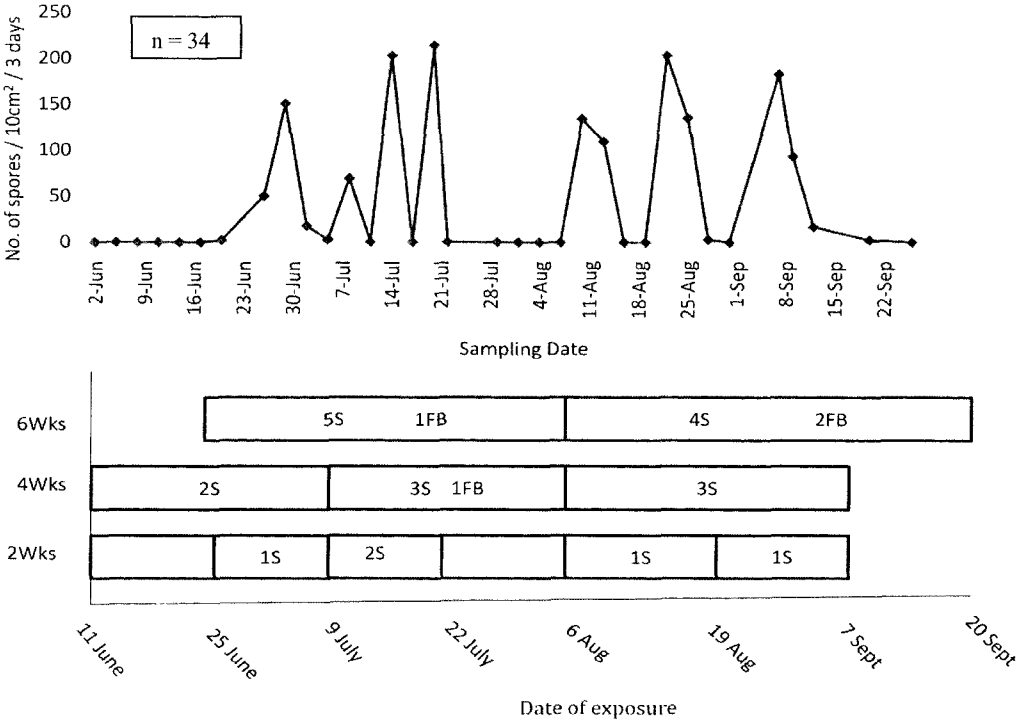


Figure 18. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean number of conidia per 10cm^2 collected on the lower slides positioned 1m horizontally from inoculum sources in three day periods over the entire period of seedling exposure at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009. Note: S = Symptoms and FB = Fruiting bodies.

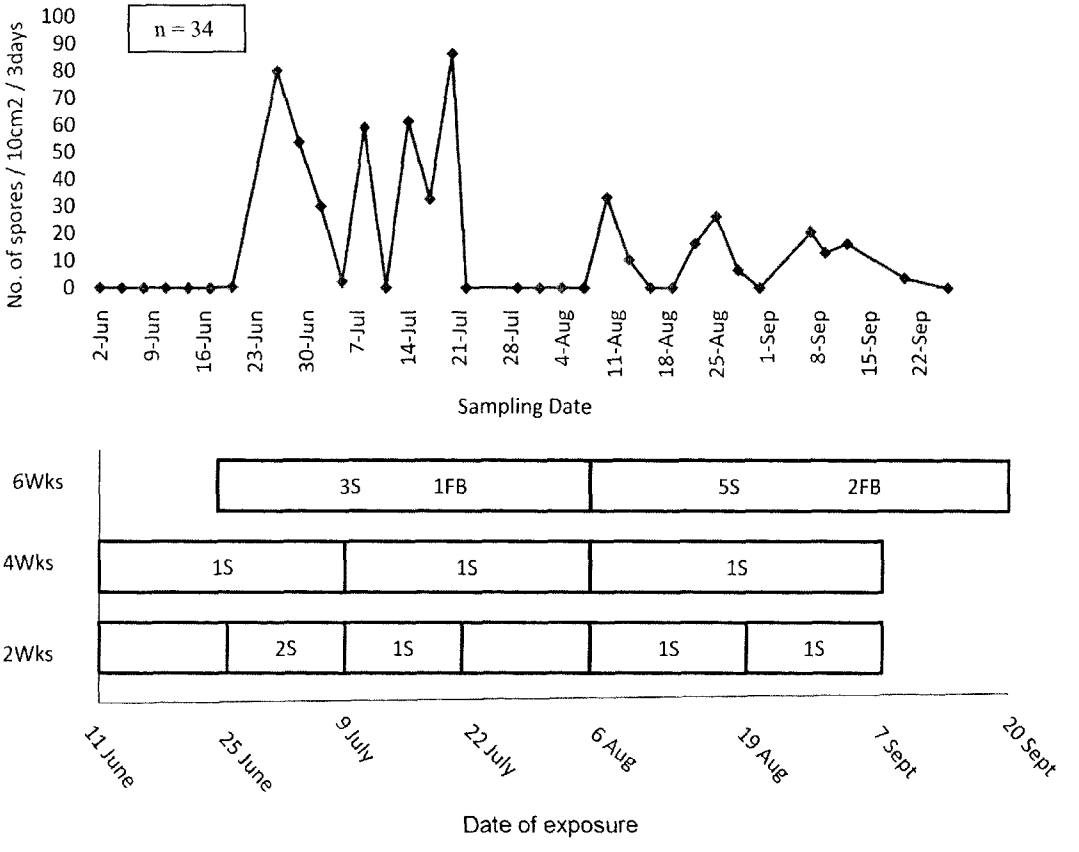


Figure 19. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean number of conidia per $10cm^2$ collected on the lower slides positioned 1m horizontally away inoculum sources in three day periods over the entire period of seedling exposure at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

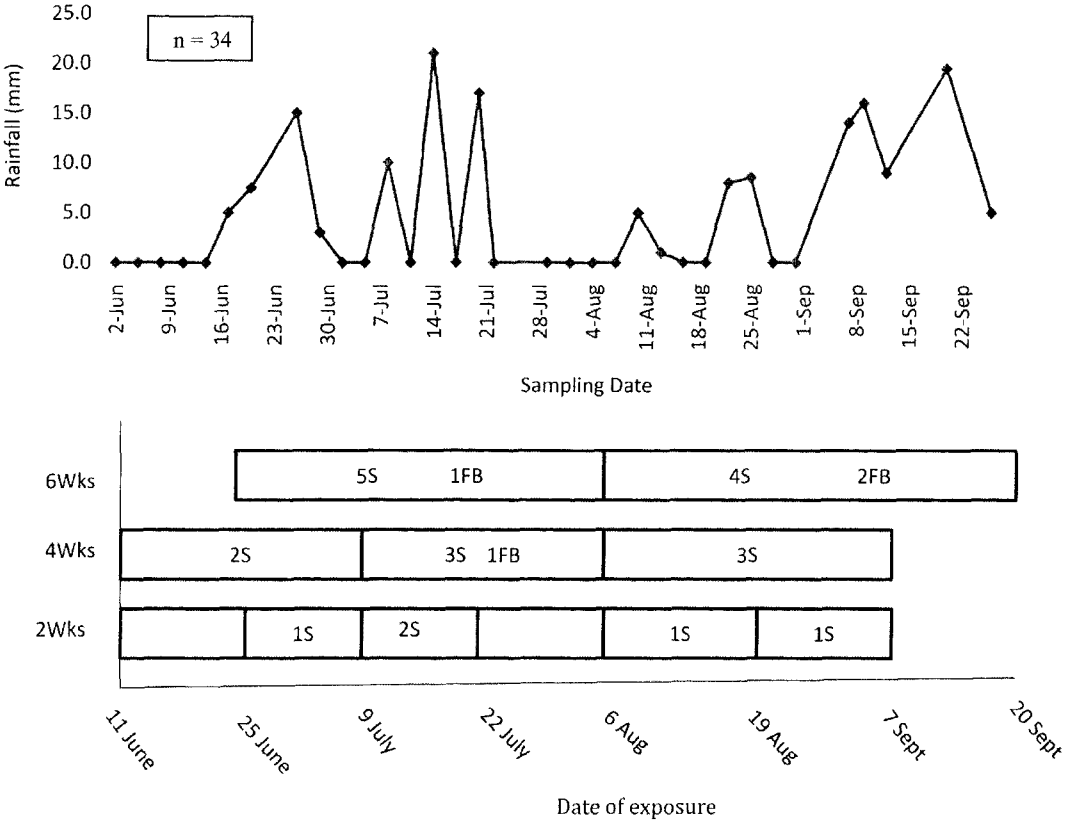


Figure 20. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean daily rainfall in three day periods over the entire period of seedling exposure at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

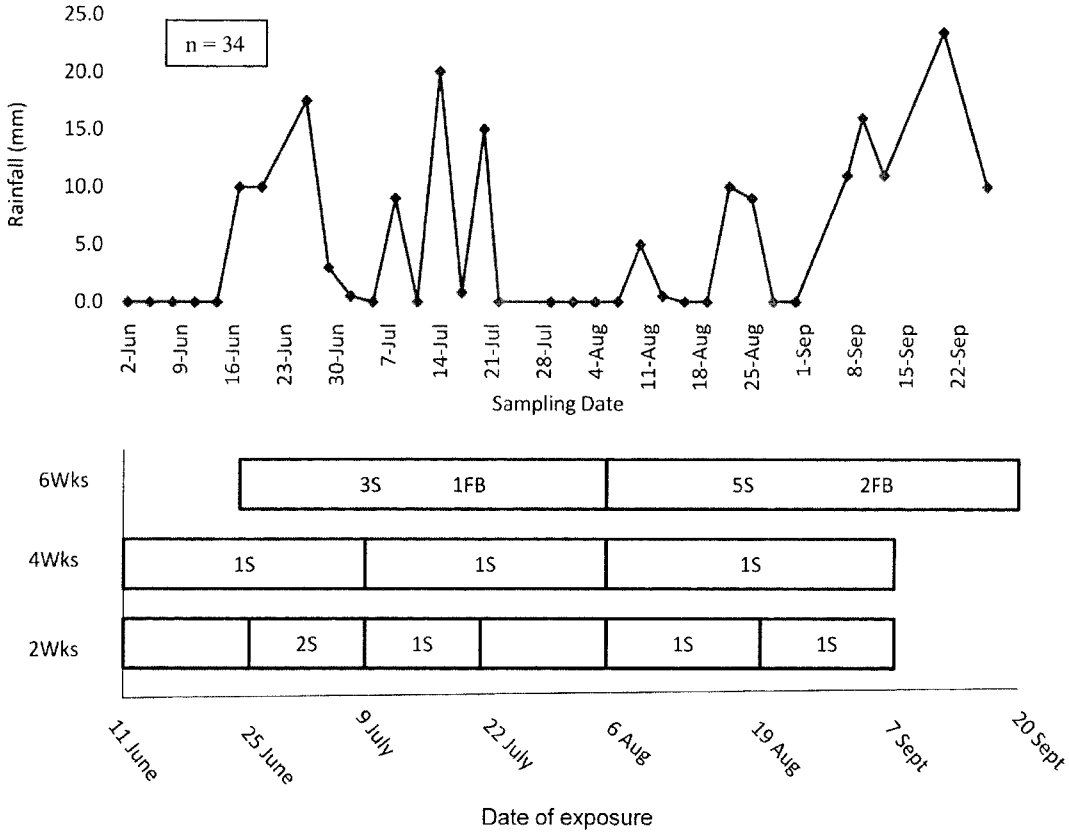


Figure 21. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean daily rainfall in three day periods over the entire period of seedling exposure at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

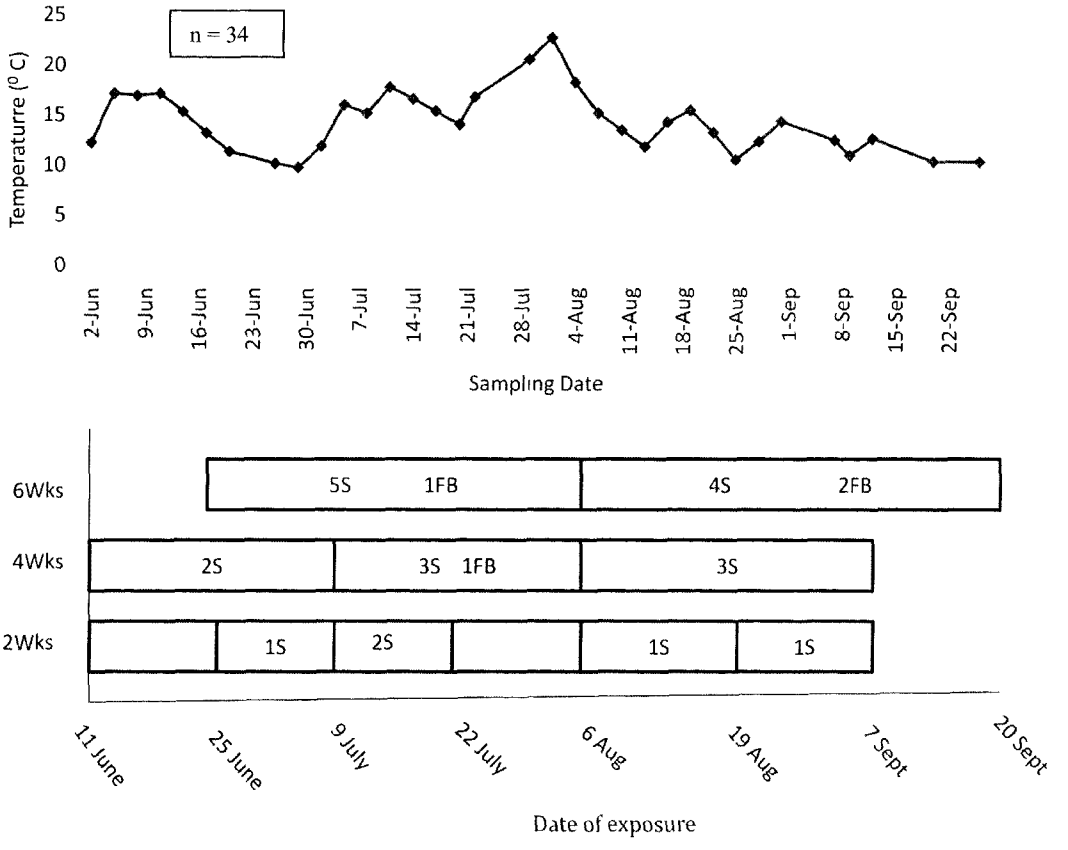


Figure 22. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean daily temperatures for 24 hours in three day periods over the entire period of seedling exposure at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

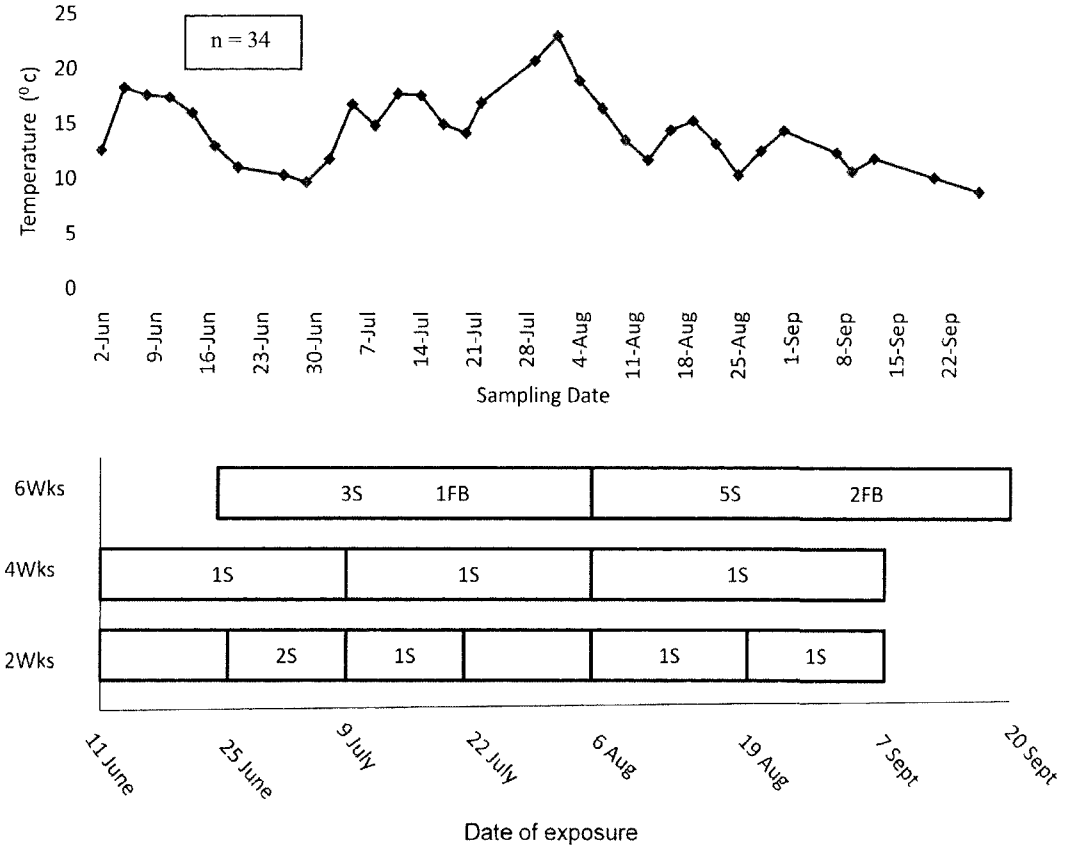


Figure 23. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean daily temperature for 24 hours in three day period over the entire period of seedling exposure at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

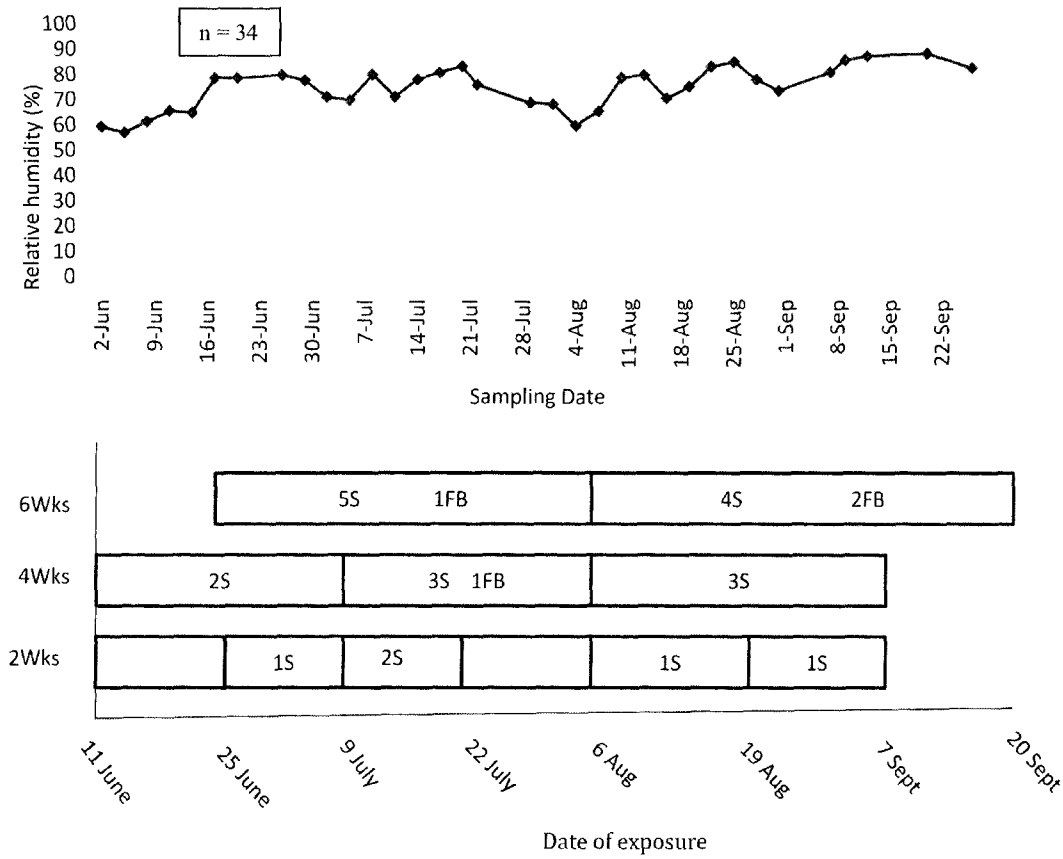


Figure 24. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean daily relative humidity for 24 hours in three day periods over the entire period of seedling exposure at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

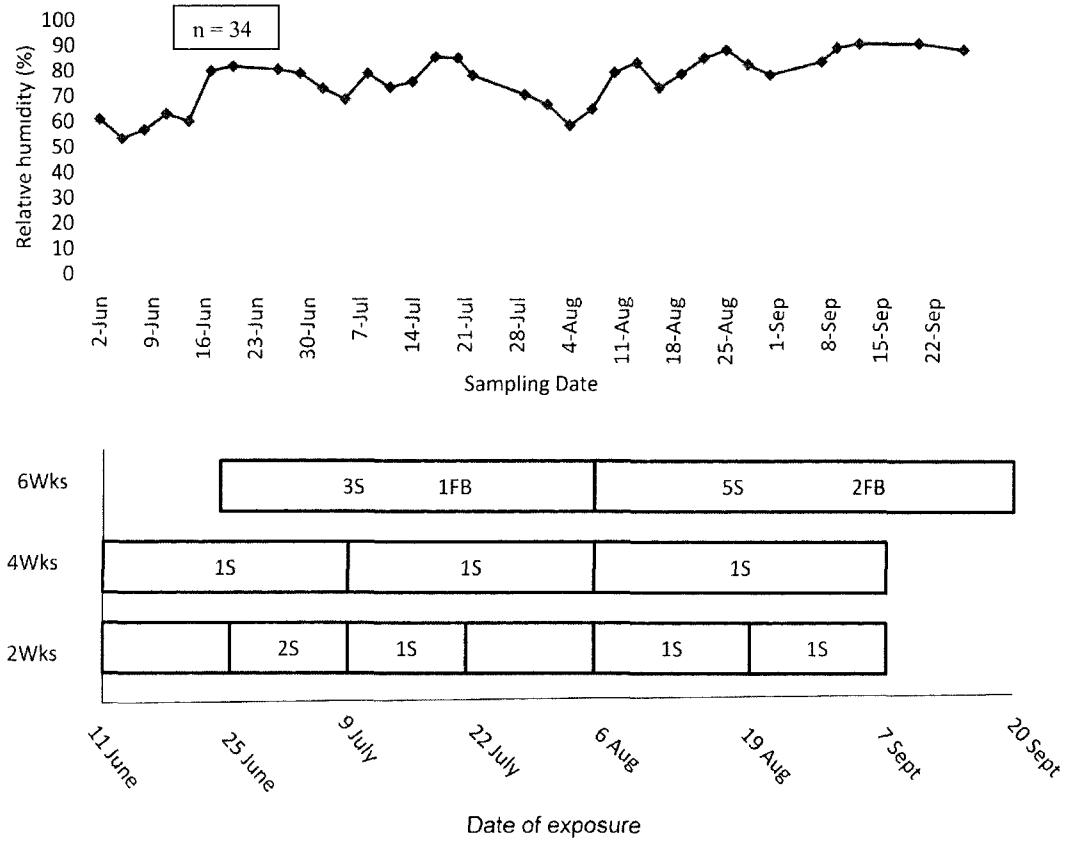


Figure 25. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the means of daily relative humidity for 24 hours in three day periods over the entire period of seedling exposure at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

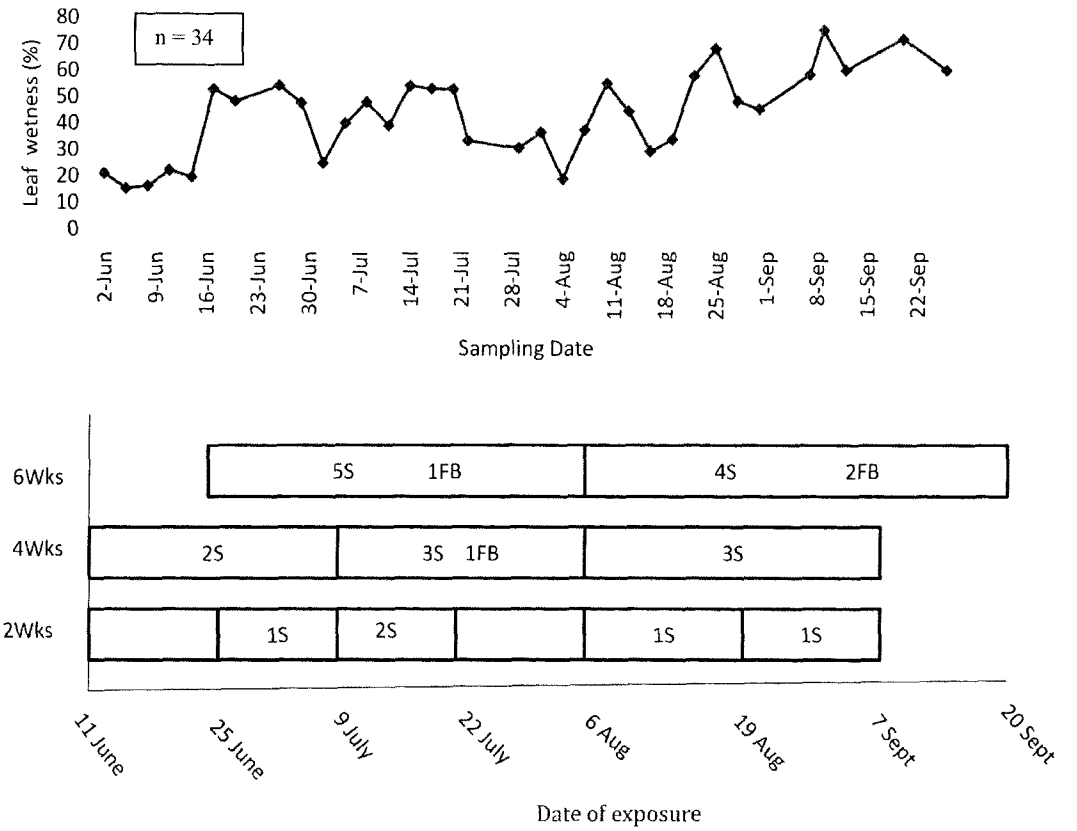


Figure 26. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the means of daily leaf wetness for 24 hours in three day period over the entire period of seedling exposure at Site 1 in the Bulkley TSA in northwest BC, Canada in 2009.

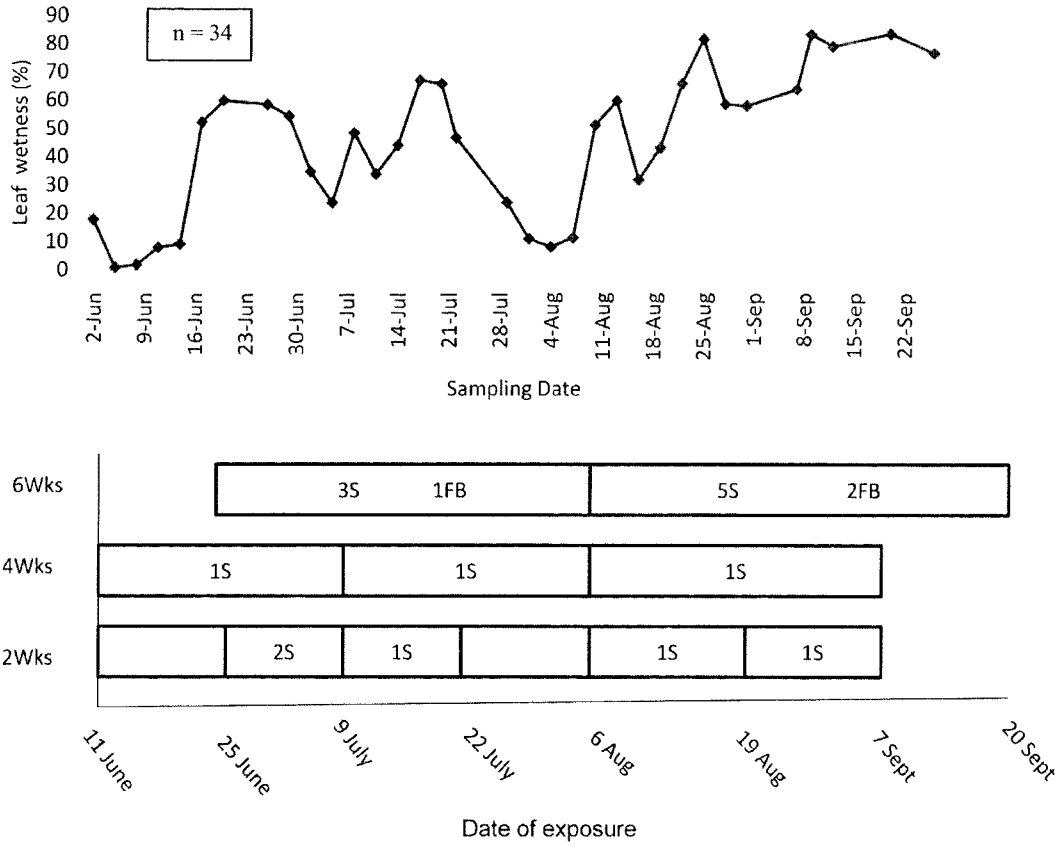


Figure 27. Number of infected seedlings with signs and symptoms of Dothistroma needle blight during each exposure period and the mean daily leaf wetness for 24 hours in each three day periods over the entire period of seedling exposure at Site 2 in the Bulkley TSA in northwest BC, Canada in 2009.

Chapter 5: Discussion

5.1 Relative frequency of ascospores and conidia

Ascospores were never observed during the study. This suggests that the sexual spores of the fungus *Dothistroma* were not common in the study area, the means of detecting the spores were not adequate, or that sexual reproduction was not taking place in the population. A study on disease development conducted by Braun (2009) in the same general area as the current study observed ascomata only twice in 2,670 fruiting body dissections. However, in 2008, Dale *et al.* (2011) found a high level of genotypic variability between isolates obtained among and within sites, and even from isolates taken from infected needles within a single tree. They concluded that sexual reproduction was the main reason for this variability, which is supported by the findings of Groenewald *et al.* (2007) which showed that both mating types of the fungus were present in isolates from the same area. Despite this evidence which supports the existance of sexual reproduction in the population of *D. septosporum* in northwest BC, only asexual spores were detected in the current study. The lack of observation of ascospores may have resulted from the sampling methods used which may not have been appropriate for collecting ascospores, or the timing of the sampling period missed ascospore production. It is also possible that during outbreak periods, the production of ascospores declines in favour of the production of conidia. The evidence from the previous studies (Braun, 2009; Dale *et al.*, 2011; Groenewald *et al.*, 2007) suggest the sexual spores of the fungus exist in northwest BC, but results from this study indicate that at least during outbreak periods, they are not as common as the asexual spores.

The situation in BC does not appear uncommon. Spore trapping investigations over a five year period by Peterson (1973) in eastern Nebraska, USA and a three year investigation by Bizhang *et al.* (1992) in China yielded no ascospores despite genetic evidence that suggested that sexual reproduction of the fungus occured in Nebraska (Groenewald *et al.*, 2007). Markovskaja and Treigiene (2009) also found no teleomorphic stage in their study to evaluate the spread of *Dothistroma* on *Pinus sylvestris* in eastern, central and southern parts of Lithuania and reported that only the anamorphic stage was responsible for the disease on needles of *Pinus sylvestris*. Barnes *et al.* (2004) reported that the teleomorph *Mycosphaerella pini* was not observed in *Dothistroma* samples collected from different areas including BC and Nebraska where evidence for sexual reproduction was observed in the isolates collected by Groenewald and colleagues (2007). Results of this study and those of previous researchers indicate that the asexual spores of *D. septosporum* are common and play a major role in the infection and spread of the disease Dothistroma needle blight (Gibson, 1974, Hildebrand, 2005). This suports the reports by Bradshaw (2004) and Karadzic (1989) that the ascospores are considered to be less important in infection and disease spread than the conidia because they are less abundant and the conidia are dispersed over longer periods. Peterson (1982) reported that the role of ascospores is not clear but undoubtedly they can cause infection as evidenced by the introduction of recombined genomes into the population. Therefore the role played by the ascopores in causing infection and disease spread can not be ruled out but they are less important in disease spread than the conidia. The finding of the current study suggests that spread of Dothistroma needle blight disease in the northwest of BC, is most likely due to the dispersal of the conidia of the fungus.

5.2 Temporal pattern of spore dispersal

According to the results of this study, conidia of *Dothistroma* dispersed between June and September during the 2009 sampling period . Although spore sampling started on May 30 and April 23 in 2009 and 2010 respectively, conidia were first trapped on June 20 in 2009 and June 10 in 2010. The peak of dispersal occurred on July 20 in 2009, a finding that was in agreement with results of a previous study conducted by Braun (2009) in the same vicinity. In that study, Braun examined development of symptoms of red band needle blight, and fruiting bodies of *Dothistroma septosporum* and found that fruiting bodies appeared on the needles in early June with peak from July to August, but spores were not detected until late June with a peak in July. In 2009 when traps were monitored from May 30 to September 26, most conidia were collected in two periods, from June 26 to July 20 and from August 10 to September 20, which suggests that *Dothistroma* spores were dispersed when conditions were conducive for spore production, release and dissemination. Results of field investigations conducted by Peterson (1973) in Nebraska, revealed that high numbers of spores were trapped in the months of June, July, August and September with no conidia trapped in October in four out of five years of the investigations despite the fact that there was significant rainfall in October for spore production. The results also agree with those of Binzhang et al. (1992) which showed peak conidia dispersal in June and July. Both Peterson (1973) and Bizhang *et al.* (1992) reported that conidia were first trapped in May but conidia dissemination started in June in both years of the current study. Although conidia were first trapped in May by Peterson during his investigations, the numbers were very low. The differences in the comencement of spore dispersal between the Nebraska and BC were presumably caused

by differences in weather conditions which affected development of the fruiting bodies and spore production. Braun (2009) found that although fruiting bodies were present on infected needles in late May, high numbers were not observed until early June and spore production began in late June. During her study, fruiting bodies dissected from the first week in June yielded no conidia until late June, suggesting a several week delay from fruit body emergence to spore production. This is consistant with Peterson (1973) that the period of first appearance of conidia in stromata and dispersal vary between two and four weeks. During the present study, the number of spores trapped at both study sites started decreasing from mid-September and no spores were collected in late September. The decrease in the number of spores collected, and the end of the spore dispersal period observed in the latter part of September was possibly caused by reduction in volume of inocula on the infected trees due to exhaustion of fruiting bodies or unfavourable weather conditions. It was observed that most of the infected needles of the lodgepole pine trees had dropped by the end of September 2009. This may be the most likely factor that contributed to the reduction of conidia dispersal. Despite high rainfall, relative humidity and leaf wetness recorded in the last two weeks of September 2009, few or no spores were observed at both study sites. Similar patterns were observed by both Bingzhang *et al.* (1992) and Peterson (1973) during their *Dothistroma* spore trapping investigations with conidia collected from May or June through September. Bingzhang and colleagues reported that conidia dispersal ended in September during their three year period investigation. Peterson observed conidia in October only once during his five-year investigation. The results of the present study suggest that spore dispersal of the fungus at the study area extends to late September.

5.3 Relationship between conidia dispersal and climatic variables

Climatic factors such rainfall, temperature, relative humidity and leaf wetness play important roles in the production, liberation and dispersal of spores of many foliar pathogens (Fitt *et al.*, 1989; Agrios, 2005). Dispersal of *D. septosporum* spores, like other foliar fungi, is strongly influence by climatic conditions (Gadgil, 1977; Peterson, 1973). During this study, spores were only observed whenever rain fell throughout the sampling period and no spores were trapped on dry days. However judging by the results, the number of conidia collected was related to rainfall but rainfall frequency was more important than the amount of rainfall. Even a small amount of rain, as low as 0.5mm triggered conidia dispersal. No conidia were observed in the first two weeks (2-17 June) of the 2009 sampling period which may be attributed to the absence of rainfall during the period. No spores were observed during a prolonged drought period from July 22 to August 7 in 2009. The trend observed in 2010 was similar, with no observation of conidia on rainless days even after the commencement of seasonal conidia dispersal. During both 2009 and 2010, conidia were observed at each plot whenever rain fell once the dispersal started and prior to the cessation of the dispersal period (late September). Similar results were observed in Nebraska (Peterson, 1973) and China (Bingzhang *et al.*, 1992), and Peterson reported that no conidia were trapped on rainless days. Bingzhang and colleagues also reported that during their three year study period, conidia were mainly dispersed by rain drops. They mentioned that no other method apart from rain splashing had caused the dispersal of the spores trapped. The results of an investigation conducted by Karadžić (1989) to define the time of production and dispersal of conidia and ascospores in Serbia also showed that conidia were intensely dispersed during wet

periods. During the present study, most of the conidia trapped on slides were observed in water droplets which had collected on the slides which was evidence that the conidia of the fungus were disseminated by rain splash. It is possible that masses of spores were released by rain water on the needle surfaces and disseminated in the water droplets (Brown and Webber, 2008; Meredith, 1973). Our results support previous research that found that the conidia of *Dothistroma* are rain splash dispersed (Peterson, 1967 and 1973; Bingzhang *et al.*, 1992; Bulman *et al.*, 2004). Wind or air might play a role in the dispersal of the spores by carrying a film of water containing the spores from the needle surfaces to new locations but the results of this study suggest that rain water is critical for *Dothistroma* spore dispersal. Based on the finding that no spores were observed during the periods with prolonged dry conditions or even a day or two without rain, the release and dispersal of the conidia were clearly not caused by dry air or any other mode of dispersal except rain water. Moreover, absence of spores on the rods which were mounted to trap spores that might be dispersed by air clearly shows that the spores were not air dispersed without rainfall. The absence of conidia on the rods during rainy periods also indicate that rain splash rather than wind-borne spores even in small droplets, is the primary means of spore dispersal of the fungus. It was observed that long distance dispersal was limited during this study (< 2 m) and this may support the fact that the spores sampled were mainly dispersed by rain but not dry air. Spores dispersed primarily by rain splash are noted to rarely travel over 2 m from their source while those dispersed by air/wind can travel several kilometers (Cooke *et al.*, 2006; Fitt *et al.*, 1989). EPPO/CABI (1997) reported that the hyaline nature of conidia makes them less adapted to exposure of ultra-violet radiation and thus less likely to survive transport by methods

other than rain-splash. Spores that are produced in mucilage are splashed dispersed and prevent dispersal by wind alone (Fitt *et al.* 1989). It has been identified that the mucilage surrounding splash-borne spores protects them from desiccation and the loss of viability during dry weather. This confines dispersal of the spores to periods of rainfall when conditions favour not only spore dispersal, but also infection because free water is available for germination on host surface (Fournet, 1969 cited in Fitt *et al.* 1989). There are reports that conidia of *Dothistroma* are dispersed over long distances by mist or fog and clouds (EPPO/CABI, 1997; Hildebrand, 2005) but no spores were trapped on foggy and cloudy days during the present study without rainfall. This suggests that moisture is needed to release the spores from the needle surfaces before they can be carried by fog, clouds or wind to different locations. Mist and fog were not measured directly during this study but if they had caused the release and dispersal of the spores, conidia would probably have been collected on rainless days, or conidia would have been collected in the rotorod traps during rainy days. Braun (2009) observed no relationship with disease severity and proximity to large water bodies (such as rivers) which would be areas with higher amounts and frequency of fog.

Percentage leaf wetness significantly influenced conidia dispersal during this investigation. The results showed that most conidia were released and dispersed when percentage leaf wetness was moderate or high (48.1 – 75.1%) and consistently few or no spores were dispersed when percentage leaf wetness was below 40%. The relationship between *Dothistroma* spore release, dispersal and percent leaf wetness may be due to the fact that the spores are formed in mucilage that requires moisture to dissolve before the

spores are released in a film of water on the needle surfaces. Meredith (1973) reported that spores that are formed in mucilage are held firmly to the plant surfaces when dry but readily released when wetted and this may best explain why no spores were observed during dry conditions in our study when needle surfaces were dried. There was a positive relationship between leaf wetness and rainfall. Periods with high percentages of leaf wetness which caused spores dispersal were only observed on days with rainfall. This excluded periods of fog or dew, as substantial enough in terms of needle wetness to cause the release of the spores.

The relationship between relative humidity and the number of spore dispersed was significant and this may be attributed to the positive relationship between rainfall and relative humidity and the fact that high relative humidity is conducive for spore production by most fungi (Agrios, 2005). High relative humidity might also have played a role in keeping the needles wet for the discharge and dispersal of the spores because during this study most spores were observed when the mean relative humidity for 24 hours was high (78.7 - 85.4%) and few or no spore were observed during periods with low relative humidity (< 65.0%). It was only on the last day of sampling (September 26) in 2009 that the presence of rainfall and high relative humidity did not result in spore dispersal at any of the sites and this could be attributed to the end of dispersal season at the study area. Although, relative humidity was positively correlated with rainfall ($r = 0.37$), it was more correlated with leaf wetness ($r = 0.65$). There were periods (July 22 to August 1) with high relative humidity (65.2 to 80.4%) during this study but no spores were collected. This suggests high relative humidity with the presence of rainfall plays

important role in the release and dispersal of the spores of *Dothistroma*. Therefore rainfall was found to be the most important factor affecting the release and dispersal of the spores. It may also explain why the combined effects of rainfall and leaf wetness fitted best in the model developed to determine the relationship between Dothistroma spore dispersal and climatic factors.

In contrast to the positive relationship between rainfall, relative humidity, leaf wetness and number of conidia collected, mean daily temperature negatively correlated with the number of spores collected and the relationship was significant. Temperature negatively correlated with relative humidity and leaf wetness during the study. This might be caused by evaporation of moisture and condensation of water vapour in the air due to the absorption of high solar radiation on sunny days. Moreover, minimum overnight temperature had no significant influence on number of spores collected. It was found that increases in temperature resulted in a reduced number of spores trapped which was assumed to have been caused by dry conditions during periods with high temperatures, which negatively affects spore dispersal (Bulman *et al.*, 2004; Gibson, 1972; Peterson, 1973). In Poland, Arseniuk *et al.* (1998) conducted a similar study on *Phaeosphaeria spp.* and *Stagonospora spp.* (fungal pathogens that cause septoria disease of wheat). They found that high air temperatures were associated with reduction in numbers of spores collected. From the results of the current study, high numbers of spores were trapped when mean daily temperature for 24 hours was between 9.7 and 17.6°C and fewer or no spores observed when the temperature was above 18°C. Sunny-dry conditions with high temperatures did not favour conidia dispersal and this could be attributed to the dryness

of the needle surfaces which caused the spores to be held firmly to the needle surfaces. Since the 1970's, the study area has shown evidence of increased precipitation during mid-summers with mean daily temperatures above 18 and 20^{0}C (Woods *et al.,* 2005). However, during the present study, no rainfall was recorded when the mean daily temperature was above 18^{0}C and no spore was collected during those periods. Temperature may contribute to maturation of spores but not directly to the release and dispersal of spores (Laflamme and Archambault, 1990). Increased frequency of warm rain in the mid-to-late 1990s has been identified to have coincided with sharp increase in the extent and severity of the current epidemic in northwest BC (Woods et al., 2005). Therefore it is possible that the spores of the fungus could have been released and dispersed with rainfall when mean daily temperature was above 18^{0}C, because spores were collected any time rain fell when the dispersal started through to the end of dispersal period on September 26.

5.4 Relationship between number of conidia dispersed, distance and height from the inoculum source

The results showed that most conidia were trapped within 1m from inoculum sources and it was rare to trap conidia beyond 2 m from inoculum sources. These results support previous findings that the dispersal distance of the conidia of *D. septosporum* is limited. In previous findings by Peterson (1973) and Podger (1978) conidia of *Dothistroma* were seldom trapped beyond 1.5 m and 2 m respectively from an inoculum source. Peterson conducted his investigation in Austrian and ponderosa pine (*Pinus nigra* Arnold and *P. ponderosa* Laws) plantations in Nebraska, USA. He placed petroleum jelly coated slides

beneath shoots of infected trees and placed Hirst traps and cans beneath the shoots of infected trees, 61 and 152 cm from the crown of infected trees. Peterson's results showed that conidia were seldom collected beyond 152 cm of infected trees. Podger conducted a similar investigation in *P. radiata and P. ponderosa* plantations in New Zealand and found that splash-dispersed conidia coming from inoculum sources were seldom trapped beyond 2 m from their sources even during storms with wind speeds up to 64 km per hour. During the current study, it was observed that dispersal of conidia of *Dothistroma* over long distances was not common. In the assessment and control of Dothistroma needle blight in New Zealand, Bulman *et al.*, (2004) reported that the dispersal distance of spores of the fungus was normally short.

It has been reported that long distance dispersal is limited in spores that are rain-splashed because in still air few inoculum-carrying droplets travel beyond 1m from the source (Fitt *et al.*, 1989). Splash-dispersed spores have smooth, thin hyaline walls and elongated shape while dry-dispersed spores often have rough surfaces, thick coloured walls and a more or less round shape (Fitt and McCartney, 1986). The rough, thick coloured walls of dry-dispersed spores are thought to protect them against harsh environmental conditions which are not needed by splash-dispersed spores because they are protected by the mucilage and water surrounding them. The elongate shape of splash-dispersed spores is thought to aid adhesion to host surfaces by reducing surface mobility (Fitt *et al.*, 1989). Conidia of *D. septosporum* have smooth, thin hyaline walls and an elongated shape and therefore are typical of conidia that are mainly splash-dispersed over short distance. This does not eliminate the possibility that some spores are deposited considerably further than

normal. Gibson (1974) reported that fog may carry conidia over long distances; however results of the current study showed no evidence of long distance dispersal by clouds. In the case of this study no spores were trapped on cloudy days without rainfall and it was rare to trap conidia even 2m from inoculum sources. Spread of the disease may have also been caused by re-splashing of the spores after they had landed on foliar surfaces from original source of inoculum because for splash dispersal, spore depositions are not permanent (Gregory, 1961 cited in Meredith, 1979). The fact that conidia of *Dothistroma* are produced in mucilage and mainly dispersed by rain splash may be the best explanation for why they are dispersed only over short distances. Most spores that are splash dispersed travel a few centimeters (Madden 1992 and 1997) and because the mucilage surrounding them prevents wind dispersal, their dispersal gradient is much shorter (Fitt *et al.*, 1989).

Ascospore dispersal over long distance may have played a role in the spread of the fungus at the study area. Although ascospores were not observed during this study, there is genetic evidence supporting the existence of the ascospores in the study area (Dale *et al.,* 2011; Groenewald *et al.*, 2007) which are believed to be wind or mist dispersed (Gibson 1974; Bradshaw 2004). Dale observed high genetic diversity within a stand and suggested that ascospores dispersal play an important role in shaping the genetic structure of populations at all levels and that ascospores dispersal can cover large distances. There is no doubt *Dothistroma* has been transported over long distances because Dothistroma needle blight disease is seen at different locations in different parts of the world. The long distance dispersal might have resulted from carrying of suspended water droplets

containing the spores by air and transportation of infected planting stocks. For instance, Jankovský *et al.* (2004) reported that Dothistroma needle blight was first observed in Czech Republic on an imported *Pinus nigra* Arnord in 1999 and it was believed that the fungus probably came in on infected planting stock obtained from import at the end of the 1980s and beginning of the 1990s. This shows that once the spores are transported to new locations they are able to spread by rain splashing and re-splashing, possibly assisted by wind to new locations. Therefore long distance dispersal which has resulted in the spread of Dothistroma needle blight to different locations has been most likely caused by movement of infected planting stock, seed mixed with small infected needle pieces and logs which have infected needles lodged in the bark crevices (EPPO/CABI, 1997).

The average numbers of conidia trapped on the lower slides were slightly higher than that of the upper slides but statistically there was no significant difference between the two heights at which spores were collected in this study. The slight difference in the number of spores trapped at the two heights was probably due to the close proximity of the lower slides to the lower crown of the infected trees where most conidia were concentrated, and the downward path of splash-dispersed conidia. *Dothistroma* infections generally occur in the lower crown and on older needles and move upwards gradually (MOF, 2002). Conidia trapped on the upper slides, 0.5m above the main inoculum sources, explains the upward vertical spread of the disease within the crowns of infected pine trees. The findings on dispersal distance and height of spores of the pathogen also support previous reports (Brown and Webber, 2008) that spread of *Dothistroma* infection occurs within and between neighbouring trees. Splash plays an important role in vertical disease spread

because it can transport inoculum vertically (Shaw and Royle, 1993). Therefore, the spores can spread vertically to a new part of an infected tree which results in the upward movement of the disease on an infected tree year after year until the entire crown is infected. The disease can also spread horizontally from an infected tree to a susceptible healthy tree. The short dispersal distance identified in this study shows that horizontal spread of the disease will be slow when crowns of susceptible tree species are more than 2 m apart.

5.5 Effects of site factors on spore dispersal at the study sites.

In general, site factors had no significant effect on the number of conidia trapped during this study. Both sites were located within the same biogeoclimatic zone with similar climate and stand characteristics (Banner *et al.*, 1993). More conidia were collected on Site 1 than Site 2 which could be attributed to the difference in the volume of inoculum on the infected needles at each site. The percentage functional live crown of lodgepole pine trees on the two sites were not statistically different but lodgepole pine trees at Site 1 had a slightly higher percentage live crown than trees at Site 2. Trees on Site 2 might have been infected first, and due to repeated years of defoliation, most of the needles were cast and therefore had a lower volume of inoculum compared to the trees on Site 1. It is known that infected needles attached to the tree are the principal source of inoculum and when needles drop to the forest floor, the fruiting bodies which contain the spores are soon overgrown by saprophytes and spore production stops within 2 months (Gadgil, 1970).

Another factor that might have contributed to the difference in the number of conidia trapped, could be the difference in densities of trees on the two sites. Site 1 was less dense than Site 2 and therefore Site 1 was more open and impaction of rain drops on the needle surfaces and movement of wind-blown rain droplets containing conidia would have been greater compared to Site 2. This may be the best plausible explanation because canopy structure has been found to affect the deposition of splashed droplets and the potential for spread of many fugal spores by secondary splash (Madden, 1997). The lower elevation of Site 1 compared with Site 2 might have also affected the volume of inoculum dispersed in a way, because flat lands or low – lying plantations where cool air can pool overnight are known to be conducive for *Dothistroma* infection and hence production of high concentrations of inoculum because of poor air circulation (Marks and Hepworth, 1986).

5.6 Infection of seedlings

Results of the study showed that only the seedlings exposed to *Dothistroma* inoculum between July and September became infected. Symptoms were observed on the seedlings exposed between June 26 and September 20 and not on the seedlings exposed between May 5 and June 25. This may be attributed to the absence of spores before June 20 and low number of spores dispersed between June 20 and 25. Similarly, none of the seedlings exposed for two weeks from July 22 to August 6 showed symptoms or signs of the disease, probably due to absence of spores during that period. The presence of a high number of conidia with favourable environmental conditions has been identified as

important for *Dothistroma* infection (Bulman *et al.,* 2004; Gadgil, 1977). Overall, evidence of infection was very low in the seedlings exposed to inoculum. Thirty eight seedlings showed symptoms and only 8 (4.17%) developed signs of the disease. It may be possible that more than 38 seedlings got infected but the conditions in the green house where the trees were kept and monitored after the inoculation in the field were not conducive for the disease development and formation of acervuli. Although the trees were watered as necessary while in the green house, the needles dried up quickly because of their smaller sizes and low relative humidity in the green house. Continuous moisture and high relative humidity are important for the development of Dothistroma needle blight disease (Colhoun, 1973).

The periods of exposure of the seedlings to inoculum and number of spores collected were strongly associated with the occurrence of the disease. Seedlings exposed for 2, 4 and 6 weeks, respectively had 9.2, 22 and 47% disease incidence as indicated by symptoms. This may be explained by the fact that more spores were deposited on the needles as they were exposed for longer period and the possibility of presence of favourable environmental conditions for infection as they were exposed longer. According to Bulman *et al.* (2004), inoculum density plays an important role in the infection by *Dothistroma* and very large numbers of spores are needed to get moderate levels of infection. The high numbers of the spores may be necessary for the pathogen to overcome the host defenses.

The amount of rainfall during the seedlings exposure had a weakly significant influence on the development of symptoms whereas mean daily leaf wetness and relative humidity highly influenced symptom development. Infection as evidenced by the development of symptoms but not necessarily fruiting bodies occurred with variable amounts of rainfall (5 to 23.5 mm), high relative humidity (72 to 84%) and infection never occurred during periods without rainfall. Spores were only dispersed when rain fell during this study and this may be one of the possible reasons why infection did not occur without moisture. Moisture is needed for the dispersal of spores of plant pathogens and also important for spore germination and penetration during infection process. Severity of *Dothistroma* infection has been shown to increase when wetness period increased (Gadgil, 1977). Results of a study conducted by Woods et al. (2005) revealed a strong spatial correlation between mean summer precipitation and infection by *Dothistroma*. The amount of rainfall in June to September has been identified as a good indicator for the severity of Dothistroma needle blight disease in the northern hemisphere (Peterson, 1973). In California, Muir and Cobs (2005) also found that the proportion of infected radiata and bishop pine needles by *Mycosphaerella pini* in infection chambers was greater in 24h/day mist spray than in 16h/day mist spray. The high relative humidity required by *Dothistroma* for infection may be attributed to its influence on the spore germination. It may also play a role in survival and viability of the spores when deposited on the needles surface before germination and penetration of the needles. The spores are hyaline and cannot survive and remain viable in warm and dry conditions for a longer period on the needles surface (Kathy Lewis[5], personal communication, January 31, 2011). The

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observations of the current study and the previous studies suggest that dry periods negatively affect infection by the fungus because of the roles played by moisture in both dispersal and germination of the spores.

Symptom development occurred at daily mean temperature of 12 to 18.1^{0}C at Site 1 and 12 to 19.2^{0}C at Site 2. This supports a report by Brown and Webber (2008) that, the optimum temperature for successful establishment of *Dothistroma* infection range between 12 and 18^{0}C under conditions of high humidity. In New Zealand, Bulman *et al.* (2004) reported that frequent rainfall with temperatures exceeding 16 to 18^{0}C was conducive for *Dothistroma* infection and severity. Gadgil (1974) also found that the most infection was obtained at day/night temperatures of 20°/12°C when continuous moisture was provided. Temperature had no significant influence on the seedling's infection during this study. This suggests temperature does not have much effect on the infection by the fungus without extended moisture period and high relative humidity. The results of the monitoring of infection pattern of *D. pini* in the field in New Zealand by Gilmour and Crockett (1972) showed that temperature was less important than humidity. According to their results, infection and development of symptoms occurred between 5 and 26 °C, although infection at lower temperature was dependent on an extended periods of high humidity. Based on the results of logistic regression with mixed effect models, the effects of climatic variables, number of spores and exposure period was modeled with the regression equation, $y = -12.978 + 0.569(EP) + 0.120\ (RH) + 0.013(NS)$, where EP = exposure period of seedlings to inoculum, RH = daily mean relative humidity during the exposure period and NS = number of spores dispersed for infection during each exposure period. This suggests, although the infection by *Dothistroma* is influenced by all the

climatic factors, greater disease incidence will occur when large number of spores are deposited on needle surfaces for a longer period under high relative humidity conditions.

5.7 Management implications and strategies

Forest management and practices in northwest BC have played an important role in the current epidemic of Dothistroma needle blight in the area. It has been noted that extensive plantations of lodgepole pine in northwest BC along with a marked increase in the frequency of weather events favourable to the disease have resulted in unprecedented levels of *Dothistroma* infection (McCulloch and Woods, 2009, Welsh *et al.*, 2009). The results of this study support the previous reports that extensive plantations of lodgepole pines might have favoured the spread of the disease because of the inability of the spores to disperse over a longer distance. If lodgepole pine plantations in the area were mixed with large component of non-susceptible trees, the spread of the disease might have been checked with the non-susceptible tree species serving as barriers against spore transport from an inoculum source to uninfected trees. The extension of branches of susceptible trees reduces spore dispersal distance from an inoculum source when there is a large component of susceptible trees in a stand (Bingzhang *et al.* (1992). An increase in the distances between susceptible trees has been reported to reduce the effectiveness of rain-splashed spores which travel only short distances (Gadgil and Bain, 1999). During this study, it was found that lodgepole pine stands at Site 2, which was denser, had low percentage functional live crown and most of the needles had already been dropped. This presumably was caused by decreased in dispersal distance and creation of moist

conditions within the forest stands. Some forest stewardship plans (FSPs) in BC have proposed a minimum stocking standard of 700 well-spaced trees per hectare averaged over a standard unit and a target stocking standard of 1200 per hectare with a minimum inter-tree distance of 2 m (BC MoFR, 2010; Bergerud, 2002). Based on the findings of the current study, adapting the proposed well-spaced stocking density with inter-tree distance of 2 m for lodgepole pine plantations in Northwest BC may help to reduce the spread of dothistroma needle blight disease in the area.

Lack of pruning of lodgepole pine trees might have also contributed to the spread of the disease at the area because the infected needles which serve as inoculum sources for new infection are not removed. These dead tissues while on the tree are found to be the chief means of dispersal of the pathogen within the trees (Gibson, 1973). The absence of pruning in forest plantations creates conducive conditions for the building up of inoculum because of the moist and high humidity conditions created within the canopy of forest stands (Villebonne and Maugard, 1999). Pruning helps to eliminate previously infected needles as a mean of reducing the volume of spores that spread the disease (Meredith, 1973). The distance of dispersal by the spores is also shortened when forest stands are not pruned. Therefore, pruning will not only create microclimatic conditions unfavourable for Dothistroma needle blight disease, but it will reduce the effectiveness of spore transport by increasing the dispersal distance and also reducing the volume of inoculum available for new infection.

The short distance dispersal of spores observed in this study suggests that increasing planting distance of lodgepole pine plantation may help to reduce the spread and severity of the disease. Most fungal spores like *Dothistroma* are found to disperse over very short distances by natural means and therefore they are dispersed from one host to another one very close to it (IICA, 2006). Therefore pruning seems promising to reduce or control the spread of the disease by increasing spore dispersal distances from inoculum sources. Moreover, since the spore dispersal at the study area has been noted to start in June, pruning of the lower branches of lodgepole pine trees before June in the growing seasons may be necessary to reduce the number of conidia available for new infection. The observations of the significant influence of spore numbers, high relative humidity and moist conditions on infection by the fungus also support the idea of pruning.
Planting tree species that are not susceptible to Dothistroma needle blight at high risk areas and the use of planting stocks that are not infected with the disease seem the most effective measures to control the disease (Gadgil and Bain, 1999, Villebonne and Maugard, 1999 and Woods *et al.*, 2005).

Chemical control through the application of copper-based fungicides has also proved to be effective in controlling the disease in countries such as New Zealand, Australia and Chile (Gadgil, 1984, Brown and Webber, 2008). In Nebraska, a single fungicide application in June was reported to have provided excellent control of Dothistroma needle blight in pine plantations (Peterson, 1973). Investigation conducted by Bingzhang *et al.* (1992) in China also showed that the average effect of chemical control was more than 60%. These suggest the effectiveness of the use of fungicide in controlling the

disease or reducing the spread of the fungus. Chemical control of the fungus in pine plantations is not practiced in BC, Canada because of its negative environmental and health effects. Therefore the use of silvicultural management strategies and planting of resistant pine tree species or non-pine tree species will be the best alternative methods to reduce the spread of the disease in the province. Based on the findings of this study, if chemical control of the fungus is considered in forest plantations in BC, late May to early June will be the best time for fungicide application. Two applications with one before current-year needle emerge and one after they have emerged may be effective.

5.8 Conclusions and Recommendations

Based on the results from this study, the conidia (asexual spores) of *D.septosporum* are the predominant source of inoculum for the spread of Dothistroma needle blight disease of lodgepole pine plantations in northwest BC, Canada. The conidia of *D. septosporum* are dispersed whenever it rains at the study area from mid-June through to September with the peak in July. It has been identified that the spores of the fungus *Dothistroma* naturally do not disperse over long distances and are rarely able to disperse over 2 m from an inoculum source. The numbers of spores dispersed decreases with increasing distance from an inoculum source. The results of this study suggest that rain splash is the main mechanism for the dispersal of the spores and the dispersal remains hugely under the control of rainfall and leaf wetness. It appears that the amount of rainfall is not as important as the frequency of rainfall in *D. septosporum* spore dispersal because small amount of rainfall as low as 0.5 mm can result in the release and dispersal of the spores.

In this study, the most conducive climatic variables for *Dothistroma* spore dispersal in the study area were identified. These include mean amount of rainfall for 3 days ranging from 1.1 to 17 mm, daily mean temperatures for 24 hours ranging from 9.6 to 17.6^0 C, daily mean relative humidity for 24 hours ranging from 72.3 to 85.4% and daily mean leaf wetness for 24 hours ranging from 48.1 and 75.1%. Rainfall, relative humidity and leaf wetness have significant positive influence on *Dothistroma* spore dispersal while temperature on the other hand has a significant negative effect on the spore dispersal.

The relationship between the number of conidia dispersed and the climatic variables during this study was modeled by the equation $\text{Log}_{10}\, y = -0.197 + 0.042x_1 + 0.030x_2$, where y is the average number of conidia per 10cm^2, x_1 is mean amount of rainfall for 3 days in mm and x_2 is daily mean leaf wetness in percentage. This suggests that a model with combination of rainfall and leaf wetness can be used to forecast and manage Dothistroma needle blight disease by ensuring less dense canopy that create microclimate with low humidity and less leaf wetness period. Moreover, the relationship between horizontal distance and spore counts was modeled best by the regression equation $\text{Log}_{10} y = 1.849 - 0.649x$, where y is number of conidia sampled and x is distance from the inoculum source. This relationship holds for numbers of conidia sampled at distances of 0, 1, 2 and 3m from inoculum sources at the study area. This suggests the number of spores that reach a healthy susceptible tree from an inoculum source decreases as the distance increases.

Although the infection by the fungus has been noted to be influenced significantly by all the climatic factors apart from temperature, exposure of susceptible tree species to high number of spores for a longer period under conditions with high relative humidity is required for infection to occur. The effects of climatic variables, number of spores and exposure period was modeled with the regression equation,

$y = -1.191 + 0.453(EP) + 0.113\ (RH) + 0.001(NS)$, where EP = exposure period of seedlings to inoculum, RH = daily mean relative humidity during the exposure period and NS = number of spores dispersed for infection.

Spore sampling in 2010 was terminated in June and this resulted in small sample size which was not enough to compare the results of the two years of the study. Complete sampling in the 2010 growing season would have been necessary to collect enough data to better understand the peaking and halting of the spore dispersal at the area during the season. It would have also been necessary to get a better understanding on the effects of the climatic factors on the spore dispersal under variable climatic conditions. This was a limitation of the study and therefore it is recommended that additional study be conducted in the area for about 3 consecutive years to get a deeper understanding of the relationship between *Dothistroma* spore dispersal, infection and the climatic variables. Such a study could also help to determine the periods of spore release and dispersal in the growing seasons under different microclimatic conditions. Based on the findings of this study, it is also recommended that lodgepole pine plantations in northwest BC should be mixed with tree species that are not susceptible to Dothistroma needle blight disease to serve as barrier against the dispersal of the spores of the fungus. Planting distances of the

susceptible tree species should be increased (2 m minimum) in order to increase the dispersal distance of the spores. This is based on the finding that the fungus is naturally dispersed over short distance. Finally, lodgepole pine trees which have been identified as the main host of the fungus should be pruned before June each year to remove the infected needles that serve as sources of inoculum for new infections, increase the spore dispersal distance and create microclimatic conditions unfavourable for *Dothistroma* spores production and dispersal. If chemical control of the fungus is considered in BC, mid- May to early June is recommended for chemical application at the area for effective results.

The findings of this study on the spore dispersal and infection of *D. septosporum*, will provide useful information for the Ministry of Forest and Range, BC in forecasting the spread of Dothistroma needle blight disease and developing effective management strategies to reduce the spread of the disease in the province.

Chapter 6: References

Agrios, G.N.1997. Plant pathology. 4th edition. Academic press, San Diago, California.

Agrios, G.N.2005. Plant pathology. 5th edition. Academic press, San Diago, California. Pp. 250-257.

Allen, E., Morrison, D. and Wallis, G. 2003. Common tree diseases of British Columbia-Conifer Needle Disease: Dothitroma needle blight. Available from: www.pfc.forestry.ca/diseases/CTD/Group/Needle/needle5e.html Accessed 2009 April 20.

Arseniuk, E., Góral, T. and Scharen, A. L. 1998. Seasonal patterns of spore dispersal of Phaeosphaeria spp. and Stagonospora spp. Plant Dis. 82:187-194.

Banner, A., MacKenzie, W., Haeussler, S., Thomson, S., Pojar, J. and Trowbridge, R. 1993. A field guide to site identification and interpretation for the Prince Rupert Forest Region. Victoria(Canada): British Columbia Ministry of Forests. Land Management Handbook 26.

Barnes, I., Crous, P.W., Wingfield, B.D and Wingfield, M.J. 2004. Multigene phylogenies reveal that red band needle blight of *Pinus* is caused by two distinct species of *Dothistroma, D. septosporum and D. pini.*Studies in mycology 50:551-565.

Bergerud, W.A. 2002. Effect of the silviculture survey parameters on the free-growing decision probabilities and projected volume at rotation. Res. Br., B.C. Min. For., Victoria, B.C. Land Manage. Handb. No.50

Bingzhang, H., Xinglin, D., Chengyu, L., Guigin, L. and Yuping, Y. 1992. The development pattern of Dothistroma needle blight and its control. J. Northeast For. Univ. vol. 3, No. 1. 43-53.

Bradshaw, R.E., Ganley, R.J., Jones, W.T. and Dyer, P.S. 2000.High levels of dothistromin toxin produced by the forest pathogen *Dothistroma pini.* Mycological Research 104, 325–332.

Bradshaw, R.E. 2004. Dothitroma (red-band) needle blight of pines and the dothistroma toxin: a review. Forest Pathology 34: 163-185

Bradshaw, R.E. and Zhang, S. 2006. Biosynthesis of dothistroma. Mycopathologia 162: 207-213

Braun, C. 2009. The role of weather and topography in the development of *Dothistroma septosporum* M.S thesis. University of Northern British Columbia, Prince George.

Brown, A. and Webber, J. 2008. Red band needle blight of conifers in Britain. UK Forestry Commission Research Note. Available from: www.forest.gov.uk/redbandneedleblight. Accessed 2009 January 21.

Brown, A., Rose, D. and Webber, J. 2003. Red band needle blight of pines. UK Forestry Commission Research Note. Available from: www.forest.gov.uk/fr/INFD-6ZHC69. Accessed 2010 March 24.

Bulman, L.S.1993. *Cyclaneusma* needle –cast and Dothistroma needle blight in NZ pine plantation. New Zealand Journal of Forestry 38(2):21-24.

Bulman, L.S., Gadgil, P.D., Kershaw, D.J., and Ray, J.W.2004. Assessment and control of Dothistroma needle blight. Forest Research Bulletin No. 229.

Campbell, C.L., and Madden, L.V. 1990. Introduction to plant diseases and Epidemiology. p.54.

Coates, K.D, Barnner, A., Douglas, S., Lepage, P. and Bartemucci, P. 1997. The Date Creek Silvicultural System Study in the Interior Cedar-Hemlock Forest of Northwest British Columbia: Overview and Treatment Summaries. Land Management Handbook 58, MOF, BC.

Colhoun, J. 1973. Effect of environmental factors on plant disease. Annual Review of Phytopathology. 11: 343-364.

Cooke, D.M., Gareth D.J., and Kaye, B. 2006. The Epidemiology of plant diseases. 2nd edition, pp. 159-192.

Cordell, C.E., Anderson, R.L., Hoffard, W.H., Landis, T.D., Smith, R.S. Jr., and Toko H.V. 1989. Forest Nursery Pests. USDA Forest Service, Agriculture Handbook No. 680. P.184.

Cox, C.S. and Wathes, C.M. 1995. Bioaerosols Handbook. CRC LLL, U.S.A. P248

Dale, A. L., Lewis, K. J. and Murray, B. W. 2011. Sexual Reproduction and Gene Flow in the Pine Pathogen Dothistroma septosporum in British Columbia. Phytopathology. 101(1): 68 – 76.

Delong, C. 2004. A field guide to site identification and interpretation for the north central portion of the Northern Interior Forest Region. B.C. Min. For., Victoria, B.C. land management hand book. No. 54.

European and Mediterranean plant protection organization (EPPO)/CAB International (CABI). 1997. Quarantine pests for Europe, 2nd edition. p.1425.

Esker, P.D., Sparks, A.H., Bates, M., Anthony, G., Acqua, W.D., Frank, E.E., Huebel, L., Segovia, V. and Garreth, K.A., 2007. Ecology and Epidemiology in R: Modeling Dispersal Gradient. Available from: www.k-state.edu/pdecology/RModules.html Accessed 2009 October 10.

Evans, H. C. 1984. The genus *Mycosphaerella* and its anamorphs *Cercoseptoria*, *Dothistroma* and *Lecanosticta* on pines. Mycol. Pap. 153:1-102.

Fitt, B.D.L. and McCartney, H.A. 1986. Spore dispersal in relation to epidemic model, in plant disease epidemiology. Vol.1: Population Dynamics and Management. Macmillan, New York, Pp. 311-345.

Fitt, B.D.L., McCartney, H.A. and Walklate, P.J. 1989. The role of rain in dispersal of pathogen inoculum. Annu. Rev. Phytopathol. 27:241-270.

Funk, A. 1985. Foliar fungi of western trees. Canadian Forest Services, Canada. Pp. 99-100.

Gadgil, P.D.1967. Infection of *Pinus radiata* needles by *Dothistroma pini.* New Zealand Journal of Botany 5:498-503.

Gadgil, P.D.1970. Survival of *Dothitroma pini* on fallen needles of *Pinus radiata.* New Zealand Journal of Botany 8:303-309.

Gadgil, P.D. 1974. Effect of temperature and leaf wetness period on infection of *Pinus radiata* by *Dothitroma pini.* New Zealand Journal of Forestry Science 4:495-501.

Gadgil, P.D. 1977. Duration of leaf wetness periods and infection of *Pinus radiata* by *Dothistroma pini.* New Zealand Journal of Forest Science 7: 83-90.

Gadgil, P.D., and Holden, G. 1976: Effect of light intensity on infection of *Pinus radiata* by *Dothistroma pini.* New Zealand Journal of Forestry Science. 6:67–71.

Gadgil, P.D. 1984. Dothistroma needle blight. Forest Pathology in New Zealand, No. 5.New Zealand Forest Service, Roturua, New Zealand.

Gadgil, P.D. and Bain, J. 1999. Vulnerability of planted forest to biotic and abiotic disturbances. New Forests **17:** 227–238.

Gäumann, E. 1950. Principles of plant infection. New York: Hafner.

Gibson, I.A.S. 1963. A further note on Dothistroma (Actinothyrium) blight of Pines in Kenya. Commw. Phytopath. News 9, 47-48.

Gibson, I.A.S. 1972. Dothistroma blight of *Pinus radiata*. Annual Review of Phytopathology 10: 51-72.

Gibson, I.A.S. 1974. Impact and control of Dothistroma blight of pines. European Journal of Forest Pathology. 4: 89-100.

Gilmour, J.W. 1981: The effect of season on infection of *Pinus radiata* by *Dothistroma pini.* European Journal of Forest Pathology 11: 265–269.

Gilmour, J.W., and Crockett, F., 1972: Dothistroma pini project: monitoring of infection patterns in the field. Rept. Forest. Res. Inst. N Z Forest. Serv. No. 53. Rotorua NZ:Forest Research Institute

Gou, X.W. and Fernando, W.G.D. 2005. Seasonal and diurnal patterns of spore dispersal by *Leptosphaeria maculans* from canola stubble in relation to environmental conditions. Plant Disease. 89:97-104.

Gregory, P.H. 1973. The microbiology of the Atmosphere, 2nd ed. Leonard Hill, London, p.377

Groenewald, M., Barnes, I., Bradshaw, R. E., Brown A. V., Dale, A., Groenewald, J. Z., Lewis, K. J., Wingfield, B. D., Wingfield, M. J., andCrous, P. W. 2007. Characterization and distribution of mating type genes in the Dothistroma needle blight pathogens. Phytopathology 97:825-834.

Groot-de, P., Hopkin, A.A. and Sajan, R. 2003. Forest Management Guidelines for Major Insects and Diseases of Spruce, Pine and Aspen in eastern Canada. Availablefrom: www.Forestresearch.canadianecology.ca/projects/opimplmt/MgmtGuidelinesForInsects$Diseases.pdf Accessed 2010 April 2.

Hildebrand, D.M. 2005. *Mycosphaerella pini.* NAFC-Exfor Pest Report. Available from: www.spfnic.fs.fed.us/exfor/data/pestreport.cfm?pestival=39&langdisplay=english Accessed 2009 January 19.

Hist, J.M and Stedman, O.J. 1971. Patterns of spore dispersal in crops. Ecology of leaf surface micro-organisms. T.F. Preece and C.H. Dickinson, eds. Academic Press, London. PP. 229-237.

Hulbary, R.L. 1941. A needle blight of Austrian pine. Illinois Natural History Survey Bulletin 21: 231-236.

Inter-America Institute Cooperation for Agriculture (IICA), 2006. Action plan for Black Sigatoka (Mycosphaerella fijiensis (Morelet). Saint Lucia. Pp 3
Available from: http://orton.catie.ac.cr/repdoc/A0967I/A0967I.PDF
Accessed 2010 September 10

Ivory, M. H. 1967: A new variety of *Dothistroma pini* in Kenya. Trans. Br. Mycol. Soc. 50: 289–297.

Ivory, M.H. 1994. Records of foliage pathogens of *Pinus* species in tropical countries. Plant Pathology 43(30):511-518.

Jankovský, L., Bednářová, M. and Palovčíková, D. 2004. Dothistroma needle blight Mycosphaerella pini E. Rostrup, a new quarantine pathogen of pines in the CR. Journal of Forest Science, 50(7): 319-326.

Karadžić, D. 1989. *Scirrhia pini* Funk et Parker. Life cycle of the fungus in plantations of *Pinus nigra* Ard. in Serbia. European Journal of Forest Pathology 19:231-236.

Karadžić, D.M. 1994. Picea omorica – a new host of *Dothistroma septospora*. European Journal of Forest Pathology 24:300-303.

Kershaw, D.J., Gadgil, P.D., Ray, J.W., van der Pas, J.B. and Blair, R.G. 1988. Assessment andControl of *Dothistroma* needle-blight. Forest Research Institute Bulletin No. 18. Forest Research Institute, Rotorua, New Zealand

Kimmins, J. P. 1997. Forest Ecology: A Foundation for Sustainable Management. Prentice-Hall Inc. 2nd ed. Upper Saddle River, New Jersey, USA.

Lacey, J. 1996. Spore dispersal-its role in ecology and disease: the British contribution to fungal aerobiology. Mycological Research 100: 641-660.

Laflamme, G. and Archambault, L. 1990. Evaluation of microclimatic factors affecting ascospores release of *Gremmeniella abietina var balsamea*. Canadian Journal of Plant Pathology. 12: 190-194.

Lotan, J.E. and Critchfield, W.B. 1990. Pinus contorta Dougl. ex. Loud. Lodgepole Pine. Agriculture Handbook 654. Silvics of North America. Washington, USA. Pp. 302-315.

Madden, L.V. 1992. Rainfall and dispersal of fungal spores. Advances in Plant Pathology. 8: 39-79.

Madden, L.V. 1997. Effect of rainfall on splash dispersal of fungal pathogen. Canadian Journal of Plant Pathology. 19: 225-230.

Marks, G.C and Hepworth, G. 1986. Effect of Certain Features of a Stand of *Pinus radiata* on *Dothistroma septospora* Needle Blight. Aust. For. Res. 16: 223-229.

Markovskaja, S. and Treigienė, A. 2009. New data on invasive pathogenic fungus *Dothistroma septosporum* in Lithuania. Botanica Lithuanica. 15(1): 41-45.

McCulloch, L. and Woods, A. 2009. British Columbia's northern interior forests: *Dothistroma* Stand Establishment Decision Aid. BC Journal of Ecosystems and Management 10(1):1–3.

McCartney, H.A., Fitt, B.D.L. and Schmechel, D. 1997. Sampling bioaerosols in plant pathology. Journal of Aerosols Science. 28: 349-364.

Meredith, D.S. 1973. Significance of spore release and dispersal mechanisms in plant disease epidemiology. Annu. Rev. Phytopathology. 11:313-342.

Ministry of Forest and Range. 2002. Red band needle blight/Dothistroma needle blight, *Mycosphaerella pini*. Available from: www.for.gov.bc.ca/hfp/publication/00198/red_band_needle_blight.htm. Accessed 2009 April 20.

Ministry of Forest and Range. 2005. Defoliation free growing damage standard for determinate growth conifers. Available from: www.for.gov.bc.ca/hfp/health/fhdata/defoliation_FG.pdf Accessed 2009 May 10.

Ministry of Forest and Range, BC. 2010. Introduction to reference guide for the FDP stocking standard. Available at: www.for.gov.bc.ca/hfp/silviculture/stocking_stds.htm Accessed 2011 February 28.

Muir, J.A., and Cobb, F.W. 2005. Infection of radiate and bishop pine *Mycosphaerella pini* in Calofornia. Canadian Journal of Forest Research 35(11):2529-2538.

Parker, A.K. and Collins, D.G. 1966. Dothistroma needle blight of pines in British Columbia. Forestry Chronicle 42: 160 – 161.

Peterson, G.W. 1967. Dothistroma needle blight of Austrian and ponderosa pines: epidemiology and control. Phytopathology 57: 437-441.

Peterson, G.W. 1973. Infection of Austrian and Ponderosa Pines by *Dothistroma pini* in Eastern Nebraska. Phytopathology 63: 1060-1063.

Peterson, G.W. 1982. Dothistroma needle blight of pines. Forest Insect & Disease Leaflet 143, USDA forest service. Available from: www.na.fs.fed.us/spfo/pubs/dothistroma/doth.htm. Accessed 2009 January 19

Podger, F.D. 1978. Studies on the effect of some climatic factors and spore dispersal on Infection of two species of pinus by Dothistroma pini. Phd thesis, University of Auckland.

Runion, G.B. 2003. Climate change and plant pathosystems – future disease prevention starts here. New Phythologist 159: 531 – 538.

Schoch, C.L., Shoemaker, R.A., Seifert, K.A, Hambleton, S., Spatafora, J.W and Crous, P.W. 2006. A multigene phylogeny of the *Dothideomycetes* using four nuclear loci. *Mycologia* 98:1041 –1052.

Schwelm, A., Barron, J.B., Dick, M., Long, P.G., Zhang, S. and Bradshaw, R.E. 2009. Dothitromin toxin is not required for Dothitroma needle blight in *Pinus radiata*. Plant Pthology 58: 293-304.

Shain, L. and Franish, R.A. 1981. Induction of Dothistroma blight symptoms with dothistromin. Physiological plant pathology 19: 49-55.

Shaw, M.W. and Royle, D.J. 1993. Factors determing the severity of epidemics of Mycosphaerella graminicola (Septoria tritici) on winter wheat in the UK. Plant pathology, 42: 882-899.

Steven, V. 1995. Wildlife diversity in British Columbia: distribution and habitat use of amphibians, reptiles, birds and mammals in biogeoclimatic zones. Res. Br., B.C. Min. For., Wildl. Br., B.C. Min.Environ. Lands and Parks, Victoria, B.C. pp. 19-29.

Vadim, L., William, T.J., Valentine, A.M., Dawn, H. and Paul, H.S.R. 2000. Single chain anti-idiotypic antibody mimics of the phytotoxin dothistromin. Protein and Peptide Letters, vol. 7. No. 3, pp. 175 – 182.

Van Der Pas, J. B. (1981) Reduced early growth rates of *Pinus radiata* caused by *Dothistroma pini.* New Zealand Journal of Forestry Science 11: 210-220.

Villebonne, D. and Maugard, F. 1999. Rapid development of Dothistroma needle blight (*Scirrhia pini*) on Corsican pine (*Pinus nigra* subsp. *laricio*) in France. *La Santé des Forêts*, Annual Report 1998, Les Cahiers du DSF **1**, DERF, Paris, Pp. 30–32.

Watt, M.S., Kriticos, J. D., Alcaraz, S., Brown, A.N and Leriche, A. 2009. The host and potential geographic range of Dothistroma needle blight. Forest Ecology and Management. 257: 1505-1519.

Welsh, C., Lewis, K. and Woods, A. 2009. The outbreak history of Dothistroma needle blight: an emerging forest disease in northwestern British Columbia, Canada Can. J. For. Res. 39(12): 2505–2519.

Westfall, J. and Ebata, T. 2008. Summary of forest health conditions in British Columbia. Forest Practices Branch BC, Aerial overview survey. Available from: www.for.gov.bc.ca/hfp/health/overview.htm Accessed 2009 April 20.

Woods, A. J. 2003. Species diversity and forest health in northern British Columbia. Forest Chronicle 79(5): 892-897

Woods, A. J., Coates, K. D. and Hamann, A. 2005. Is an unprecedented Dothistroma Needle Blight Epidemic Related to Climate Change? Bioscience 55(9): 761-769.