The Role of Bark Beetles as Vectors in the Colonisation of Windthrown Timber by Fungi

---

A thesis submitted in partial fulfilment of the

requirements for the Degree

Master of Science in Ecology

at the University of Canterbury

by James K. McCarthy

University of Canterbury

2011

Abstract

The increasing frequency and severity of windthrow events affecting the forestry industry in New Zealand have raised important management issues surrounding the rate of colonisation of fallen trees by sapstain fungi and the time available for salvage harvesting before sapstain degradation limits potential economic returns. These fungi are known to be spread by a multitude of factors including wind, rain splash, harvesting processes and insect vectoring. Apart from the ecological interest in these interactions between fungi, plants and insects, sapstain fungi are also economically important because their hyphae discolour the sapwood and reduce the overall quality of the timber. The amount of time available to salvage harvest damaged trees is unknown, especially on seasonal and regional scales. Manipulative experiments were established in *Pinus radiata* forests to examine this seasonal and regional variation in sapstain attack following windthrow, and to investigate the importance of bark beetles as vectors of sapstain fungi. A range of methods were implemented to assess the role of bark beetles as vectors and to ascertain which sapstain fungi are associated with them. Experimental billet logs were caged to exclude beetles and subsequently analyse fungal attack in comparison with identical logs left exposed to beetles. In addition, individual beetles were sampled directly to determine whether they carried spores of particular fungal species and to assess the degree of association in vector-fungal dynamics. Finally, a novel application of DNA melt peak analysis was developed to investigate variation among the fungal communities associated with beetles potentially involved in vectoring sapstain spores.

The moisture content of fallen trees was found to be the main factor regulating sapstain development, and when moisture content drops below 100% (on dry weight basis) sapstain fungi grew rapidly. The speed at which this level drops depends on the season, with much faster drying occurring in the warmer months of spring and summer. As a result, trees that fell in the previous winter or autumn did not develop significant sapstain levels until temperatures rose in the following summer, suggesting that storm-damaged trees that fall in winter can be left safely until just before the next summer before they are no longer suitable for salvage harvest. In New

Zealand, the bark beetle species acting as vectors of sapstain fungi are not behaviourally adapted to colonisation of logs that are not in contact with the ground. Following windthrow events in pine forests, trees generally lie with their stems suspended above the level of the ground by their branches. As a result, under these circumstances, beetle colonisation of windthrown timber was low, and bark beetles were not a significant vector of stain. The caged and un-caged experimental log billets, however, were in contact with the ground, resulting in colonisation of the un-caged logs. In this case, bark beetles did play an important role in contributing to sapstain intensity, and the stain distribution within the logs mirrored that of the stain distribution. However, this effect may be due to the provision of access points for wind- or water-borne spores of the non-insect vectored stain fungus *Diplodia pinea*, or to the spread of hyphae through the tunnelling and feeding activities of beetles within the tree, rather than by bark beetles acting as vectors of spores. Bark beetles were confirmed as sapstain vectors with the isolation of seven different ophiostomatoid stain fungi from them, five of which were also found in wood. Finally, the development of a laboratory based, rapid species identification method was developed to identify fungal DNA. Melt peak analysis allowed the species-specific DNA melt temperatures to be compared with the melt temperatures of known species to be able to rapidly, and cheaply, identify an unknown species.

Bark beetles are vectors of sapstain fungi in *P. radiata* forests, however the bark beetle species naturalised in New Zealand prefer to colonise wood when it is in contact with the ground. Following windthrow, trees are generally not attacked by beetles as they are held from the ground by their branches, leaving them to be stained predominantly be wind and rain dispersed stain fungus *D. pinea*. Stain did not occur until the moisture content of fallen trees dropped below 100%, which only happens in the warm months of summer and spring. In New Zealand, there are interactions between trees and bark beetles, and bark beetles with fungal pathogens from all around the globe resulting in a unique novel assemblage of species together for the first time. Understanding the dynamics of these species in their novel environment is crucial to effectively responding to potential pest threats.

Table of Contents

List of Figures

List of Tables

Acknowledgements

It is a pleasure to thank those who made this thesis possible; over the last two years many people have been generous enough to offer their time and expertise to contribute to this thesis. In particular, I would like to thank the following people and organisations for their support.

First, I would like to show my deepest gratitude to my primary supervisors Prof. Raphael Didham (University of Canterbury) and Dr Ecki Brockerhoff (Scion). I was very lucky to have two supervisors so knowledgeable and dedicated, and this thesis wouldn't be the same without their input. In addition, the knowledge and experience of Ian Hood (Scion) has also been invaluable, and this project would not exist without him driving it from the start.

Mark Forward of Nelson Forests Ltd. was instrumental in this project by alerting us to the initial issue, and supporting funding applications. Our contractor in Nelson, Rex Mitchell, has been involved with this study from its beginning, and his interest and dedication to the project was a fantastic help when field work was at its toughest! Likewise in Christchurch, Marcel van Leeuwen of the Selwyn Plantation Board (SPB) assisted us from the beginning providing study sites, experimental trees, access, and even his personal chainsaw assistance. This invaluable assistance was continually offered during the sale of the SPB forests in Canterbury, at a time that must have been personally difficult. I am extremely grateful for this.

Many academic and technical staff from the School of Biological sciences at UC have offered their assistance to this thesis. This extensive list of people include: Craig Galilee for assistance in the lab, as well as Dr David Collings Neil Andrews, Matt Walters and Jan McKenzie who all assisted with microscopy and imagery. Toward the end of this thesis I entered the world of molecular biology for the first time, and learnt a lot. Dr Arvind Varsani made this possible, and brought me in from the trees to his lab. His depth of knowledge and experience pushed this project further than I imagined it ever would. I am also grateful to his students (and others who happened to be near-by): Simona Kraberger, Daisy Stainton, Davon Callander, Anisha Dayaram and Katherine van Bysterveldt, whose advice and assistance have contributed immensely.

I have been fortunate enough to be involved with Scion (NZ Forest Research Institute) since my third year of undergraduate studies. I have learned and experienced an huge amount with this organisation and cannot thank them enough. Specifically I would like to thank Heather Flint, Katrin Walbert and Margaret Dick for the immense amount of lab work they offered. Lindsay Bulman and Brian Richardson always made sure funds were available, I am obviously grateful for this. Mark Kimberly was kind enough to provide statistical analysis assistance, even on his holiday, and Colleen Carlson assisted with getting me started and provided advice on all

things numbers! Assistance with fieldwork was generously offered by Dave Henley, Alan Leckie, Graham Coker, and Jessica Kerr. Joy Wraight was able to solve almost any issue, and Steve Pawson was a great source of practical advice.

Following the earthquake of 2011, Lincoln University and Kelvin Nicolle were kind enough to offer their facilities to process some urgent samples. The earthquake was a difficult and busy time for everyone, and it was kind of them to offer us a workshop and their assistance at this time.

This project was funded by a Tertiary Education Commission enterprise scholarship, the New Zealand Forest Research Institute, the New Zealand Foundation for Research, Science and Technology, and the Brian Mason Scientific and Technical Trust.

Finally, I would like to thank my friends and family. The coffees, lunches, dinners, random conversations, and trips away gave me much needed rest and relaxation and time to forget about my thesis! Andrew Barnes has been a fantastic fountain of knowledge and knows more about statistics than a computer. Alwin Sky was a great field-hand and was very good at approaching issues and ideas from angles I hadn't considered. And finally, Josh Van Vianen's lunchtime yarns about his life always reminded me either that there is a brighter side to life than just a thesis, or that things could be much, much worse.

My girlfriend Emma, and my family have always been supportive of my endeavours, and have showed continual interest in what I am doing and would always help in whatever way they could. I am very grateful for this.

This is an extensive list, but this would not have been possible without all of you – thank you.

Chapter 1 – General Introduction

1.1 Introduction

In New Zealand, commercial plantation forests represent ca. 22% of the total forest cover (Foley et al. 2005, Brockerhoff et al. 2008), and 7% of the total land area (Anon 2011b). In addition to their economic profitability through the sales of production timber, the establishment of plantation forests can reduce or eliminate the need to exploit natural forest for timber production, and consequently the land area of plantation forestry is increasing world-wide (Brockerhoff et al. 2008). The planting and subsequent management of exotic forests in New Zealand has created habitats that are also suitable for a wide range of native plants, insects, microbes and other organisms, many of which can be injurious to the plantation itself.

The stand management regime of plantation forests, and the fact that they are usually planted on hills, makes them highly susceptible to damage by wind (Finnigan and Brunet 1995). Harvesting of individual block and stands of trees allows strong winds to funnel through corridors causing damage to trees standing at the perimeter of the harvested stand, and hills create turbulent airflow that speed up as they rise in altitude and cause significant damage at the hill crest (Finnigan and Brunet 1995). Following large-scale windthrow events, a salvage harvest strategy is usually implemented where damaged trees are harvested, and the land either replanted or left to fallow until adjacent stands are harvested. One major unknown with salvage harvesting involves the timeframes available until staining of the wood by sapstain fungi becomes extensive, causing the value of the timber to drop considerably (Grayson 1989). These sapstain species are known to be transported by bark beetles (Reay et al. 2005, 2006a, Romon et al. 2007), however there are also species that disperse independently by wind and rain-splash (Bihon et al. 2011).

Increases in frequency and intensity of extreme storm events are occurring world-wide as temperature extremes (both low and high) become more common, and as wind and rainfall events increase in intensity (Easterling et al. 2000, Emanuel 2005). Changes in these weather

patterns also has profound effects on plant and animals in the form of climate-induced extinctions, species range shifts, and phenological changes (Easterling et al. 2000). Impacts of these changes on species can be from one end of the spectrum where habitat loss can lead to mass extinctions or sensitive species (Brooks et al. 2002, Brook et al. 2003), or the other where they can alter the climate sufficiently enough to allow a species to overcome historical geographic barriers to expand their range at alarming rates (Thomas et al. 2001).

The mountain pine beetle (*Dendroctonus ponderosae*) is a native pest bark beetle of North America whose populations periodically erupt into large scale outbreaks (Kurz et al. 2008). The current outbreak of this species is an order of magnitude larger in area and severity than all previously recorded outbreaks (Taylor et al. 2006). Previous barriers preventing the beetles spread (latitude and climate) have been overcome as a result of a changing climate, and the beetles have expanded their range to areas not previously recorded (Taylor et al. 2006). This has allowed the pest beetle species to come into contact with host trees un-adapted to beetle epidemics, where the beetles have higher reproductive success (Cudmore et al. 2010).

The increase of beetle outbreaks (Kurz et al. 2008), and increased extreme weather events (Easterling et al. 2000), are predicted to alter the "disease triangle" between plant hosts, plant pathogens, and environmental change where unlikely combinations of species, coupled with environmental variability, result in unpredicted outcomes (Grulke 2011). As exotic and native species mix in the global homogenisation of species, and as pests and pathogens meet novel hosts, outcomes of major disease and pest outcomes will become more difficult to predict (Hulcr and Dunn 2011). With additional complexities added to the three-factored triangle, such as pathogen vectors, this issue of unpredictability and increased virulence becomes even more difficult to manage when attempting to protect vulnerable ecosystems (Parker and Gilbert 2004).

Plantation forests in New Zealand are vulnerable to multiple threats ranging from fungal diseases such as Dothistroma needle blight (Watt et al. 2009, Watt et al. 2011) and Armillaria (Hood 1989, Kimberley et al. 2002), to bark beetle attack of seedlings (Reay et al. 2001, Reay

and Walsh 2002), in addition to abiotic events such as windthrow (Childs 1966, Nieuwenhuis and Fitzpatrick 2002). Windthrow is caused by high speed winds, and is one of the most frequent and severe forms of damage to forests world-wide (Grayson 1989, Usbeck et al. 2010). There is not a single generalised relationship between wind speed and damage intensity, the extent of damage is a result of a variation of factors such as soil moisture content, soil depth, soil/bedrock acidity, tree species, and stand height (Usbeck et al. 2010). This stochasticity and unpredictability of these storm events makes it challenging to adequately prepare a response, and predict the downstream-effects resulting from the damage, including insect and fungal outbreaks. The abundance of freshly felled and damaged trees greatly exacerbates pest abundances that respond to the sudden abundance of host material with population explosions (e.g. bark beetles - Wichmann and Ravn 2001, Wermelinger 2004).

These issues are not unique to New Zealand, and forest managers in many regions of the world face similar challenges. Although on the surface this might seem advantageous, as solutions to common pest issues may be readily available, this is not always the case as biotic interactions and community assembly dynamics in novel, or 'emerging', ecosystems can be unpredictable (Hobbs et al. 2006, Hobbs et al. 2009). Even interactions between the same organisms under differing environmental contexts can vary greatly (Cramer et al. 2008), which alters the potential management responses to pest threats in novel environments.

Windthrow is a significant source of damage and disturbance in both natural (Kramer et al. 2001, Martin and Ogden 2006, Panayotov et al. 2011) and production forests (Childs 1966, Grayson 1989, Nieuwenhuis and Fitzpatrick 2002, McCarthy et al. 2010) world-wide, which can cause damage at the scales from individual trees, to stands, or even landscapes (Kuuluvainen 1994). When such events occur over large spatial scales, salvage harvest operations are often implemented to recover as much value as possible from the damaged wood (Childs 1966, Lang et al. 2009). Controversy arises in the salvage-harvesting of natural forests where disturbances can be viewed as key ecosystem processes, rather than ecological disasters that require human repair (Lindenmayer et al. 2004).

Salvage harvest of natural forests can have negative impacts on many taxa due to the removal of critical habitats for specialised species (Lindenmayer and Ough 2006), or the interactive effects of two events in succession (initial storm or fire, followed by salvage logging) on species not adapted to high rates of disturbance (Paine et al. 1998). Salvage logging in natural forests can also facilitate the colonisation of invasive species, impair natural regeneration, and have detrimental effects on soil properties (Lindenmayer and Noss 2006), and the implementation of salvage logging in such areas has been discouraged. Conversely, there can also be negative effects of leaving windthrown timber unsalvaged. For example, following severe storms damage of spruce forests in Central Europe, outbreaks of the spruce bark beetle, *Ips typographus*, rose to levels where they infested living spruce trees – a behaviour only displayed under epidemic conditions (Wichmann and Ravn 2001, Wermelinger 2004). In the case of plantation forestry, it is advantageous to harvest not only to salvage the value of damaged trees, but also to protect those that remain. In New Zealand, pine plantations have been demonstrated to be valuable alternate habitats for native species (Brockerhoff et al. 2005, Brockerhoff et al. 2008, Pawson et al. 2008). With this being said, salvage harvest operations are unlikely to have major negative environmental impacts relative to normal plantation management, which involves practices such as clearfelling.

Aside from the loss of trees before reaching their full size in terms of growth, there are concerns following windthrow as to the available timeframes of salvage before the quality and value of the timber is reduced (Childs 1966, Hood and Ramsden 1997, Wylie et al. 1999). There are multiple agents that can reduce the value of damaged stands such as sapstain fungi, decay fungi, and insects. It is also possible for these agents to interact, and occur collectively with synergistic losses on quality and value. Sapstain is a major issue and costs the forest industry upward of NZ$100 million per year in spoiled timber (Wakeling 1997), a significant loss of revenue for the forestry sector.

Sapstain fungi are aerobic organisms, so the main factor limiting their growth is the water saturation of the conducting tissues of a living tree. Sapstain becomes evident once the moisture

content of felled trees falls below 100% (dry weight basis), and oxygen levels begin to rise (Seifert 1993, Beal et al. 2010). Fungal inoculum can be dispersed through methods such as wind and rain splash (Farrell et al. 1997, Uzunovic et al. 2004), however bark beetles have also been shown to be vectors (Leach et al. 1934, Paine et al. 1997, Reay et al. 2001, Romon et al. 2007). This study aims to identify the principal determinates of the timing and rate of sapstain onset in windthrown timber in New Zealand. This will be investigated with particular reference to the importance of bark beetles as vectors of the staining fungi. Timeframes available to salvage harvest damaged timber before sapstain develops extensively will be experimentally tested, depending on the seasonality and regionality of the storms occurrence.

1.2 Bark beetles

Bark beetles (Coleoptera: Curculionidae, Scolytinae) are one of the most economically important forest insect groups (Paine et al. 1997). Bark beetles can be described as either primary ("aggressive") or secondary ("non-aggressive") pest species, which generally conforms to their relative ability to colonise and kill healthy living trees as opposed to stressed, dying or dead trees, respectively (Raffa et al. 1993). Relatively few species of bark beetle are highly aggressive. For example, of the 411 species of bark beetle found in the United States, only 7 to 10 species commonly kill otherwise healthy trees (Six 2005). Most bark beetle species are secondary bark beetles, capable of colonising trees that are already stressed, weakened, or recently killed (Raffa et al. 1993, Paine et al. 1997). Causes of stress to a host tree can be due to factors such as disease, drought or disturbance. Other sources of material for secondary bark beetles are recently harvested trees, logging residue such as stumps and branches, or storm damaged trees (Mausel et al. 2007).

New Zealand has 11 exotic Scolytinae species established within its forests (Brockerhoff et al. 2003) with two species, the pine bark beetle (*Hylastes ater* Payk.) and the golden haired pine bark beetle (*Hylurgus ligniperda* Fabr.) being the most abundant and widespread (Reay and Walsh 2001, Brockerhoff et al. 2003, Brockerhoff et al. 2006a). As both are introduced pests in an introduced ecosystem, few of their natural enemies exist in this novel environment to control their populations (Ciesla 1988). Both are considered secondary pest species in that they do not attack healthy living trees, and are generally not considered major forest pests (Ciesla 1988). Both species use fresh stumps and slash as material for breeding sites, and are generally considered more harmful to the New Zealand forest industry as quarantine pests (Lanfranco et al. 2004, Zhang et al. 2004). As a result of this, export timber requires fumigation with the gas methyl bromide, to kill bark beetles and other insects before it leaves the country (Pawson and Watt 2009, Pawson et al. 2009). In some cases, continuous harvesting of large areas year round ensures an ample supply of breeding habitat allowing bark beetle populations to persist at epidemic levels for longer periods of time than what would be expected in a natural forest

environment (Örlander et al. 1997, Leather et al. 1999).

Hylastes ater was first detected in the North Island of New Zealand in 1929 (Clark 1932), and is now established wherever pine forests occur. It is native to Europe, and in addition to New Zealand has been introduced to Australia and Chile (Milligan 1978, Leahy et al. 2007). In New Zealand, *H. ater* adults are known to feed predominantly on *P. radiata*, but also attack *Picea* (spruce), *Abies* (fir), *Larix* (larch), and *P. menziesii* (Douglas-fir) (Milligan 1978). The most troublesome destruction caused by *H. ater* is due to its feeding on the stem of young pine seedlings, sometimes resulting in death (Milligan 1978, Ciesla 1988, Reay et al. 2005). Up to 12 *H. ater* adults have been found on the root system of a single seedling (Ciesla 1988). Evidence suggests that seedlings are able to survive all but the most severe attacks (Reay and Walsh 2002), but the impact of sub-lethal damage to seedlings is unclear as these beetles are also known to vector sapstain fungi which also adversely affects seedling growth (Reay et al. 2005). *Hylastes ater* often co-occurs with *H. ligniperda* (Ciesla 1988), and is usually suppressed in numbers with the other species present (Kerr 2010), possibly resulting in a reduction of seedling damage overall due to reduced numbers.

Hylurgus ligniperda was first detected in the North Island of New Zealand in 1974 (Bain 1977a), and has recently been detected as far south as Dunedin (Kerr 2010). It is native to Europe, the Mediterranean area, and Atlantic islands and is established in North America, Australia, Japan, South America, and South Africa, in addition to New Zealand (Bain 1977a, Ciesla 1988, Haack 2006). It is classically thought to be restricted to pine trees (Bain 1977a), however recent studies linked it to colonisation of Douglas-fir and other species, though breeding among these hosts has not been observed (Vanhanen, unpublished data). Like *H. ater*, adults breed in the inner bark of recently felled logs, as well as the remnant stumps and roots (Bain 1977a, Ciesla 1988). *Hylurgus ligniperda* contrasts *H.* ater in that it is not known to attack seedlings in New Zealand (Bain 1977a).

The New Zealand native wood borer *Pachycotes peregrinus* (Chapuis) (Coleoptera: Curculionidae, Scolytinae) colonises freshly felled logs, and will attack most softwoods,

including *P. radiata* (Bain 1977b). Their attack can be distinguished by distinctive holes about 2.5 mm in diameter with white frass evident at the opening, materials such as logs can become heavily and repetitively attacked when left in contact with the ground (Bain 1977b). This beetle occurs throughout New Zealand, except some dry zones in the Canterbury region (Bain 1977b), and has been documented at sites following windthrow (McCarthy et al. 2010). *Pachycotes peregrinus* may be mistaken for *H. ater* and *H. ligniperda*, but can be distinguished from each other by their setae (hairs) and pronotum (Figure 1.1) (Bain 1977b).

Both *H. ater* and *H. ligniperda* are primarily stump and root feeders, and are therefore behaviourally adapted to attacking trees at ground level; or where there is some form of contact with something solid; rather than sections of wood suspended in the air (McCarthy et al. 2010). This behaviour is common among many *Hylastes* species (Flechtmann et al. 1999). This reduces their impact on storm damaged trees, as these tend to be held above the ground by their branches, or by resting on other fallen trees. In addition to the physical beetle damage caused by tunnelling into the phloem and sapwood of susceptible trees (Paynter et al. 1990, Mausel et al. 2007), bark beetles and wood borers play an important role as vectors of fungal spores (Paine et al. 1997, Suckling et al. 1999, Reay et al. 2005, 2006a, Romon et al. 2007). The importance of this relationship to the colonisation of trees by sapstain fungi has not been thoroughly studied in New Zealand.

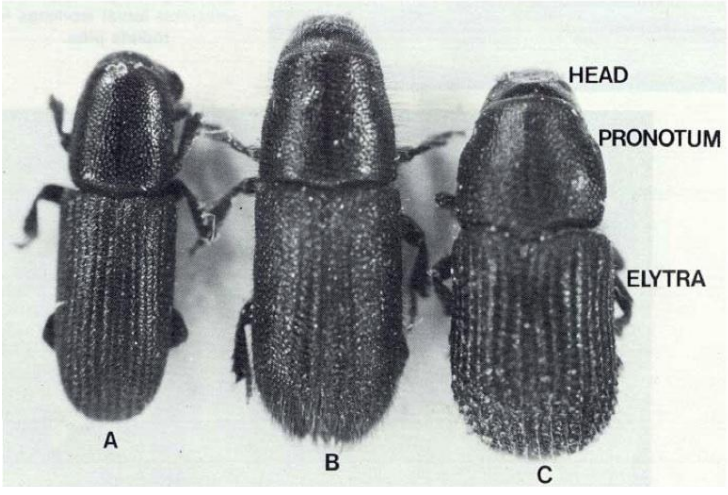


Figure 1.1. (A) *Hylastes ater*, (B) *Hylurgus ligniperda*, and (C) *Pachycotes peregrinus*. Note the differences in shape of the pronotum, also the distribution and abundance of setae (from Bain 1977b).

1.3 Sapstain fungi

Sapstain is the discolouration of wood caused by fungi, affecting both conifers and broadleaved trees (Gibbs 1993), and has caught the attention of foresters and scientists since the middle of the 19th century (Von Schrenk 1903). There are at least 14 species of sapstain fungi present in New Zealand (Thwaites et al. 2005) and *P. radiata* is highly susceptible to attack from sapstain which greatly reduces the cosmetic value of the wood and associated products (Francke-Grosmann 1967, Farrell et al. 1997). This staining is caused by a dark pigment called melanin, which is present in small granules on the outer surface of the fungal wall (Zimmerman et al. 1995, Eagen et al. 1997, Reay et al. 2002). It has been shown that this pigment may enhance competitive interactions by conferring resistance to UV and desiccation, and may be required for the growth of appressoria (a hyphal "pressing" organ) that fungi use to actively penetrate their hosts (Zimmerman et al. 1995, Eagen et al. 1997). Some ophiostomatoid fungi are colourless, but the species that cause staining range across shades of blue, brown, and black depending on the pigment of the fungal hyphae (Seifert 1993). Sapstain is generally caused by three types of fungi (Seifert 1993, McCarthy et al. 2010):

1. Species of *Ophiostoma*, *Ceratocystis*, *Ceratocystiopsis* and *Grosmannia* (sexual teleomorphs), and their asexual anamorphs, all collectively known as "ophiostomatoid fungi" (Zipfel et al. 2006, Bueno et al. 2010). The genus *Ophiostoma* has many anamorphs classified in at least four genera: *Pesotum*, *Leptographium*, *Hyalorhinocladiella* and *Sporothrix* (Aghayeva et al. 2004).
2. Black yeasts such as *Hormonema dematioides*, *Aureobasidium pullulans*, *Rhinocladiella atrovirens* and *Phialophora* spp.
3. Dark moulds such as *Alternaria alternata*, *Cladosporium sphaerospermum*, *C. cladosporioides* and *Diplodia pinea*.

The darkly pigmented sapstain fungal hyphae first grow into the sapwood, then extend radially via the parenchyma of the xylem rays, and finally into the tracheids of the axial water-

conducting system (Ballard et al. 1982). In live trees, this disrupts conduction of water and results in stress on the tree (Ballard et al. 1982). In dead trees, sapstain simply affects the cosmetic value of the wood, as the structural integrity is not reduced (Von Schrenk 1903, Gibbs 1993, Hood and Ramsden 1997). In the case of felled timber, stain growth is initially hampered by the natural moisture levels of the wood physically occupying the space where fungi grow and reducing oxygen levels in the conducting tissues, but as the wood dries out space is created allowing the fungi to grow, and discolour the wood (Seifert 1993, Beal et al. 2010). This was evident in a study by McCarthy et al. (2010) investigating the onset of sapstain following windthrow in the Nelson region of New Zealand. Felled trees were separated into two classes, severed or rooted, where snapped trees were killed instantly and rooted trees fell with some of the root system remaining intact in the soil. Increases in sapstain proceeded more rapidly in snapped trees, which also dried quicker, than in trees that toppled but remained rooted (Figure 1.2).

Moisture content levels required to suppress fungal growth are reported to be between 100 and 120% (Liese 1984, Beal et al. 2010), or below 20% (Colley and Rumbold 1930). In addition, there is an optimal temperature of between 22 and 30°C (Seifert 1993). With knowledge of some of these factors affecting fungal growth, a model called the "Sapstain Danger Index" (SDI) was created to track the time taken for felled logs to be spoiled due to sapstain (Zeff 1999). The model was developed based on the critical growth factors of fungi: nutrient level, temperature, and wood moisture content.

The SDI model is shown below (Eq. 1) (Zeff 1999).

$$\mathrm{SDI} = \frac{8.0}{(\mathrm{F1} \times \mathrm{F2} \times \mathrm{F3})} \quad (1)$$

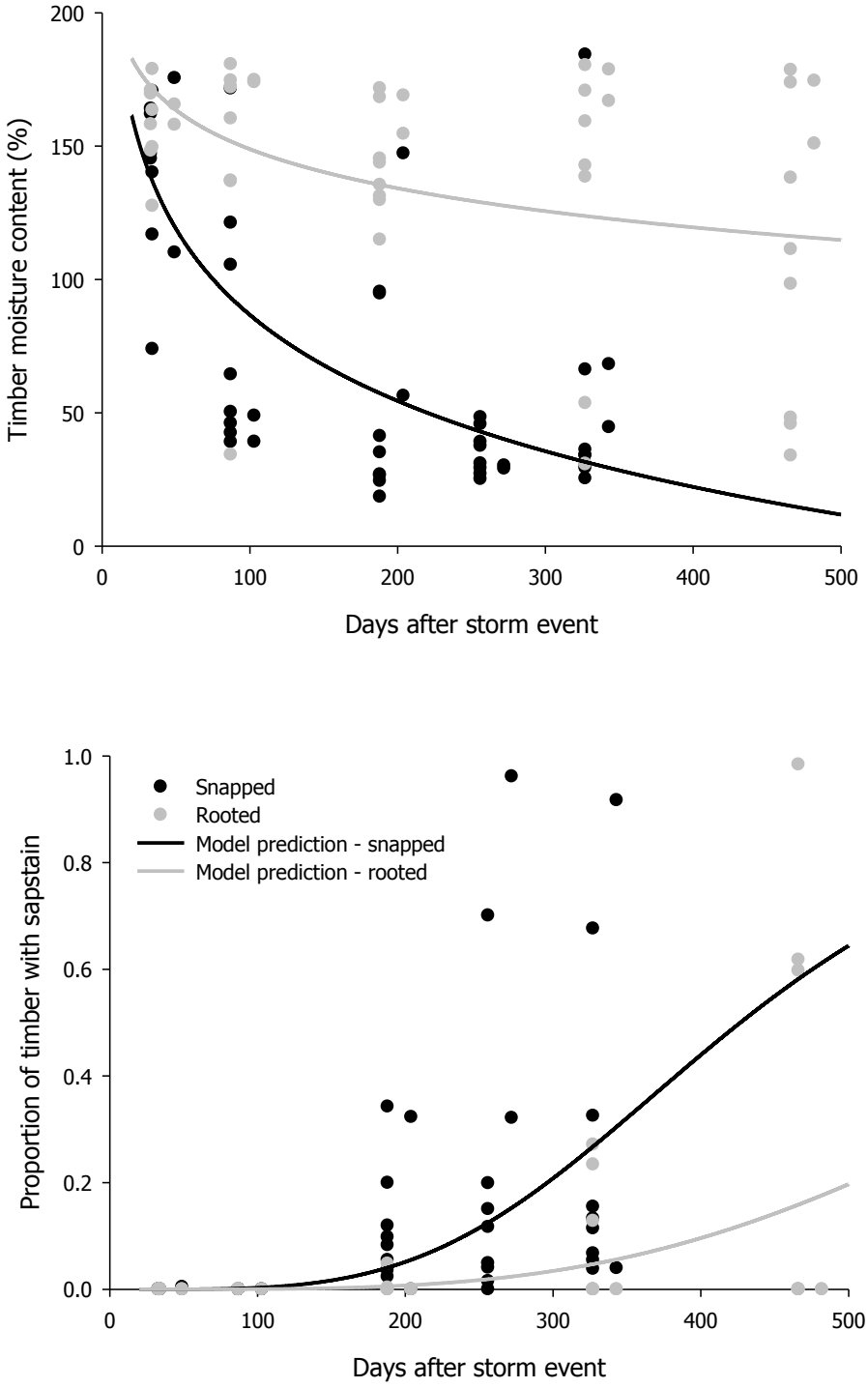


Figure 1.2. Comparisons of model predictions for timber moisture content and sapstain in snapped (severed, black) and rooted (grey) trees. Symbols indicate observed data points, with the curve being the predicted values from a model developed to predict the rate of sapstain accumulation over time (adapted from McCarthy et al. 2010).

The numerator 8.0 is used as a constant as the maximum proposed number of days between felling and application of anti-sapstain treatment (Cooper 2000). F1 is time of year, used as a surrogate for nutrient level – highest in spring and summer (0.2), and lower in autumn and winter (0.1) (Cooper 2000). F2 is midday temperature, and F3 is time since last significant rainfall, a surrogate for moisture content. Therefore, the SDI takes into account the major growth limiting factors of sapstain fungi to create a model allowing foresters to determine the maximum number of days between the felling of a log and when anti-sapstain treatment should be applied (Figure 1.3). A high SDI value indicates that anti-sapstain treatment is not required for a number of days, whereas a low value shows that log turnover should not exceed one or two days.

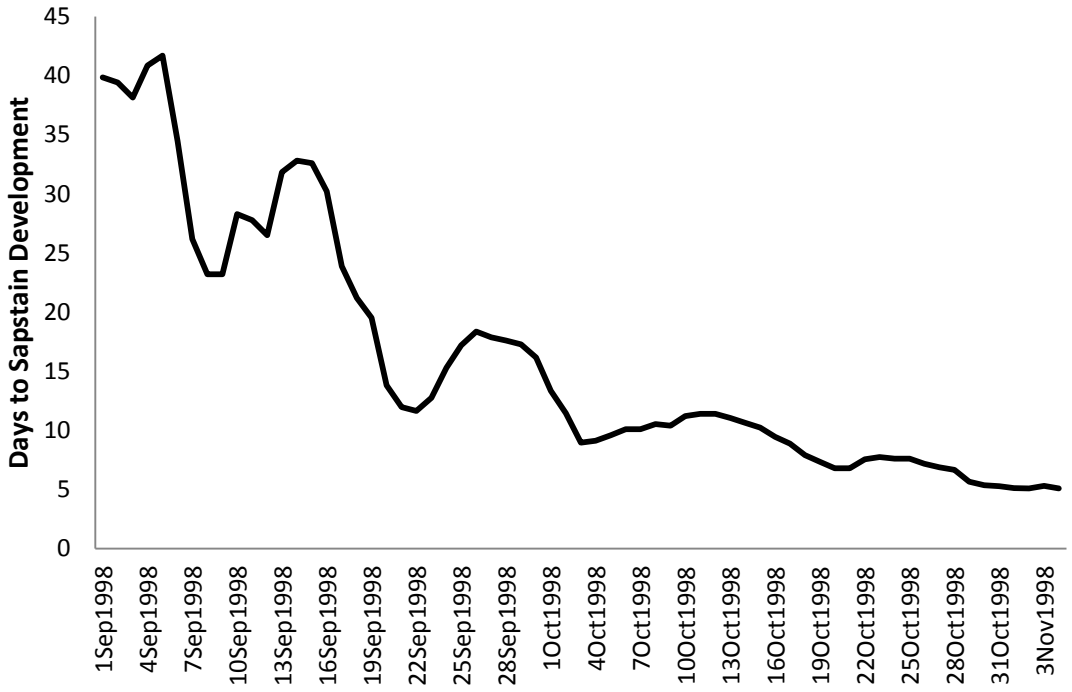


Figure 1.3. Sapstain Danger Index for Spring 1998 with a 7-day average to remove day-to-day variations in the index (adapted from Zeff 1999).

The effect of sapstain on wood strength is generally thought to be negligible (Seifert 1993). A study by Schirp et al. (2003a) investigated the effect of sapstaining fungi on the strength properties of *P. radiata* in New Zealand. Samples of pine were inoculated with isolates of stain fungi (*Ophiostoma floccosum*, *O. pluriannulatum*, *O. ips*, *O. piceae*, *Leptographium procerum*, and *Diplodia pinea*) with tests conducted on the effects of sapstain on toughness and dry weight compared to controls. They reported no significant differences between wood specimens inoculated with sapstaining fungi and the controls for all species except *O. ips* which caused an 18% reduction in strength after 16 weeks (Schirp et al. 2003a). There were, however, significant decreases in strength when decay fungi were present, with as much as a 61% reduction in toughness caused by *Gloeophyllum trabeum* after 16 weeks (Schirp et al. 2003a). This needs to be taken into account as recent studies have indicated that it is common to have sapstain and decay fungi co-existing within the same piece of wood (McCarthy et al. 2010), meaning that there would be a decrease in strength, even if not directly attributable to the stain fungus. McCarthy et al. (2010) reported repeated isolations of decay fungi such as *Phlebiopsis gigantea* and *Schizophyllum commune* which caused toughness reductions of 16% and 32%, respectively (Schirp et al. 2003a). Sapstain fungi have been shown to preferably metabolise readily accessible, non-structural wood components such as starch, with little secretion of cell wall degrading enzymes (Schirp et al. 2003b) explaining the minimal effect on wood strength.

Multiple methods for the control of sapstain fungi have been investigated. Following serious events such as fire and windthrow, there is often an influx of material salvaged from the damaged stands flooding the timber market with an oversupply or product. A major concern during such events is the salvage and sale of affected logs before deterioration occurs (Childs 1966, Liese 1984, Hood and Ramsden 1997, Wylie et al. 1999, McCarthy et al. 2010). One method of protecting logs from beetle and fungal attack is to maintain moisture content levels by storing logs under wet storage, either using sprinkler systems, or storing the logs directly in large bodies of water such as lakes (Clifton 1978, Liese 1984). This option has been implemented successfully for up to two years (Clifton 1978), though serious attack by beetles

and fungi is still possible so this option is not recommended for anything longer than six months (Wylie et al. 1999).

Multiple studies have named *D. pinea* (syn. *Sphaeropsis sapinea*) as the most significant and important stain fungus in New Zealand (Farrell et al. 1997, Thwaites et al. 2005, McCarthy et al. 2010). It is known that *D. pinea* sometimes acts as a latent endophyte in healthy *Pinus* trees (unlike ophiostomatoid fungi), often becoming obvious once a tree becomes damaged or stressed (Smith et al. 1996, Flowers et al. 2001, Reay et al. 2006b). Infection of live trees by *D. pinea* can result in leader dieback, crown wilt and whorl cankers in many *Pinus* species (Chow 1984). Short-distance dispersal of this pathogen is generally attributed to wind and rain splash (Thwaites et al. 2005), however, the methods of long distance dispersal are unknown (Brookhouser and Peterson 1971, Palmer et al. 1988). There is limited evidence suggesting a possible relationship between *D. pinea* and bark beetles (Wingfield and Knox-Davies 1980, Romon et al. 2007, Whitehill et al. 2007), but this species is generally thought to disperse independently of insects and was not found to be present on any Diptera or Coleoptera in a New Zealand survey (Suckling et al. 1999). It is thought that the main method of dispersal of this fungus is through wind and rain splash of spores originating from infected mature trees nearby (Bihon et al. 2011). Three morphotypes, A-C, have been described for *D. pinea* (de Wet et al. 2002), with morphotype B assigned the species name *Diplodia scrobiculata* (de Wet et al. 2003). Morphotypes A and C retain the *D. pinea* classification, with morphotype A being widely distributed across the southern hemisphere, and the more virulent morphotype C thus far known only in the north central United States (de Wet et al. 2003).

In contrast to *D. pinea*, the ophiostomatoid fungi have small, sticky spores that are elegantly adapted for insect dispersal (Francke-Grosmann 1967, Beaver 1989, Paine et al. 1997). These fungi are not particularly virulent pathogens, with the exception of those that cause Dutch elm disease and black stain root disease (Harrington 1993, Harrington et al. 2001), but they do cause significant staining of timber (Figure 1.4) (Seifert 1993, Uzunović et al. 1999b, Thwaites et al. 2005). A major advantage this fungal group has over other species, is its method of dispersal

that not only depends on wind and rain, but is commonly carried on the bodies of insects (Gibbs 1993, Paine et al. 1997, Harrington 2005, Romon et al. 2007).

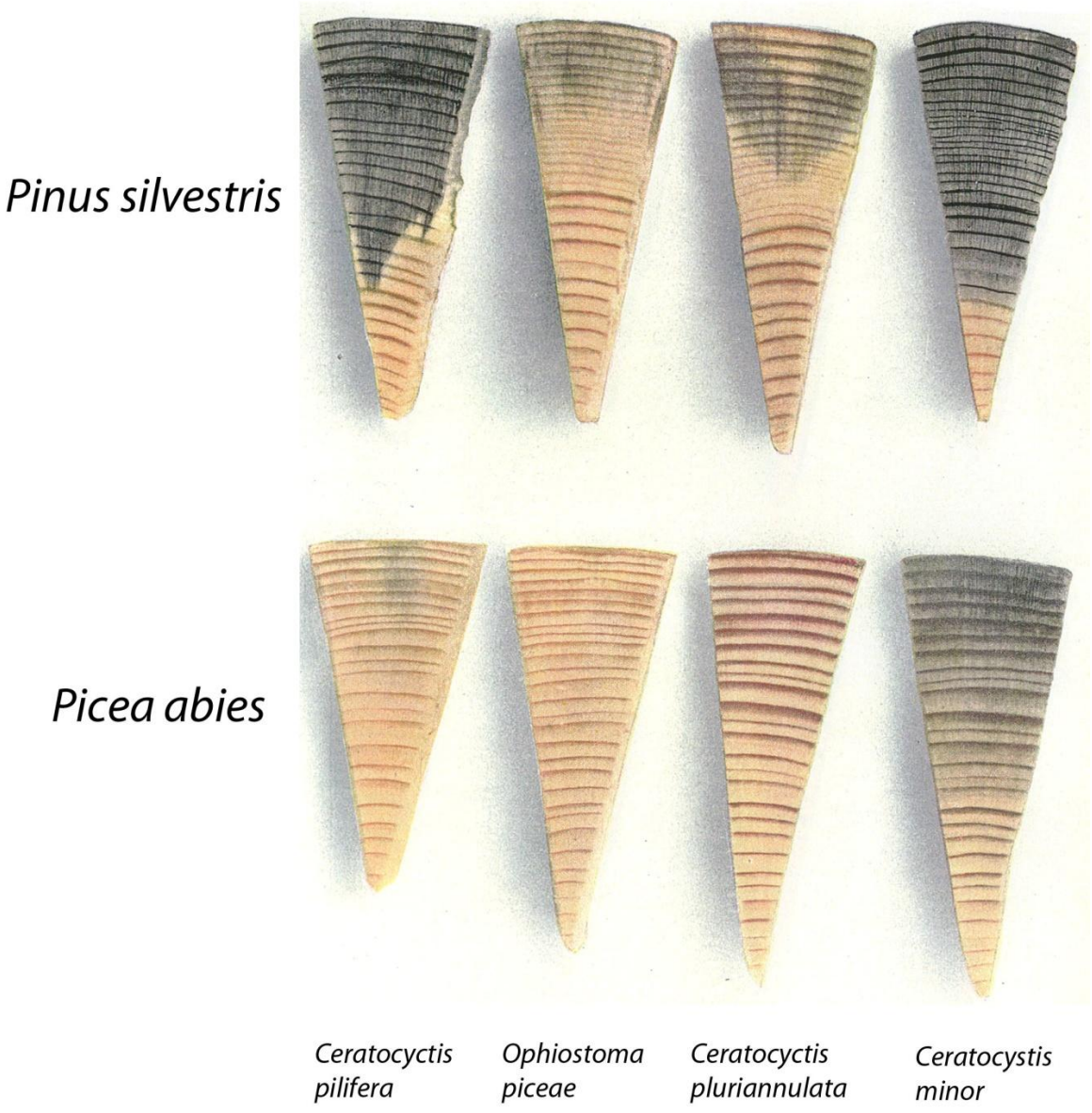


Figure 1.4. Comparison of multiple Ophiostomatoid fungal stain species on two species of coniferous tree, Scots Pine (*Pinus silvestris*) and Norway Spruce (*Picea abies*) (adapted from Lagerberg et al. 1927).

1.4 Beetle-fungus interactions

There is substantial evidence indicating that many of the sapstaining fungi, especially ophiostomatoid fungi, are intimately linked with bark beetles that act as vectors (Gibbs and Inman 1991, Paine et al. 1997, Farrell et al. 2001, Harrington 2005, Romon et al. 2007). The association was first reported in 1903 at the Black Hills Forest Reserve (South Dakota and Wyoming), but after unsuccessful attempts at isolating the fungus from the beetle it was concluded that the spores were wind-blown ("blown about by the wind in countless thousands"), merely entering the holes made by the beetles (Von Schrenk 1903). The links were then re-examined in detail in 1934 in freshly cut logs of Norway pine (*Pinus resinosa*), under a variety of treatments to exclude both insects and fungi (Leach et al. 1934). These experiments showed that bark beetles (*Ips pini* and *Ips grandicollis*) introduced blue-staining fungi into the logs, and that the fungi were rarely, if ever, introduced in any other way. They also suggest that mites, frequently introduced to the logs by beetles, may be moving about the tunnels and spreading blue stain fungi as they travel (Leach et al. 1934). They went on to report that although there was no nutritional symbiosis present between the two organisms, the insects benefited from the fungi inhibiting the flow of sap, and the fungi obviously benefitted from the introduction to the inner bark of susceptible trees, indicating the presence of a broad symbiosis (Leach et al. 1934).

There is a wealth of fungi, mainly ascomycetes, that can be found in the galleries of coniferous bark beetles (Harrington 2005). Many of these fungi show adaptations for insect dispersal, such as the formation of spores at the tip of fruiting structures which aid in their being picked up by passing beetles (Malloch and Blackwell 1993). *Ophiostoma* species and their anamorphs are common associates of bark beetles (Paine et al. 1997, Harrington 2005). The *Ophiostoma* genus is thought to have a late cretaceous origin (Farrell et al. 2001). Bark beetles are also believed to have origins in the late cretaceous, where their ancestors shifted from angiosperm hosts to *Araucaria* species, and coinciding with the evolution of the Pinaceae family and the genus *Pinus* (Sequeira and Farrell 2001). It has been hypothesised that either the

diversification of *Ophiostoma* followed that of the coniferous bark beetles who provided access to uncolonised phloem, or that the pathogenic *Ophiostoma* allowed bark beetles to attack the defensive resin canal system of the conifer host (Farrell et al. 2001, Harrington 2005).

Ophiostomatoid fungal spores can be either carried on the outside, or through the gut passages of a beetle (Francke-Grosmann 1967). These fungi produce a sticky mucilage which helps to hold together spore masses, aids adhesion to the beetle exoskeleton or the bark, and helps protect the spore from desiccation (Beaver 1989). This mucilage also helps to protect the spore from digestion while in the gut of the beetle (Francke-Grosmann 1967), so that epizoic (living nonparasitically on the exterior of a living animal) and endozoic (living within, or passing through, an animal) dispersal is be possible (Beaver 1989).

Some bark beetle species have special structures on their bodies that are adapted for the transport of symbiotic fungal spores, called mycangia (Figure 1.5) (Batra 1963, Beaver 1989). Mycangia may have evolved as hollow glands, or pocket-like internal expansions of existing exoskeleton (Beaver 1989). There are two types of mycangia that have been described for bark beetles – oral, and prothoracic. Oral mycangia are small pouches on or around the mandibles, whereas prothoracic mycangia are glandular with secretions allowing for growth of the fungi within the mycangium (Harrington 2005). Mycangia have evolved independently several times in bark beetles, suggesting that they play a role in the continuity of bark beetle-fungus interactions between generations (Bleiker et al. 2009). It has been suggested that actively growing hyphae from mycangial fungi may provide a continual (regenerating) source of fungal inocula over the extended periods of gallery development (Bleiker et al. 2009). Having mycangia is by no means a requirement for a relationship with fungi – there are many bark beetles such as *Dendroctonus pseudotsugae*, *D. rufipennis*, and *Ips avulses* known to have consistent associations with particular fungi, but none appear to have mycangia (Beaver 1989). Of all bark beetle species, only nine are known to have well-developed mycangia (Table 1.1). Most of the known mycangia-bearing bark beetles are near-obligate parasites of pines and are within the genus *Dendroctonus*. This is a relatively old genus, estimated to have diverged in the

Table 1.1. Species of bark beetles known to have mycangia, their hosts, mycangial types, and principal fungi (ascomycetous or basidiomycetous) upon which they feed or carry (adapted from Harrington 2005).

Bark beetle	Tribe	Principal Plant Hosts	Mycangial Type	Ascomycete Associates	Basidiomycete Associates
Dendroctonus frontalis	Tomicini	*Pinus* spp.	Prothoratic, glandular	*Ceratocystiopsis ranaculosus*	*Entomocorticium* sp. A
D. brevicomis	Tomicini	*P. ponderosae*, *P. coulteri*	Prothoratic, glandular	*C. brevicomis*	*Entomocorticium* sp. B
D. approximates	Tomicini	*Pinus* spp.	Prothoratic, glandular	Unknown	*Phlebiopsis gigantea*
D. adjunctus	Tomicini	*Pinus* spp.	Prothoratic, glandular	*Leptographium pyrinum*	Unknown
D. ponderosae	Tomicini	*Pinus* spp.	Maxillary	*Ophiostoma clavigerum, O. montium*	*Entomocorticium dendroctoni, E.* sp. D, E, F, G, H, *P. gigantea*
D. jeffreyi	Tomicini	*P. jeffreyi*	Maxillary	*O. clavigerum*	*Entomocorticium* sp. E
Ips acuminatus	Ipini	*Pinus* spp.	Mandibular	*O. clavatum, A. macrospora*	Unknown
Pityborus comatus	Corthylini	*Pinus* spp.	Prothoratic, pubescent	Unknown	*Entomocorticium* sp. C

early cenozoic, relatively close to the divergence of bark beetles and the *Ophiostoma* genus (Sequeira and Farrell 2001).

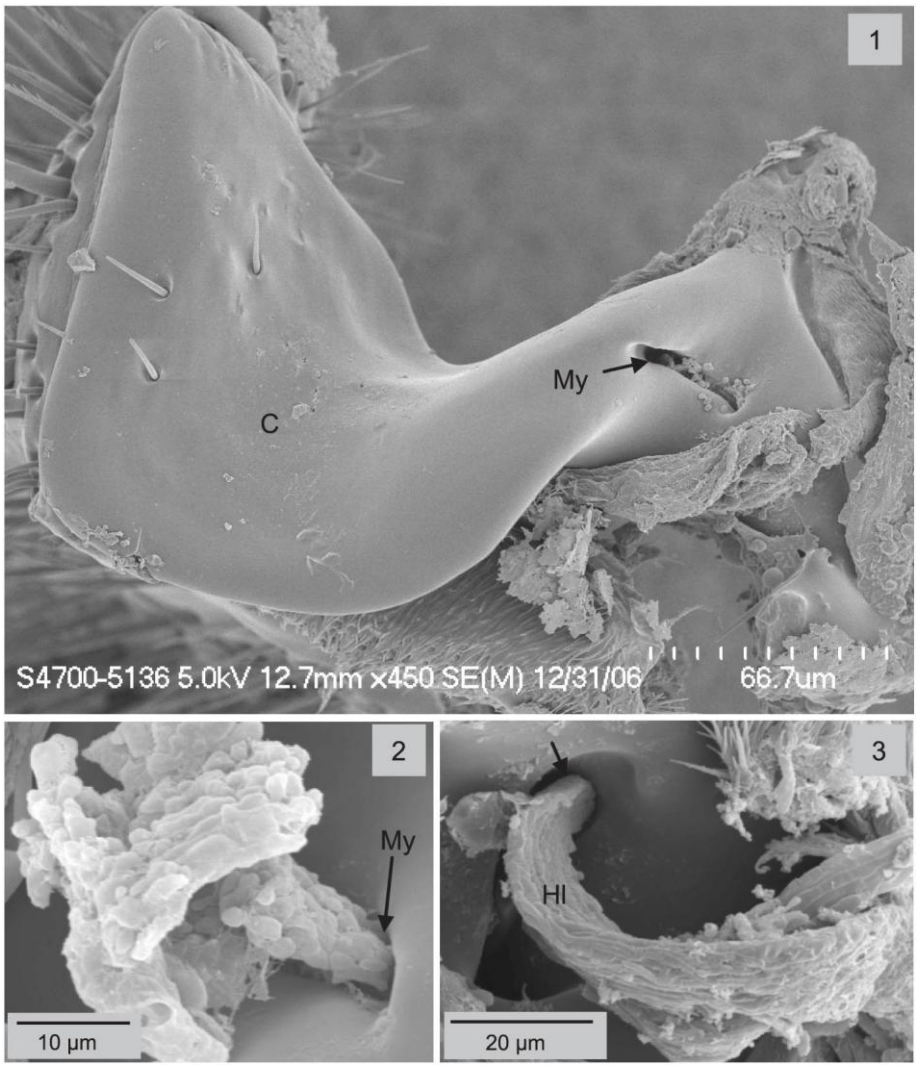


Figure 1.5. Mycangia on the Mountain Pine Beetle, *D. ponderosae*: 1 - mycangial opening (My) on the maxillary cardine (c), 2 – yeasts and spore-like objects protruding from a mycangial opening, 3 – hypha (HI) extending from a mycangial opening (adapted from Bleiker et al. 2009).

Highly developed mycangia are not essential for the transportation of fungi by bark beetles, and it is likely that all coniferous bark beetles at least occasionally carry ophiostomatoid fungi. As an example, the nonmycangial beetle *Hylastes ater* has been demonstrated to commonly carry ophiostomatoid fungi such as *Leptographium guttulatum*, *Ophiostoma quercus*, *O. ips* and *Grosmannia galeiformis* (Zhou et al. 2004c, Reay et al. 2005, Romon et al. 2007). This

transport can involve the carrying of spores on the exoskeleton, primarily in pits and asperites below setae (Figure 1.6). For the mountain pine beetle, *Dendroctonus ponderosae*, there is evidence suggesting that the external transport of fungi is incidental rather than adaptive, and that no one species is better adapted for external transport than another (Bleiker et al. 2009). It has been suggested that mycangia have evolved for carrying specific beneficial fungi. All fungi listed in Table 1.1, with the exception of *O. clavigerum*, are not known to be tree pathogens, therefore mycangial fungi seem to be a source of nutrition for the beetles (Harrington 2005).

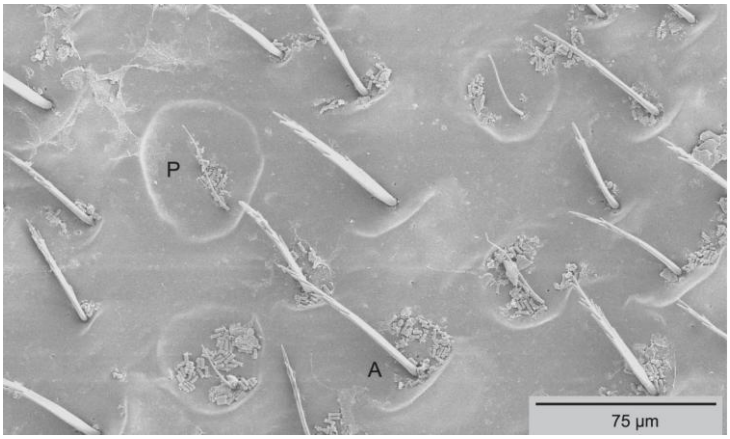


Figure 1.6. Fungal spores in the exoskeletal pits (P) or asperites (A) of the Mountain Pine Beetle, *D. ponderosae* (adapted from Bleiker et al. 2009).

Currently only four genera of bark beetles (that all attack conifers) are thought to be feed on fungi, including *Dendroctonus*, *Ips*, *Tomicus*, and *Pityoborus* (Harrington 2005). Both ascomycetes and basidiomycetes are fed upon by bark beetles, and both are carried in mycangia (Table 1.1). Overall, mycophagy seems to be relatively uncommon, possibly because the inner bark of trees are rich in nutrients (Harrington 2005). It is probable that the fungi provide many essential nutrients and other benefits for beetles (Ayres et al. 2000), though this is not usually obligatory (Harrington 2005).

The bark beetles present in New Zealand, *Hylastes ater* and *Hylurgus ligniperda*, are known to have multiple fungal associates, though the importance of this to sapstain accumulation of sensitive logs is not known, especially in a New Zealand context.

1.5 Management of sapstain in timber production systems following windthrow

Windthrow events are frequent in managed forests, and are the major cause of forest destruction world-wide (Grayson 1989, Ulanova 2000, Nieuwenhuis and Fitzpatrick 2002). There is a large body of information suggesting methods to manage stands in a manner that reduces the chance and severity of potential damage (Grayson 1989, Usbeck et al. 2010), though information on the effective retrieval of damaged trees before insect attack, sapstain and decay become prevalent is limited (Childs 1966). Severe wind and snow storm events in July and August of 2008 (Figure 1.7) caused significant damage to several production forest estates near Nelson (South Island, New Zealand). These storms raised questions about the time available for the salvage harvest of damaged trees before extensive damage by insects, sapstain, and decay fungi reduced the value of the affected timber. *Pinus radiata*, the predominant species of New Zealand's plantation forests, is highly susceptible to attack from sapstain and decay fungi which damage the wood both cosmetically and structurally, respectively (Childs 1966).

Following the July - August 2008 severe storm events, a trial was set up in the Golden Downs region (near Nelson) to assess the time available to salvage harvest timber from affected stands. Both "rooted" trees (that had toppled with their roots still in the ground) and "snapped" trees (that had snapped boles) were assessed for sapstain over a 12 month period at intervals of 1 to 3 months . The findings indicated that sapstain and decay fungi colonised and developed at a considerably faster rate in snapped trees than in rooted trees. However, even in snapped trees, the onset of sapstain took longer than expected, probably because the storms occurred in winter when sapstain progresses more slowly (McCarthy et al. 2010).

Climatic conditions have a strong effect on the activity levels of insects that may vector potential sapstain and decay fungi, with their prevalence usually heightened during the warmer months of the year (Reay and Walsh 2001, Mausel et al. 2007, Kerr 2010). This has been demonstrated for sapstain with harvested logs, indicating that in the warmer months sapstain can develop in less than two weeks in some regions (Zeff 1999). However, the study in Nelson showed that the colouration of timber by sapstain can take a much longer period of time

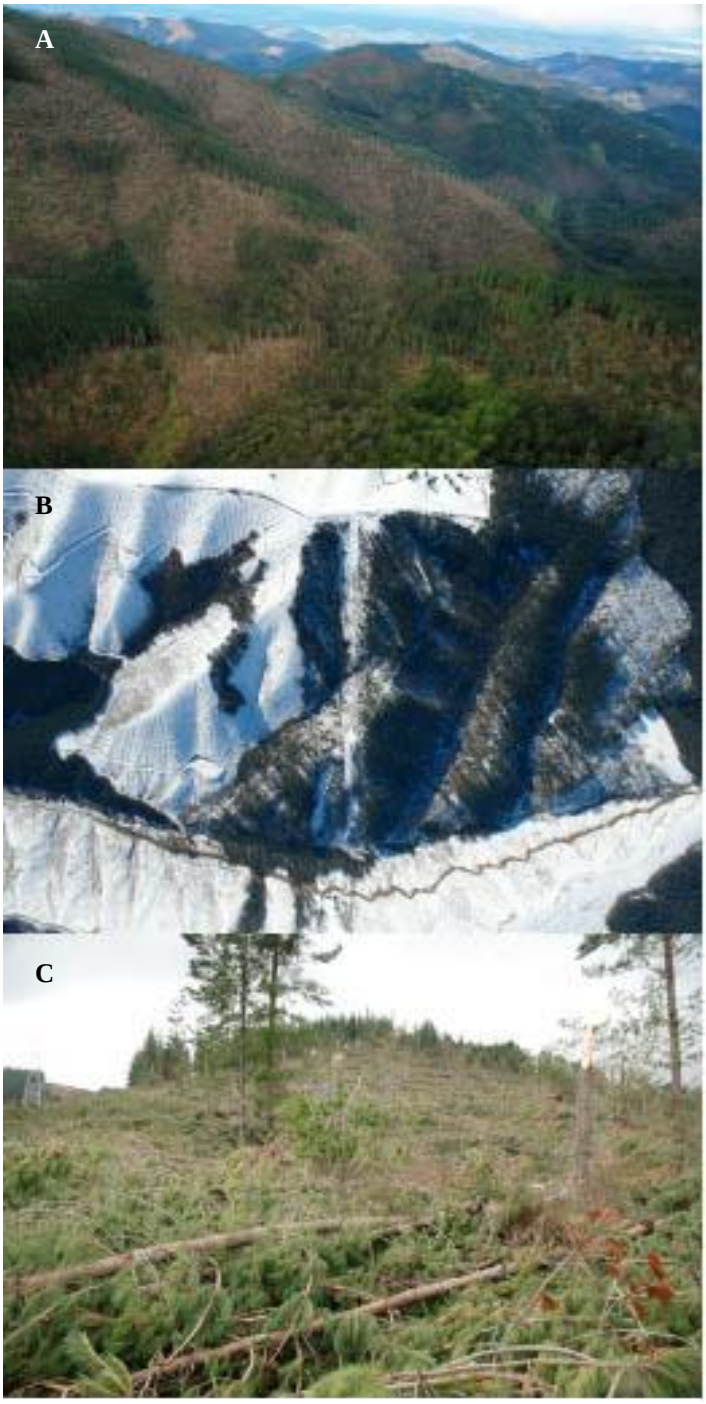


Figure 1.7. Images of damage caused by extreme weather events in *P. radiata* plantations in New Zealand. (A) Typical damage to forests following the Nelson windstorm of July 2008. (B) Snow cover of forests following the Nelson snowstorm of August 2008. (C) Image depicting typical damage sustained with snapping of trees and branches.

(McCarthy et al. 2010). This may be due to a lower incidence of bark damage, and also because many of the toppled trees remained rooted. These factors both contribute to maintenance of higher log moisture content, slowing fungal development. In addition, the windthrow in Golden Downs occurred in winter when there were fewer active adult bark beetles that could potentially vector fungal spores. Although an index, based on an empirical study of seasonal nutrient level, midday temperature, and wood moisture content, is available indicating the probability of sapstain onset across seasons (for harvested wood, Zeff 1999), information on relative determinants of intensity and distribution of this stain, with regards to fungal species and insect vectors, is limited, especially in a salvage harvest situation. These knowledge gaps restrict the ability of New Zealand's forestry industry to develop reliable protocols that can be adopted following severe weather events such as wind or snow damage.

1.6 This thesis

This thesis will test the hypothesis that bark beetles (vectors) facilitate the colonisation of damaged/windthrown trees by sapstain and decay fungi, and that the strength of this interaction intensity varies through time. Activity levels of bark beetles vary through the seasons, along with fluctuating temperatures and rainfall, this is usually as a response to temperature (Reay and Walsh 2001, Mausel et al. 2007). For example, as temperature increases, bark beetle activity does also, likely affecting the rate of attack on vulnerable wood and subsequently increasing fungal spore distribution and colonisation.

The general association between sapstain fungi and insects has long been known (Von Schrenk 1903, Leach et al. 1934), though the importance of the sapstain fungi they introduce, as opposed to those dispersed by wind and rain, is unknown. I will quantify the importance of bark beetles as vectors of stain fungi, in comparison with those dispersed by other means. In addition, I will assess the timeframes available to salvage harvest windthrown trees following events of severe windthrow, taking both seasonal and regional variations into account.

The ecological effects of spatial and temporal variation in inter- and intra-specific interactions between multiple vectors and pathogens are poorly understood. It is known that the insects vector sapstain spores, however the importance and overall effect of this is uncertain relative to wind-dispersal of spores. Pine trees are introduced to New Zealand, and consequently most of their pathogens and insect pests are introduced also. The resulting assemblage is what could be termed a novel ecosystem, as only a subset of what would be a 'natural assemblage' for these species is present, and they predominantly interact with a novel suite of species native to New Zealand. Key features of novel ecosystems are the formation of new species combinations, potential changes to ecosystem functioning (Hobbs et al. 2006), and virulence of pathogens can be altered (Parker and Gilbert 2004). The bark beetles examined in this study would not have had associations with many of the fungal species in their native distributions that they have come into contact with in New Zealand. The uncertainty behind the consequences of these novel associations requires targeted research in order to manage them adequately.

From an applied perspective, the results of this research will provide a solid knowledge base for effective harvest and stand recovery from wind and snow damage, and detail the importance of the interaction between insects and decay fungi and sapstain fungal spores. This knowledge base will allow forest managers to better prioritise salvage-harvesting efforts following such events, and recover maximum value. This becomes increasingly important as climate patterns are changing and extreme weather events become more likely (Easterling et al. 2000).

In Chapter 2, I experimentally test the rate of sapstain onset following simulated windthrow events with both seasonal and regional variation. In total, 288 trees were experimentally felled in this trial, in order to test the effects of: time since felling, wood moisture content, bark beetle colonisation and fungal species identity on the intensity and distribution of sapstain attack. As temperature is a one of the factors driving moisture loss from logs, it was predicted that the seasonality of the storm simulations would alter the rate of sapstain development. Likewise, differences in regional climates were also predicted to have an effect.

Chapter 3 tests whether bark beetles vector fungi to experimental billet logs, half of which were caged to prevent them from beetle attack. This experiment was undertaken in order to assess the effect of insects alone on stain development, while keeping all other factors (ground contact, temperature, wind-blown sapstain spores) constant. A controlled experiment such as this one allows the measurement of beetle attack alone as determinants of stain development, by comparison of logs that were caged and un-caged.

In Chapter 4 sapstain fungi are isolated from the bark beetles *H. ater* and *H. ligniperda*, and the wood borer *P. peregrinus*, as well as sections of wood in order find out what (if any) sapstain species are vectored by them, and also to compare these with isolations taken from stained wood. The specificity of vector-fungi associations was determined from over 5000 individual isolations, identifying previously unrecorded associations.

Finally, in Chapter 5 I draw together evidence of the of the individual thesis chapters to describe the importance of bark beetles as vectors of windthrown timber in New Zealand. The factors driving the success of bark beetles as fungal vectors to windthrown pine trees in

particular, are also described. Management implications of the development rate of sapstain are discussed, with timeframes for the salvage harvest of wind damaged stands described depending on the seasonality and regionality of the storm.

Chapter 2 – Temporal and spatial patterns of sapstain following simulated windthrow

2.1 Introduction

One of the most frequent and severe causes of damage to forests world-wide is the snapping and breaking of trees caused by windthrow (Franklin et al. 2002, Gandhi et al. 2008, Usbeck et al. 2010). When these storms occur within production forests, financial losses can be compounded with losses from trees deemed uneconomical to salvage (Grayson 1989, Mitchell 1995) due to damage or immaturity (Nieuwenhuis and Fitzpatrick 2002), and attack by insects and sapstain fungi (Childs 1966, Hosking 1977, Schroeder 2001, Komonen et al. 2011). The ability to limit economic damage following storm events relies on accurate information regarding the management of damaged stands. One major uncertainty following both large- and small-scale windthrow events is the timeframe available to salvage-harvest logs before the process becomes uneconomical due to fungal staining, decay, and insect infestation.

Sapstain is the discolouration of wood caused by the darkly pigmented hyphae of some groups of fungi. This staining is caused by a dark pigment called melanin present on the outer surface of the fungal cell wall (Zimmerman et al. 1995, Eagen et al. 1997, Reay et al. 2002). Stain is generally caused by three groups of fungi (Seifert 1993), the insect-dispersed ophiostomatoid fungi (Zipfel et al. 2006), black yeasts, and dark moulds. The darkly pigmented hyphae grow first into the sapwood, then extend radially through the rays, and finally into the tracheids of the water conducting system (Figure 2.1, Ballard et al. 1982). Although the structural integrity of stained wood is not significantly reduced (Gibbs 1993, Schirp et al. 2003a), the colouration reduces the cosmetic quality of the timber and generally limits it to low-grade uses (Childs 1966). One of the main regulating factors on the extent of sapstain growth is the change in moisture content of the timber following tree death, whereby values greater than 120% (dry weight basis) maintain low oxygen levels preventing the growth of many sapstain fungi (Clifton 1978, Liese 1984, Seifert 1993, Hood and Ramsden 1997).

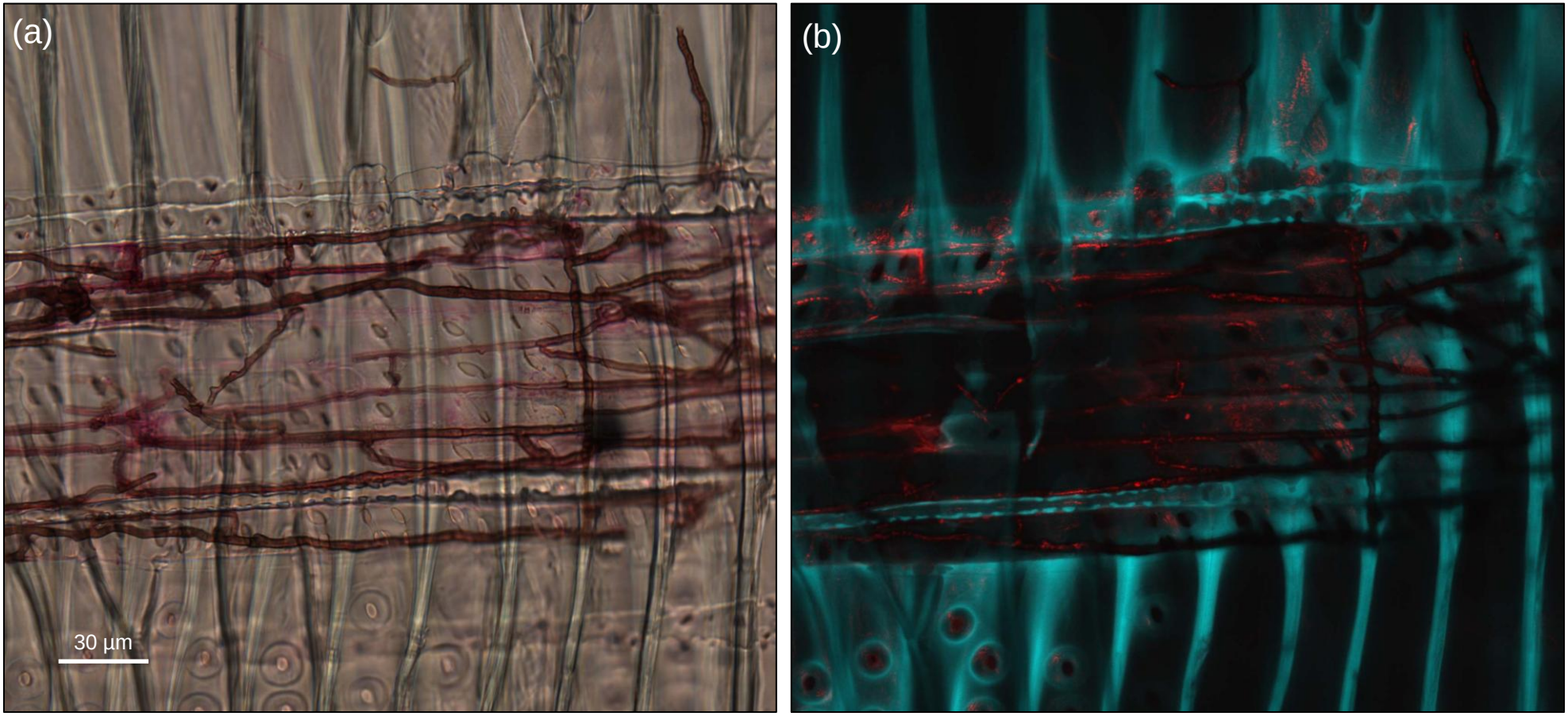


Figure 2.1. (a) a false colour image depicting sapstain (*D. pinea*) hyphae (arrows) in the ray cells of *P. radiata* sapwood. Fixed material was microtomed into 60 µm thick sections and stained with 0.03% lactofuschin. (b) lactofuschin was also imaged by confocal microscopy using excitation at 561 nm (green light) with florescence collected from 570 to 620 nm (yellow and red light). Scale bar = 30 µm (image: David Collings and James McCarthy).

Sapstain degradation of windthrown timber costs New Zealand's forestry industry upwards of NZ$100 million per year (Wakeling 1997). Recently, successive wind and snow storms during the winter of 2008 caused extensive uprooting and breakages of *Pinus radiata* trees in plantation forests in the Nelson region of the northern South Island of New Zealand. More than 2000 ha of plantation forest were affected in this event, and salvage harvesting operations were implemented immediately to salvage as much from the damaged stands as possible, with no reliable quantitative information as to how much time was available before the logs became uneconomic to harvest. A study was initiated in the areas affected by the storm to obtain quantitative information on the rate of sapstain development on the fallen trees. This study found that in a storm at that time of the year, trees that toppled but remained rooted would retain minimum stain levels for up to one year, whereas severed stems would succumb to extensive staining after just 4 months (McCarthy et al. 2010 - see appendix A). Sapstain development coincided with a reduction of moisture content over time that was accelerated if the tree was severed from its roots.

Large-scale damage of production forests caused by storms is a common event around the world. In 1997 a major storm occurred in the south of Ireland damaging close to 500 000 m^3 of timber (Nieuwenhuis and O'Connor 2001). Salvage operations commenced shortly afterwards, but the profit made from wind-thrown timber was on average 10.5% less than timber harvested by standard clearfell methods. A small portion of this reduction of profit was attributed to increased harvesting costs, but most was due to the downgrading of sawlog material due to structural and cosmetic damage to the timber (Nieuwenhuis and O'Connor 2001). In New Zealand, Littlejohn (1984) described a salvage harvest operation following a storm in the central North Island where very little degradation occurred until spring, six months after the storm, when sapstain started to spread rapidly in the fallen trees and cause substantial degradation of timber quality. Climatic conditions appear to have a strong effect on the activity levels of insects that vector sapstain fungi, with their activity usually heightened during the warmer months of the year (Reay and Walsh 2001, Mausel et al. 2007). The warmer temperatures of

summer also accelerate the rate of moisture loss from the logs (Zeff 1999). Knowledge of these factors affecting sapstain dispersal and growth have led to further questions regarding the impact of seasonality and regional locality on the rate of onset of sapstain degradation, in order to more effectively manage salvage harvesting following the impacts of such storms.

The aim of this study was to experimentally test the rate of decline of timber moisture content, and increase in sapstain cover with time since felling under experimentally controlled conditions, discriminating the temporal (successional) effects from seasonal effects on sapstain cover in two climatically different regions of New Zealand. Windthrow was experimentally simulated across two regions of the south island of New Zealand – Golden Downs Forest, Nelson region and Bottle Lake Forest, Canterbury. Both sites were selected as they are among large areas of plantation estate, and sit in differing climates and altitudes where Nelson is generally warmer with higher precipitation than Canterbury (Figure 2.2). The windthrow simulations were staggered across summer, autumn, winter and spring in order to track the effect of storm seasonality on sapstain growth, and determine the degree to which sapstain growth is a time-dependent process versus a season-dependent process. Moisture content of the wood, insect attack, insect phenology, and fungal species were all determined in order to fully understand the dynamics within this system. In an applied sense, the goal of this research is to provide guidance of timeframes available for salvage harvest following windthrow before wood quality is significantly reduced.

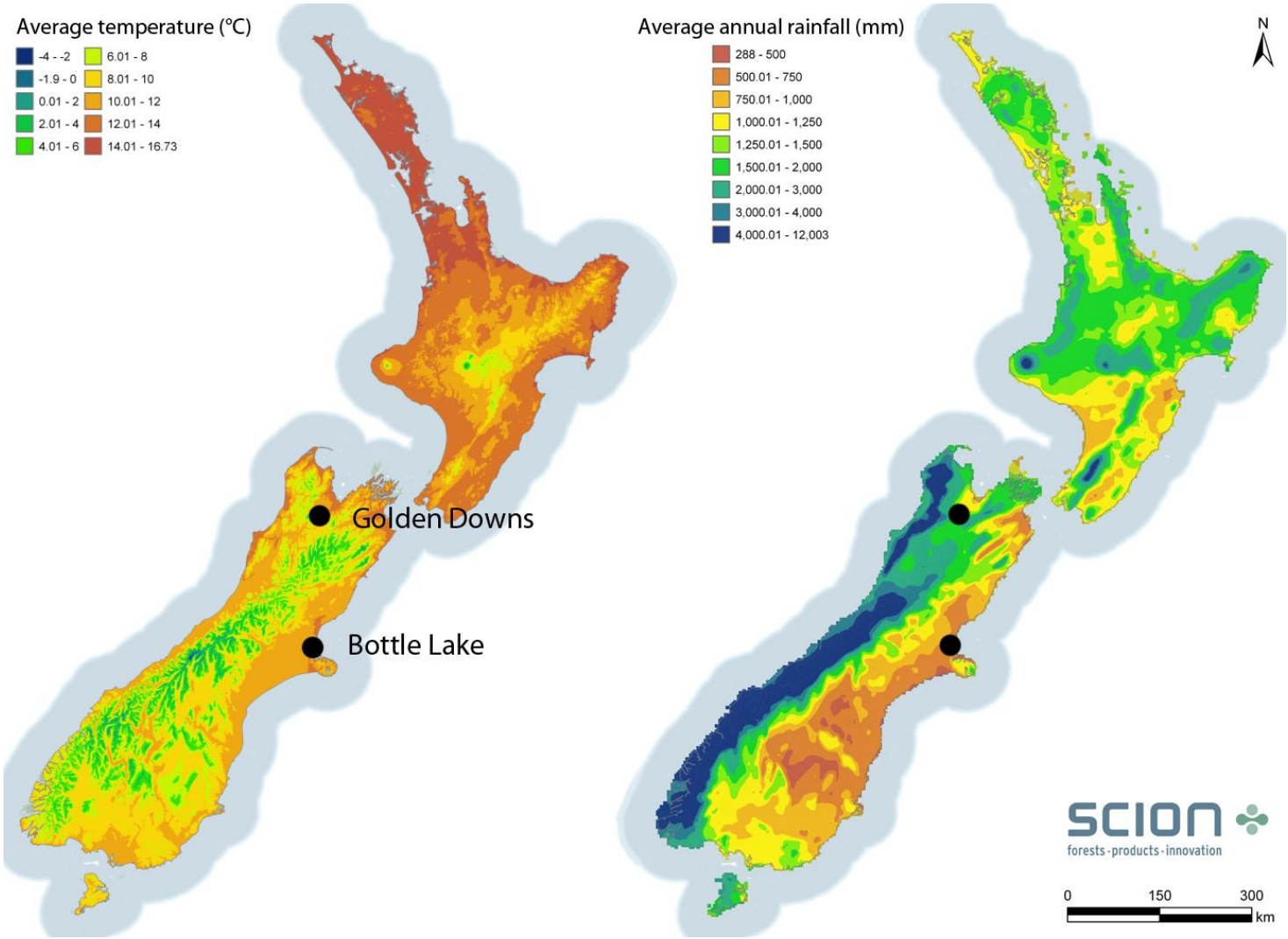


Figure 2.2. Location of the two study regions with rainfall and temperature characteristics. From NIWA 500 m resolution gridded interpolated virtual climate station annual rainfall data for the period 1980-1999.

2.2 Materials and methods

2.2.1 Study sites, and establishment

The field study was conducted over 21 months in *P. radiata* stands in Canterbury (Bottle Lake) and Nelson (Golden Downs), in the South Island of New Zealand. Canterbury was chosen as a cooler region (av. max. temp 16.8°C, av. min. temp. 7.3°C (Anon 2011a)), with a relatively low rainfall (av. 524 mm p/a) and low elevation (2.5 m above sea level), whereas Nelson has a higher temperature (av. max. temp 17.6°C, av. min. temp. 4.8°C), higher rainfall (av. 1385 mm p/a), and higher elevation (approx. 550 m above sea level). Four study sites were established in each region, each approximately 200 m by 200 m, and ranging in spatial separation from 2-16 km apart in Nelson, and 2-3 km apart in Canterbury.

Study sites were established in December 2009 (Nelson) and January 2010 (Canterbury), in stands ranging in age from 9 to 10 years (Table 2.1). This project used experimentally simulated windthrow, created in a controlled fashion by felling trees at each site with a chainsaw. In Nelson, windthrow was simulated in all four seasons (summer, autumn, winter, spring), and monitoring continued for 12 months following each simulation to determine the onset and progression of sapstain fungi and beetle attack. Due to logistical constraints, windthrow at Canterbury was only simulated in summer and winter, and the final assessment of Nelson's autumn windthrow simulation was not undertaken due to high amounts of stain being present in the third assessment. In order to control for within-site heterogeneity, all sites were set up in a randomised block design where trees were arranged in a grid (ca. 20 m × 20 m) , and all trees inside were randomly assigned a number corresponding to when they would be felled, and sampled – 48 trees per site in Nelson, and 24 trees per site in Canterbury. All of these trees were identified and tagged at the beginning of the study.

The study was initiated in summer when 12 trees were cut down at every site. After three months – in autumn – three of these trees were sampled from each site (for sampling details see section 2.2.2. tree and disc processing), and in Nelson where there was an autumn sampling,

Table 2.1. Details of study sites in Canterbury and Nelson.

		Coordinates (NZTM)		Summer felling		Autumn felling		Winter felling		Spring felling	
Location	Site	E	N	Date when felled	Age when felled (yr)	Date when felled	Age when felled (yr)	Date when felled	Age when felled (yr)	Date when felled	Age when felled (yr)
Canterbury	1	1574109	5187816	14 Jan 2010	10	N/A		15 Jul 2010		N/A	10
	2	1575693	5187139		10						10
	3	1576190	5189861		9						9
	4	1575694	5188566		10						10
Nelson	1	1591963	5404592	11 Dec 2009	10	13 Mar 2010	10	24 Aug 2010	10	10 Sept 2010	10
	2	1588580	5397031		9		9		9		9
	3	1588357	5395148		9		9		9		9
	4	1587032	5411993		9		9		9		9

another 12 trees were cut down. After six months, another three trees from the summer simulation were sampled, as well as the first three trees from the trees cut in autumn. This was repeated for every season sampled, with three trees collected quarterly from each seasonal simulation, until all twelve trees from a season were collected, one year following the initial cutting. For a detailed summary see Table 2.2.

2.2.2 Tree and disc processing

Sapstain development and bark beetle infestation were monitored by cutting discs from sample trees. Five 3 cm-thick discs were taken from each tree at intervals of 1.5 m, offsetting slightly if this position intersected with a branch node. The initial disc was cut 0.2 m from the base, with 1.5 m intervals measured from there. The edge of the disc that was facing upward in the field was marked. The discs were then immediately labelled and sealed in plastic bags, and within ca 12 hours were moved to a cold room at 4°C in order to minimise moisture loss and prevent further fungal growth before measurement. Due to this destructive method of sampling, each tree could be sampled only once.

Sapstain was recorded by photographing the cut face of each disc using a specially constructed stage to keep the background colour constant. Care was taken to ensure that the disc was positioned in the correct vertical alignment in the photo, in order to allow accurate placement of a digital grid over each image when making stain measurements. In order to measure the amount of stain in each segment, the entire image was imported into Adobe Photoshop ® (CS4 Extended, Version 11.0) and the top half of the disc was separated from the bottom by placement of a line through its centre. Each half of the disc was extracted and saved as a single-coloured (black) bitmap image (one image file per half-disc) to determine its total area. This process was then repeated, but this time drawing around the stain-covered areas only. The software package ‘Image J’ (version 1.43u) was then used to calculate the number of pixels in the stain-covered area versus the total half-disc area. The pixels were summed from the two halves of each disc, and the stain cover from the halves, to calculate a total percentage cover.

Table 2.2. Sampling strategy for each region. Numbers indicated were randomly assigned to trees. This was repeated for all four sites in each region. Trees cut, indicate the trees that were cut down during the particular season, and trees sampled were those cut into discs and assessed.

		2009 Summer	2010 Autumn	2010 Winter	2010 Spring	2010 Summer	2011 Autumn	2011 Winter
Nelson	Trees cut	1-12	13-24	25-36	37-48	-	-	-
	Trees sampled	-	1-3	4-6, 13-15	7-9, 16-18, 25-27	10-12, 19-21, 28-30, 37-39	22-24, 31-33, 40-42	34-36, 43-45
Canterbury	Trees cut	1-12	-	13-24	-	-	-	-
	Trees sampled	-	1-3	4-6	7-9, 13-15	10-12, 16-18	19-21	22-24

Moisture content of the inner and outer sapwood of each disc half was determined (dry weight basis) within 36 h of sampling. Two small wooden blocks were cut from each disc half, at what was the top and bottom parts of the disc edge when the tree was in the field. From each half, one small block was taken from the outer edge, and the other block was taken from the inner portion of the disc along the same radius (a total of four small blocks per disc). Blocks were weighed at room temperature to constant mass before and after drying at 80°C in a ventilated oven.

2.2.3 Bark beetle sampling

Bark beetle infestation was measured on all study trees from the beginning of the experiment. During field sampling, each 1.5 m section of tree between the experimental discs was examined and the position of all external evidence of beetle attack was recorded (exuded frass, bore holes etc.) and counted within each 0.5 m third of every length. Beetle colonisation, or evidence thereof (insect galleries), was also recorded if it occurred on any of the discs brought back to the laboratory.

In order to measure the seasonal abundances of beetles thought to be potentially important vectors of sapstain, the phenologies of the scolytines *Hylastes ater*, *Hylurgus ligniperda*, and *Pachycotes peregrinus* (Coleoptera: Curculionidae: Scolytinae), and the longhorn beetles *Arhopalus ferus* and *Prionoplus reticularis* (White) (Coleoptera: Cerambycidae) were determined using eight-unit Lindgren funnel traps (PheroTech, Delta, BC, Canada) (Figure 2.3). Two general attractants for pine-infesting bark beetles and wood borers, α-pinene and ethanol, were used as lures as described in Brockerhoff et al. (2006a). Two traps were installed at each site, and beetles were collected fortnightly during autumn, summer and spring, and monthly during winter.

2.2.4 Fungal isolation and identification

Isolations attempts to identify and quantify sapstain fungi were undertaken within 10 days of sampling, from discs 1 and 4 only, on all study trees for each sampling time. All isolations were

Figure 2.3. Lindgren-type funnel trap, with dispensers for attractant (α-pinene and ethanol) on the side (see text).

attempted on two agar media, one general malt medium for the isolation of most stain fungi present, and one selective medium with the addition of the eukaryote antibiotic cyclohexamide, which ophiostomatoid fungi tolerate (Harrington 1981, Jacobs and Wingfield 2001). The standard media consisted of malt agar (3% malt extract, and 2% agar) with 100 µg/ml streptomycin to inhibit bacterial growth. The selective media consisted of malt agar and streptomycin as above, as well as 400 µg/ml cyclohexamide to inhibit growth of non-ophiostomatoid fungi.

An arbitrary wedge was taken from each disc using a small axe. This was bisected aseptically along the radial longitudinal plane using an axe and mallet to initiate the split, and then separated manually, avoiding any external contact of the newly exposed surfaces. Five small chips were cut along the radial line from the newly exposed face using a sterile scalpel (as

in McCarthy et al. 2010), the 1st and 5th of which were directly below the cambium, and above the pith, respectively. The remaining three were taken at equidistant lengths between the 1st and 5th chips. This was repeated twice per wedge, once inoculating a standard medium, and once inoculating the ophiostomatoid selective medium. After incubation periods of up to 10 weeks, emerging isolates were sub-cultured in tubes of malt agar (2% malt extract, and 2% agar). Occasionally, isolations were taken directly from insect hole in the wood. Bacterial colonies were recorded, but not isolated or identified further.

Emerging isolates were sorted into groups, and identified where possible from their vegetative morphology and fruiting structures. All isolates believed to be ophiostomatoid from morphological characteristics were resolved using sequence analysis of the internal transcribed spacer (ITS) using the primers ITS1F (Bruns and Gardes 1993), and β-tubulin regions using the primers Bt2a and Bt2b (Glass and Donaldson 1995). Isolates were sub-cultured on sterile GelAir cellophane (BIO-RAD Laboratories, Hercules CA, USA) that was spread over 1.5% malt agar plates. Mycelium was scraped directly from the cellophane and placed into a sterile 1.5 mL microcentrifuge tube for DNA extraction, which was processed using the REDExtract-N-Amp™ plant PCR kit (Sigma, St Louis, Missouri, USA), following the manufacturer's instructions. If this DNA failed to amplify, it was re-extracted using the ChargeSwitch® gDNA plant PCR kit (Invitrogen, Carlsbad, California, USA), following the manufacturer's instructions. DNA was amplified using the 5 × HOT FIREPol® Blend Master Mix, Ready to Load (dnature, Gisborne, New Zealand). Amplifications were performed in an Eppendorf Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany) under the following conditions for ITS and β-tubulin regions: (1) ITS - initial denaturation 95°C for 15 min (to activate the HOT FIRE polymerase), followed by 35 cycles of 95°C for 30 sec, 60°C annealing for 40 sec and 72°C extension for 40 sec, followed by a final extension period of 72°C for 5 min, (2) β-tubulin - initial denaturation 94°C for 15 min, followed by 30 cycles of 94°C for 60 sec, 58°C annealing for 60 sec and 72°C extension for 60 sec, followed by a final extension period of 72°C for 10 min.

PCR products were visualised by electrophoresis and purified using the ExoSAP method (Exonuclease I, *Escherichia coli* and Shrimp Alkaline Phosphatase, SAP) according to the supplier's instructions (Fermantas Life Sciences, Lithuania). Sequence analysis was carried out at Macrogen Inc., Korea. Sequences were edited and aligned using Sequencher version 5.0 (GeneCodes Corp., Ann Arbor, Michigan, USA) and identities were determined by Genbank BLASTn search (Altschul et al. 1990). For identification, a minimum of 95% sequence identity to an ITS sequence of at least 450 bp from a known specimen in the database was required. For β-tubulin sequences, the required sequence length was 350 bp. Those samples with 97-100% identity match to a known species were considered a match and named to the species level, provided the morphological characteristics of the isolate (sourced from the scientific literature) matched that of the name derived from sequence data.

2.2.5 Statistical analyses

Mixed model analyses of variance (ANOVAs) were used to test the significance of experimental factors including site, felling time, time of assessment, disc location within tree (Discs 1-5) and position within disc (upper versus lower) on moisture content and sapstain cover. These analyses included tree and disc within tree as random effects in the analysis as appropriate.

A test of association between sapstain with presence of insect damage was made using a variable consisting of insect borer count in the stem. This variable was included as a covariate in an ANOVA performed on stem-level sapstain data.

Prior to analysis, sapstain percentage was transformed using the angular transformation (arcsine(sqrt(proportion)) as appropriate for a variable assessed as proportional cover. Moisture content was strongly positively skewed and was normalised using a $\log_{10}$ transformation.

Pairwise comparisons between relevant means were performed using the least significant difference (LSD) test at the 5% level of significance. All analyses were performed using the SAS Version 9.2 MIXED procedure (SAS 2008).

2.3 Results

2.3.1 Regional and seasonal effects on sapstain

In this study, 288 trees were cut down for windthrow simulations across sites in Nelson and Canterbury. From these trees, 1154 discs were analysed for stain and moisture content levels. The linear mixed effects model indicated that there was no significant difference in sapstain cover between upper and lower halves of each disc ($F_{1,2307} = 0.00$, $P = 0.99$). All subsequent analyses therefore used sapstain data at the disc or tree level.

There was a significant effect of site, season, and time following harvesting on stain development at both regions (Table 2.3). There were also multiple significant interaction terms involving disc location, with variation in stain development at different sites, felling seasons, and sampling times all being dependent on disc location within trees (Table 2.3). It was evident that the disc location interaction effects were predominantly due to differences in sapstain onset between disc 1 and the remaining four discs (Figure 2.4). Percent sapstain in disc 1 was much higher than discs 2-5 in Canterbury, but much lower than discs 2-5 in Nelson (Figure 2.4a). However, there were no significant differences in sapstain between discs 2-5 at either location. Moisture content varied significantly between discs being lowest for disc 1, then higher for disc 2, and declining again from there up to disc 5 (Figure 2.4b). Because of the inconsistent behaviour of disc 1 compared with discs 2-5, the remaining analysis was performed only using data from discs 2-5. When the sapstain analysis was repeated with disc 1 removed, there were no longer any significant disc location effects (Table 2.4).

The ANOVA of data from discs 2-5 (Table 2.4) showed significant effects for most terms in the model and their interactions other than disc location indicating that site, season and sample time, and their interactions, all have an effect on stain, whereas disc locality (omitting disc 1) did not. Overall, trees at the Nelson sites had significantly more stain than in Canterbury, and there was generally more stain during the months of spring and summer (Table 2.4). In Nelson, there were significant differences in sapstain development for each felling time (Table 2.5).

Table 2.3. ANOVA table for sapstain percentage (angular-transformed) testing the significance of the model terms for discs 1-5. Fixed effects are listed, and a random effect for tree was included in each model. Significance codes indicate *P*: < 0.001 "***", 0.001 – 0.01 "**", 0.1 – 0.05 "*", 0.05 - 0.1 "+", 0.1 "+".

Fixed effect	Numerator degrees of freedom	Denominator degrees of freedom	F Value	Pr > F	Significance
site	1	46	342.40	<.0001	***
season	3	46	162.66	<.0001	***
site * season	1	46	68.77	<.0001	***
sample_time	3	46	79.26	<.0001	***
site * sample_time	3	46	13.64	<.0001	***
season * sample_time	8	46	12.68	<.0001	***
site * season * sample_time	3	46	7.49	<.0001	***
disc_loc	4	1166	0.50	0.7390	
site * disc_loc	4	1166	44.03	<.0001	***
season * disc_loc	12	1166	4.24	<.0001	***
site * season * disc_loc	4	1166	4.61	0.0011	**
disc_loc * sample_time	12	1166	3.89	<.0001	***
site * disc_loc * sample_time	12	1166	2.30	0.0069	**
season * disc_loc * sample_time	32	1166	1.83	0.0034	**
site * season * disc_loc * sample_time	12	1166	1.57	0.0942	+

Sapstain developed fastest in trees felled in summer, followed in order by spring, winter and autumn. Levels in Canterbury were significantly lower than in Nelson but again, development was significantly faster for trees felled in summer than in winter.

At the one time when all treatments were assessed more or less simultaneously (i.e., in the autumn following felling), there was a clear distinction between Nelson and Canterbury, but little difference between felling times at each site (Table 2.6). The only intra-regional difference being at Nelson where trees felled in the previous autumn (i.e., that had been felled longest) somewhat surprisingly had a significantly lower sapstain intensity than those felled in winter, spring or summer. This suggests that sapstain varies according to the season of the damage, more than with the time passed since trees have been felled. This is seen clearly by comparing Figure 2.5 (a) and (b) which shows sapstain development versus time from felling with

Table 2.4. ANOVA table for sapstain percentage (angular-transformed) testing the significance of the model terms for discs 2-5 (omitting disc 1). Fixed effects are listed, and a random effect for tree was included in each model. Significance codes indicate *P*: < 0.001 "***", 0.001 – 0.01 "**", 0.1 – 0.05 "*", 0.05 - 0.1 "+", 0.1 "+".

Fixed effect	Numerator degrees of freedom	Denominator degrees of freedom	F Value	Pr > F	Significance
site	1	46	467.65	<.0001	***
season	3	46	144.82	<.0001	***
site * season	1	46	81.94	<.0001	***
sample_time	3	46	90.38	<.0001	***
site * sample_time	3	46	18.73	<.0001	***
season * sample_time	8	46	16.23	<.0001	***
site*season * sample_time	3	46	8.65	0.0001	***
disc_loc	3	922	0.61	0.6116	
site * disc_loc	3	922	0.72	0.5405	
season * disc_loc	9	922	0.49	0.8786	
site * season * disc_loc	3	922	0.61	0.6089	
disc_loc * sample_time	9	922	0.26	0.9855	
site * disc_loc * sample_time	9	922	0.44	0.9152	
season * disc_loc * sample_time	24	922	0.39	0.9962	
site * season * disc_loc * sample_time	9	922	1.26	0.2524	

Table 2.5. Percentage sapstain in discs 2-5 assessed at 30, 180, 270, and 360 days after felling at Canterbury and Nelson for various felling times. Values in each column followed by the same letter do not differ significantly (LSD test, *P* = 0.05).

Site	Felling time	Time after felling (days) 90		180		270		360	
Canterbury	Summer	11	a	18	a	6	b	29	b
	Winter	0	c	0	c	15	bc	24	b
Nelson	Autumn	1	c	18	a	22	c	49	c
	Spring	23	a	82	d	91	d		
	Summer	66	d	87	d	91	d	91	d
	Winter	2	c	1	c	69	a	79	a

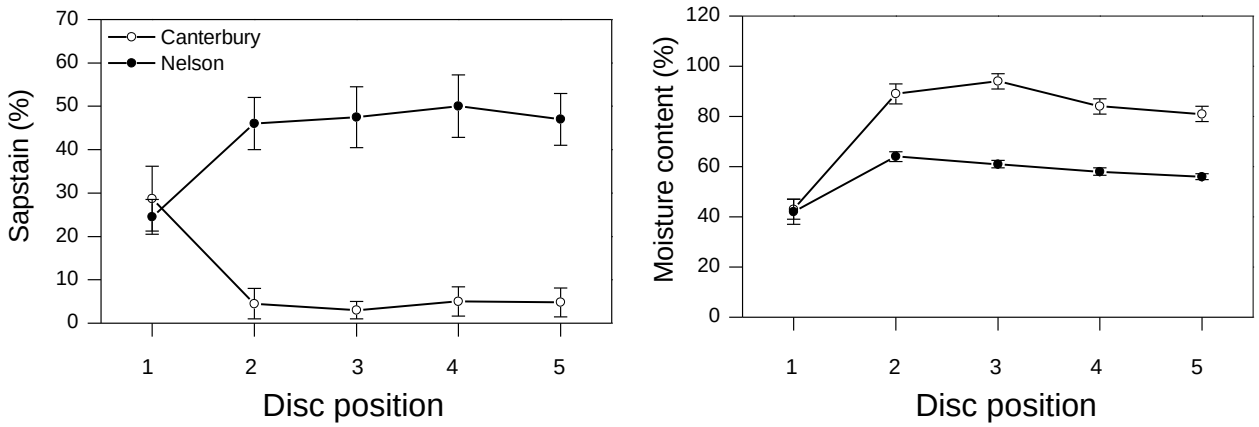


Figure 2.4. (a) Sapstain percent, and (b) moisture content percent in discs assessed by disc position (1 at the cut end/base of the tree, running sequentially up its length) 180 days after felling for the two regions. Error bars show standard errors.

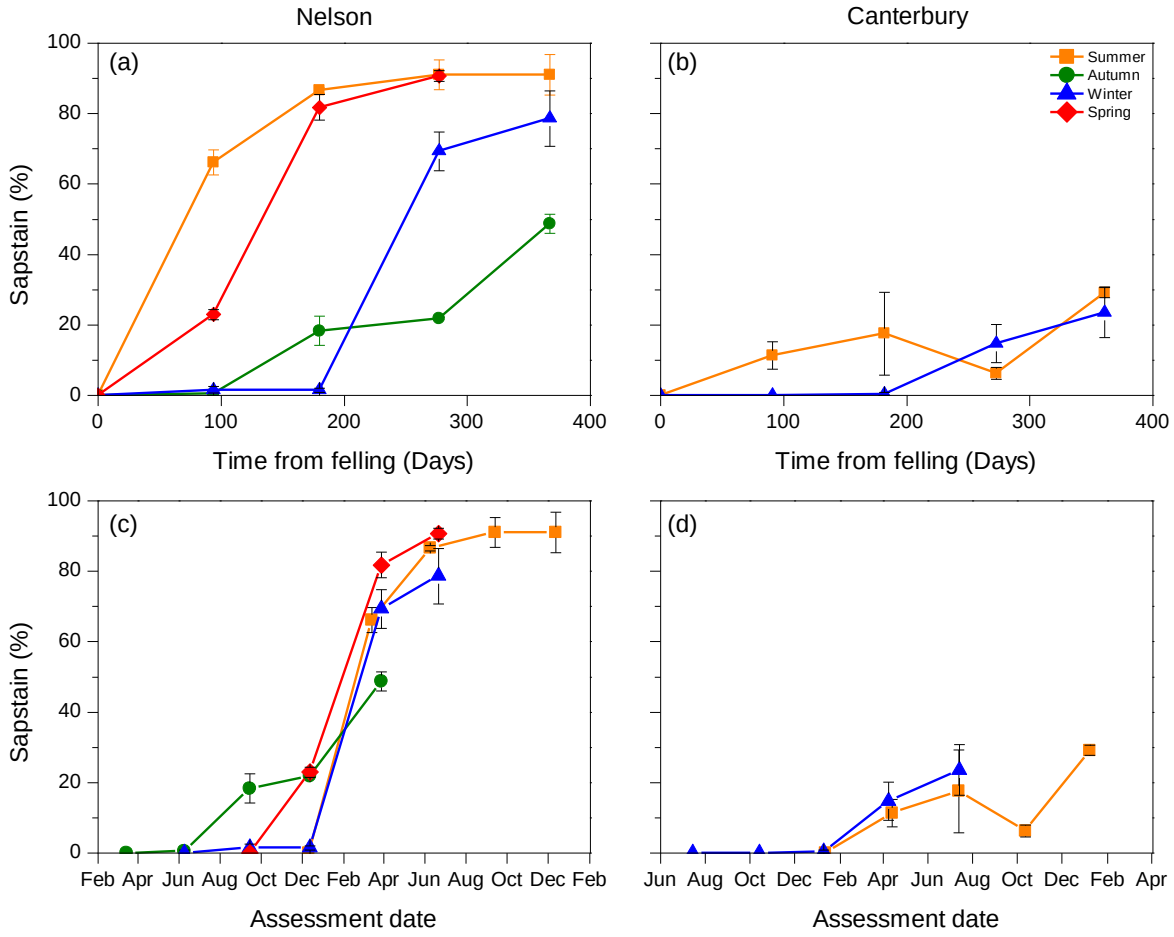


Figure 2.5. Percentage sapstain development over time for trees felled seasonally at Golden Downs and Canterbury. (a) & (b) percentage over time, and (c) & (d) date felled/assessed. Error bars show standard errors.

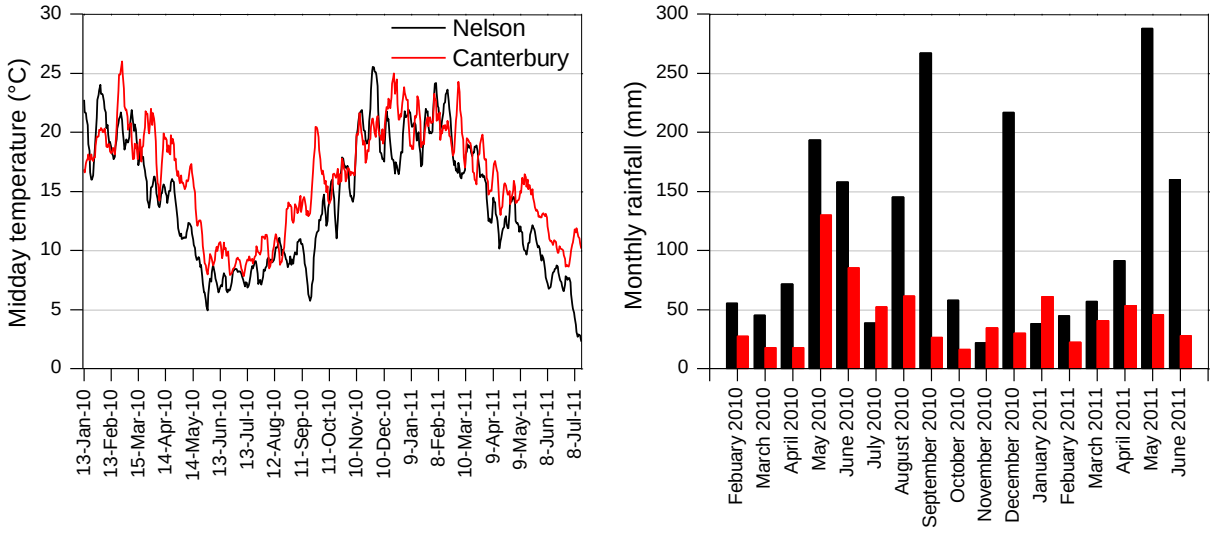


Figure 2.6. Daily temperature (at 1300 hrs) and rainfall data over the duration of the study, from weather stations located nearby in each study region. Data sourced from National Rural Fire Authority (Anon 2011a).

Table 2.6. Percentage sapstain in discs 2-5 assessed in March/April at two sites for various felling times. Values in each column followed by the same letter do not differ significantly (LSD test, $P = 0.05$).

Site	Felling time	Sapstain (%)	
Canterbury	Summer	11	a
	Winter	15	a
Nelson	Autumn	49	b
	Spring	82	c
	Summer	66	c
	Winter	69	c

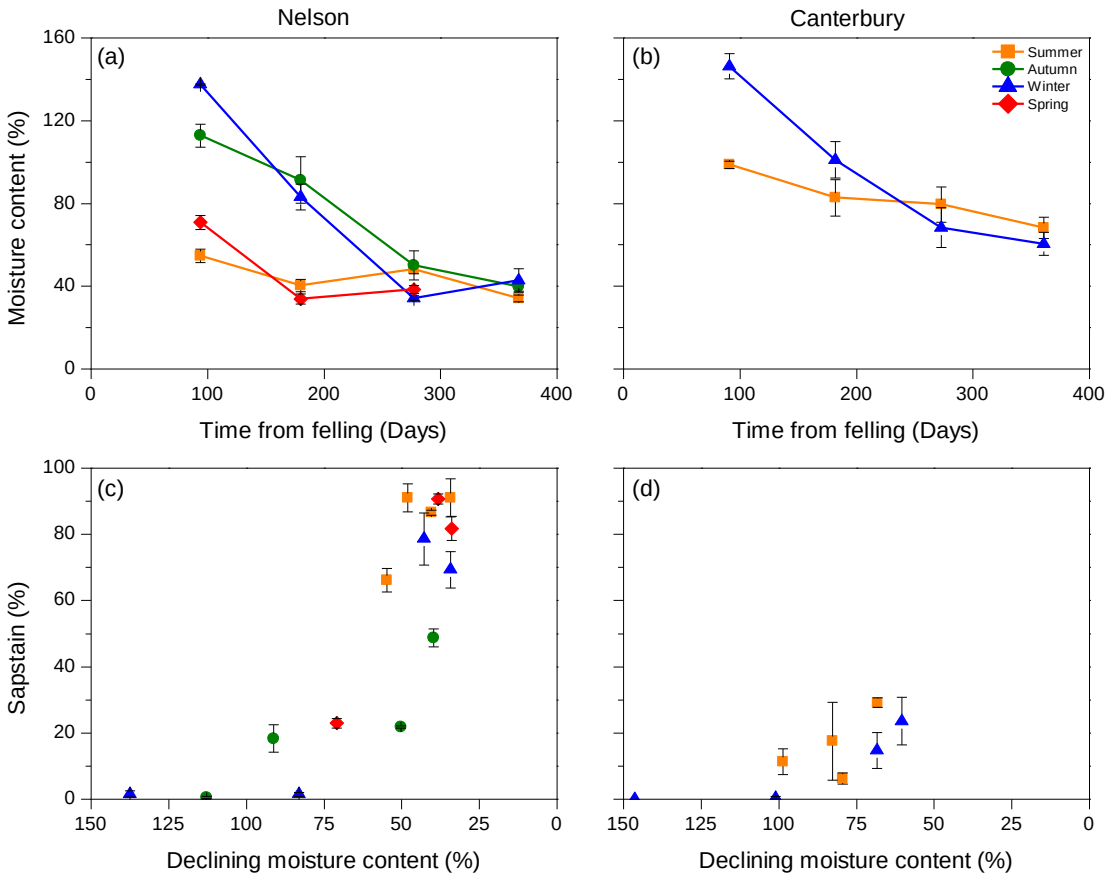


Figure 2.7. Loss of moisture content over time, depending on season of windthrow simulation, and region, and their effect on stain. Note, (c) and (d) have the x-axis reversed to display declining moisture content. Error bars show standard errors.

Figure 2.5 (c) and (d) which shows sapstain development against time-within-year. In the latter case it is clear that sapstain development was similar at the same season regardless of when during the previous year, the trees had been felled.

In all cases sapstain developed rapidly during summer, even in trees felled just previously. Development was much more rapid in Nelson than Canterbury. Splined calculations derived from interpolation calculations from met-stations allowed us to gain long term climate means for the precise locations of all sites. The mean annual temperature for the Nelson sites is 10.4°C, lower than that of Canterbury, at 12.2°C. Mean annual rainfall of 1352.2 mm was higher at Nelson than that of Canterbury which only reaches 564.5 mm (Anon 2011a). This overall trend is reflected in the data over the exact course of the study (Figure 2.6).

2.3.2 Moisture content effects on sapstain

The degree of association between sapstain and moisture content was tested, and it was shown that sapstain responds closely to, and can be predicted from, moisture content (Figure 2.7a,b). A relationship was present, where minimal sapstain was consistently found at above 100% moisture content, and stain developed rapidly once it had fallen below this level (Figure 2.7c,d, Table 2.7). Moisture content levels in both Nelson and Canterbury fell sharply following summer felling simulations, however they fell at a much faster rate in Nelson. After a simulated storm in winter, moisture content fell at a slow rate in both Nelson and Canterbury, however, after six months, by the beginning of summer, the moisture content levels matched those of summer felled trees.

Table 2.7. ANOVA table for percentage moisture content (log-transformed) testing the significance of the model terms. Fixed effects are listed, and a random effect for tree was included in each model. . Significance codes indicate *P*: < 0.001 "***", 0.001 – 0.01 "**", 0.1 – 0.05 "*", 0.05 - 0.1 "+", 0.1 "+".

Fixed effect	Numerator degrees of freedom	Denominator degrees of freedom	F Value	Pr > F	Significance
site	1	46	109.42	<.0001	***
season	3	46	41.18	<.0001	***
site * season	1	46	17.07	<.0001	***
sample_time	3	46	133.42	<.0001	***
site * sample_time	3	46	0.28	0.8368	
season * sample_time	8	46	22.73	<.0001	***
site * season * sample_time	3	46	4.88	0.005	**
disc_loc	4	1166	46.81	<.0001	***
site * disc_loc	4	1166	8.20	<.0001	***
season * disc_loc	12	1166	2.00	0.0213	*
site * season * disc_loc	4	1166	2.31	0.0559	+
disc_loc * sample_time	12	1166	8.64	<.0001	***
site * disc_loc * sample_time	12	1166	1.08	0.3774	
season * disc_loc * sample_time	32	1166	1.25	0.1578	
site*season * disc_loc * sample_time	12	1166	0.65	0.7977	

2.3.3 Bark beetle phenology and relationships with sapstain

A test of association between sapstain with presence of insect damage was made using a variable consisting of insect hole count in the stem included in an ANOVA as a covariate (Table 2.8). This showed there was no significant association between presence of insect damage and the level of sapstain at the tree scale ($P = 0.54$), after accounting for other experimental factors.

Seasonal abundance of beetle populations was monitored by trapping with funnel traps as an indicator of their ability to infest study trees. In Nelson, all beetle species, with the exception of *P. reticularis*, were trapped in large numbers during the summer months of January – March of 2010 (Figure 2.8). One bark beetle species, *H. ater*, displayed a second large peak in May and June. Numbers then fell for all species over the winter months before picking up again for the summer from December 2010 through to March 2011. This is with the exception of *P. peregrinus*, which was present during the summer of 2009/10, but not at all during the second summer.

Table 2.8. ANOVA table for percent sapstain (angular-transformed) performed at the tree-level and including insect hole count from the stem as a covariate. Fixed effects are listed, and a random effect for tree was included in each model. . Significance codes indicate *P*: < 0.001 "***", 0.001 – 0.01 "**", 0.1 – 0.05 "*", 0.05 - 0.1 "+", 0.1 "+".

Fixed effect	Denominator degrees of freedom	Numerator degrees of freedom	F Value	Pr > F	Significance
site	1	45	301.93	<.0001	***
season	3	45	109.43	<.0001	***
site * season	1	45	42.71	<.0001	***
sample_time	3	45	47.59	<.0001	***
site * sample_time	3	45	11.01	<.0001	***
season * sample_time	8	45	12.68	<.0001	***
site * season * sample_time	3	45	3.94	0.0140	*
insect presence	1	45	0.38	0.5413	

Phenologies of beetle species in Canterbury largely mirrored that of Nelson with clear summer peaks and winter troughs (Figure 2.8). The major differences being the complete absence of *P. peregrinus* in Canterbury, which was only found in Nelson, and noticeable double peaks of the bark beetles *H. ligniperda* and *H. ater*, and the longhorn beetle *A. ferus*, in October 2010 (spring) and March 2011(autumn), with a slight dip during summer. This suggests there were two generations of *H. ater* and *H. ligniperda* for that year during the summer months, with periods of low activity during the cold winter months.

The summer peaks of these species matched the period of extensive staining in both Nelson and Canterbury. It is likely that they did have some effect on stain during these high-flight periods, however the growth of wind and rain dispersed fungi were likely to be more important for stain development in this case.

2.3.4 Stain fungi

In this study, 20 isolations were attempted from two discs from each tree, on two different agar mediums (ophiostomatoid-selective, and non-ophiostomatoid-selective) for a total of 5760 isolation attempts. The most frequently isolated, and clearly dominant, stain fungus in this study was *D. pinea*, which was abundant in both regions. This species of stain fungus constituted 666 of the 691 (96.4%) positive isolations for stain fungi from Nelson, and 205 of the 215 (95.3%) from Canterbury. It was also present in all harvesting periods that yielded any discs with stain, and conversely was absent whenever all discs from a particular season had no stain at all (Table 2.9). Cultures from this species were recognised from their morphology, with a small subset verified with sequence analyses.

Isolates of other stain fungi included *Sporothrix inflata* (once in Nelson), *Ophiostoma nigrocarpum* (seven in Nelson, six in Canterbury), *O. sparsiannulatum* (once in Nelson), *O. pallidulum* (eight in Nelson, once in Canterbury), *O. radiaticola* (four in Nelson), *O. setosum*, *O. breviusculum*-like, *O. piliferum*, and *Hormonema dematioides* (all once in Nelson).

Table 2.9. Percentage of isolation attempts from each sampling time yielding the most common stain fungus, *Diplodia pinea*.

Months since windthrow	% isolations yielding *Diplodia pinea*											
	Summer			Autumn			Winter			Spring		
	Total%	Disc 1	Disc 4	Total%	Disc 1	Disc 4	Total%	Disc 1	Disc 4	Total%	Disc 1	Disc 4
(a) Nelson												
3	50.0 (55)[a]	25.5	74.5	40 (48)	66.7	33.3	0 (0)	0	0	49.2 (59)	52.5	47.5
6	66.7 (80)	26.3	73.7	8.3 (10)	10	90	5 (6)	16.7	18.3	56.7 (68)	30.9	69.1
9	36.7 (44)	34.1	65.9	17.5 (21)	4.8	95.2	46.7 (56)	16.1	83.9	65 (78)	37.2	62.8
12	45.0 (54)	24.1	75.9	33.3 (40)	27.5	72.5	39.2 (47)	4.3	95.7	-	-	-
(b) Canterbury												
3	41.7 (50)	50	50	-	-	-	4.2 (5)	100	0	-	-	-
6	35 (42	59.5	40.5	-	-	-	0 (0)	0	0	-	-	-
9	11.7 (14)	50	50	-	-	-	12.5 (15)	46.7	55.3	-	-	-
12	33.3 (30)[b]	33.3	66.7	-	-	-	23.3 (28)	10.7	89.3	-	-	-

[a] 110, and [b] 90 isolation attempts due to missing trees, all other isolation attempts total 120

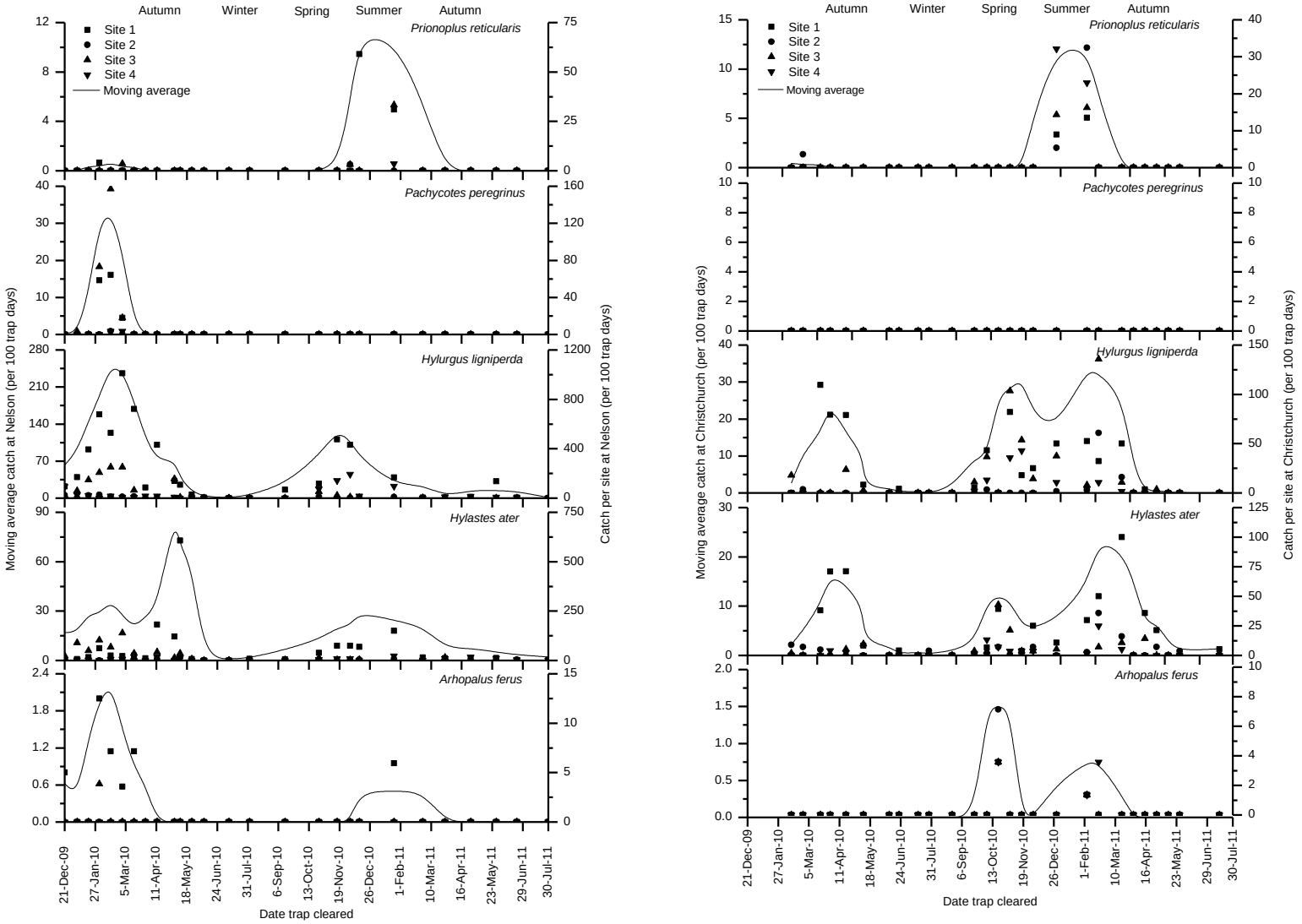


Figure 2.8. Phenology of *Prionoplus reticularis*, *Pachycotes peregrinus*, *Hylurgus ligniperda*, *Hylastes ater*, and *Arhopalus ferus* across four trial sites of simulated windthrow in Nelson (a), and Canterbury (b). Mean per trap collections at the individual sites (symbols) are plotted against a smoothed 3 week moving phenology 'average' of the same data. Note, no *P. peregrinus* were present at the Canterbury sites.

2.4 Discussion

This study of the effect of sapstain development following storms shows a substantial difference in development time and extent depending on where and when the storm occurred, with stain growing rapidly during warmer seasons and in warmer climates. Generally, this was due to a rapid loss of moisture content caused by heightened rates of desiccation in the warmer temperatures. There was no statistical effect of bark beetles and wood borers on sapstain development in this case, and this is backed up by the fungal species isolated from wood samples, where the overwhelming majority (~95%) of stain species were the non-insect vectored *Diplodia pinea*. This is likely due to low beetle colonisation of the cut trees that did not lie with much ground contact up the length of the stem – a factor that increases the colonisation rate of *H. ater* and *H. ligniperda* (Mausel et al. 2007).

This study shows that the time elapsed since windthrow is less important than the time within the year that the windthrow happened. The findings suggest that stain develops rapidly in trees felled during warm temperatures, however those felled in the cooler months are likely to remain largely stain free until the next summer.

2.4.1 Inter-disc differences

The disc taken from the base of the tree at the exposed cut face (disc 1) behaved atypically of the remaining discs taken from each tree, in both Nelson and Canterbury. The mean percentage of stain in both sites for this disc 180 days after felling was 20-30% (with a moisture content of 40-50%), whereas the remaining discs (discs 2-5) from Nelson were much higher than in Canterbury. It is likely that the cut face near disc 1 dries quickly after felling (largely regardless of the location or temperature), reaching the optimum moisture content for fungal growth (60-80%, Seifert 1993) but then rapidly falling below optimum levels of moisture content conducive to fungal growth, where continued fungal growth may again be inhibited. This resulted in higher sapstain being present in disc 1 compared to discs 2-5 at Canterbury (where stain was low overall), and decreased levels of sapstain in Nelson where stain is more prevalent. Disc 1 is

equivalent to the part of a fallen tree that would be assessed in a windthrown forest for stain cover before salvage harvesting begins, and therefore assessing this end of a log is unlikely to be a reliable representation of the log as a whole. The remaining four discs are more accurate representations of the regional sapstain prevalence overall.

2.4.2 Regional and seasonal effects

There was a clear distinction in sapstain onset between the two regions tested in this trial that could not be explained by the meteorological data alone. Both Nelson and Canterbury logs developed sapstain; however after 12 months, even the summer felled trees at Canterbury had an average of just 29.2% sapstain cover, with 11.3% after three months (90 days). Summer-felled trees from Nelson reached an average of 91.0% sapstain after 12 months, with 66.2% after three months – significantly more than Canterbury. This regional difference was also evident with the winter felled trees, however neither region began to show significant amounts of stain for six months, until the following spring/summer. The higher rainfall at Nelson could have been conducive to increased fungal dispersal by rain splash. Further research is needed to validate the importance of rain as a dispersal method for fungi.

In both regions there was an increase of stain over the summer months, regardless of when the storm occurred. When mean stain percentage was plotted against assessment date, it was clear that stain onset occurs in the summer months, regardless of when the storm occurred before then. If, in Nelson, there was a major storm event in summer it can be assumed salvage harvest would be required within a matter of days or weeks before wood quality was affected. This is consistent with results from winter felled trees in the same region near Nelson, where severed trees began to show signs of stain after 180 days, which also coincided with summer (McCarthy et al. 2010).

2.4.3 Moisture content

The moisture content levels described in the literature suggest that a water content of 100-120% is necessary to prevent sapstain in pine (Seifert 1993, Zeff 1999, Beal et al. 2010). This study

shows that trees felled in Nelson during summer and spring rapidly fall below this tolerance, to 55% and 71% respectively, within three months. When the moisture content levels of the wood were high, there were negligible levels of sapstain intensity. This trend was evident in both regions studied.

Stain generally began to increase when moisture content levels dropped below 100%, and would be extensive below 50%. After 1 year in Canterbury, there were no occasions where the average moisture content levels dropped this far, explaining the low levels of stain in this region. Unfortunately, the reasons behind this regional difference is unknown. Intuitively, one would expect the warmer and dryer region to accumulate sapstain at a greater rate; however this was not the case.

2.4.4 Bark beetle colonisation and phenology

Bark beetles are known to be vectors of some of the fungal species that cause sapstain (Paine et al. 1997, Reay et al. 2006a, Romon et al. 2007). These beetles have long been known as important dispersal agents for stain species (Leach et al. 1934), and can also cause physical damage to timber in their own right. Although there was evidence of bark beetle colonisation in this study, there was no apparent significant correlation between beetle presence and sapstain. Beetle attack was low overall with an average of 9.2 attacks per tree in Nelson (with a maximum of 45), and an average of 5.2 in Canterbury (with a maximum of 26). It is likely these beetles did vector stain fungi to the fallen trees, but their effect on sapstain as a whole was minimal compared to the stain caused by wind dispersed and endophytic fungi.

Funnel traps were placed at all sites to monitor bark beetle flight activity throughout the trial. The results are consistent with other studies where there is little activity during winter (McCarthy et al. 2010), with high numbers over the months of spring, summer and autumn (Reay and Walsh 2001, Mausel et al. 2007). There were large differences in numbers between the Nelson and Canterbury sites, with catches of *H. ater* and *H. ligniperda* at Canterbury 40% and 18% of that at Nelson, respectively. It is possible that the increased bark beetles numbers at Nelson contributed to the increased level of staining, however this is not supported by the

species of sapstain present, and further research would need to be completed on this aspect.

Both *H. ater* and *H. ligniperda* are known to preferentially colonise logs where there is ground contact, rather than logs elevated above the ground (Mausel et al. 2007). In windthrow situations such as those simulated here, trees are suspended above the ground by their branches, and only small portions of the tree are in contact with the ground. Only 8.6% of discs from Nelson were in any form of contact with the ground, with 12.7% in contact in Canterbury. It is likely that if the logs were in more direct ground-contact, then stain levels would be a larger issue due to additional stain fungal species introduced from the beetles . The only way to effectively address the role of bark beetles in stain development would be to experimentally exclude beetles in order to make direct comparisons between sapstain levels resulting from beetle vectored fungi versus spores dispersed by wind and rain (see Chapter 3).

2.4.5 Fungal species and dispersal

In both regions, the most commonly isolated stain fungus from sapwood in this study was *Diplodia pinea*. This species is a well known agent of sapstain of *P. radiata* (Uzunovic et al. 2004, Thwaites et al. 2005), and has previously been found as the primary staining fungus of windthrown pines in New Zealand (McCarthy et al. 2010). The dominance of this particular stain species is consistent with the weak correlation between bark beetle attack and stain distribution in this study. The ophiostomatoid fungi, another common group of stain fungi, are primarily dispersed by bark beetles (Gibbs 1993, Paine et al. 1997, Harrington 2005), whereas *D. pinea* is possibly a latent endophyte of healthy pines, often becoming prevalent when a tree is otherwise damaged or stressed (Smith et al. 1996, Flowers et al. 2001, Reay et al. 2006b).

Eight species of ophiostomatoid fungi were isolated from sapwood in this study, but were present in low numbers. This pattern differs from previous studies in Australia (Hood and Ramsden 1997, Wylie et al. 1999) where insect-vectored fungi were important after fire. Generally, sapstain becomes an issue during harvesting where substantial amounts of bark is dislodged by mechanical equipment removing a defence against sapstain spores and desiccation (Lee and Gibbs 1996, Uzunović et al. 1999a, Yang and Beauregard 2001, Uzunovic et al. 2004).

Compared to typical harvesting practices, very little bark was damaged or removed in this study, as is the case with windthrow in general.

2.4.6 General conclusions

Environmental variables need to be taken into account if the negative effect of sapstain is to be minimised or avoided during the salvage harvest of wind damaged trees. Damaged stands will deteriorate more rapidly if the damage occurs during the warm months of spring or summer, and should be salvaged within days. This may, however vary depending on the regional locality of the damage. Moisture content could be monitored within the central portions of logs (not the butt end) as an indicator of potential for stain development, where trees are safe as long as the moisture level stays above 100%. If unsure about the rate of onset in a particular area, adoption of a "better safe than sorry" regime should be implemented, and stands salvaged immediately. In New Zealand, the bark beetle species acting as vectors of sapstain fungi are not behaviourally adapted to colonisation of logs that are not in contact with the ground (Mausel et al. 2007). In other countries with aggressive bark beetles such as the mountain pine beetle (*Dendroctonus ponderosae*), ophiostomatoid fungi are likely to play a more important role in staining damaged trees.

Chapter 3 – Bark Beetles as Vectors of Sapstain Fungi

3.1 Introduction

Sapstain is the discolouration of wood caused by darkly pigmented fungal hyphae (Zimmerman et al. 1995, Eagen et al. 1997) growing through the sapwood and water conducting cells of susceptible trees and timber (Ballard et al. 1982). This reduces the cosmetic quality of the wood, and is of concern to forest managers world-wide. Sapstain fungi are generally aerobic species, and therefore staining generally occurs when sapwood moisture content has reduced to a point where the fungi are able to grow (Seifert 1993, Bale et al. 2002). As a result, cut trees and milled timber can be at risk within as little as five days after cutting, given optimal conditions for fungal growth which for most species are typically temperatures of around 20-24°C, and wood moisture content of 45-135% of dry weight (Seifert 1993, Zeff 1999, Beal et al. 2010). Ophiostomatoid fungi are the major stain-causing species in many parts of the world (Gibbs 1993, Uzunović et al. 1999b). Although many species can be pathogenic to trees, most are not particularly virulent, with the exception of those that cause Dutch elm disease and black stain root disease (Harrington 1993, Harrington et al. 2001). Instead, sapstain colonisation is primarily a problem in dead or damaged trees, in particular because many ophiostomatoid fungi, such as species of *Ophiostoma*, *Ceratocyctis*, *Grosmannia*, and their asexual anamorphs, are vectored by bark beetles.

A large body of literature has identified and described the associations between bark beetles and ophiostomatoid fungi, both in New Zealand (Reay et al. 2002, Reay et al. 2005, 2006a), and in other regions of the world (Zhou et al. 2004a, Chung et al. 2006, Romon et al. 2007, Lu et al. 2010, Kim et al. 2011). The intensity of sapstain in dead and damaged trees has been shown to be higher with the presence of ophiostomatoid-vectoring bark beetles, and stain was rarely extensive when beetles were not present (Leach et al. 1934). The complex of sapstain-causing species includes the ophiostomatoid fungi as well as non-ophiostomatoid fungi such as darkly-pigmented yeasts and moulds that are not typically thought to be vectored by insects (Seifert

1993). For example, recent studies have suggested that fungal species such as *Diplodia pinea* (syn. *Sphaeropsis sapinea*) can be among the most important stain-causing fungi in some systems (Farrell et al. 1997, Thwaites et al. 2005, McCarthy et al. 2010). There is only limited evidence linking *D. pinea* with transport by bark beetles (Wingfield and Knox-Davies 1980, Romon et al. 2007, Whitehill et al. 2007), and it is thought that the main method of dispersal of this fungal species is abiotic (e.g. wind and rain splash), and therefore independent of insects (Bihon et al. 2011).

From an applied perspective, there is a growing concern of the uncertainty regarding timeframes available to salvage harvest following storm- or fire-damage of production forests (Hood and Ramsden 1997, McCarthy et al. 2010) before sapstain reduces their value. The interactions between environmental conditions, the composition of the complex of sapstain-causing species, and the potential for rapid colonisation of damaged timber by beetle-vectored fungi are all thought to be important in harvest management decisions. This is the first study quantitatively measuring the effect of bark beetle vectored fungi in comparison with those dispersed through wind and rain-splash alone.

Increases in the frequency of extreme weather events, causing severe windthrow (McCarthy et al. 2010) and promoting bark beetle outbreaks (Cudmore et al. 2010), are predicted to alter the "disease triangle" between three crucial factors affecting host-plant damage – plant pathogen, plant host, and environmental change (Grulke 2011, Hulcr and Dunn 2011). In the case of sapstain, this complexity is exacerbated by significant variation in a fourth factor, pathogen-vector dynamics, driven by variation in bark beetle abundance and propensity to attack susceptible trees under differing environmental conditions. Although sapstain colonisation of wind-thrown trees is a secondary cause of tree damage, the complexities of vector-pathogen and pathogen-host relationships are nevertheless central to improved understanding of the onset and development of tree damage. The key problem with predicting and managing sapstain attack on windthrown timber is that there is only a general qualititative understanding of the major drivers of sapstain onset and development, such as the fact that bark

beetles can vector ophiostomatoid stain fungi, and stain development is related to environmental conditions. At present, there is little understanding of the quantitative relationship between the activity of beetle vectors and the eventual outcome of stain development within a colonised log. This is the first study to quantitatively measure the effect of bark beetle vectored fungi alone in comparison with those dispersed through wind and rain-splash.

Very few studies have attempted to directly test the degree to which variation in bark beetle abundance, distribution and attack rates determines the extent and distribution of sapstain. There have been cases of wind, snow, and fire damage to valuable forests where the role and importance of bark beetle as vectors of stain causing fungi were, and still are, unknown (Hood and Ramsden 1997, Wylie et al. 1999, McCarthy et al. 2010). Knowledge of the role of bark beetles, and its relative importance, to the spread and extent of sapstain in damaged logs would provide decision-makers with tools to act appropriately in the face of insect attack on their damaged resource. There is clearly a need for an increased understanding of vector-pathogen dynamics and the role of bark beetles in the onset of sapstain.

This study aims to test whether colonisation by bark beetles increases the extent of sapstain in logs, and to compare this with stain caused by fungi that disperse via wind and rain-splash. I experimentally excluded the abundant non-native bark beetles *Hylurgus ligniperda* and *Hylastes ater* from experimental log billets in New Zealand *Pinus radiata* plantations. This was to test whether stain is influenced predominantly by bark beetle vectoring, or whether its distribution is determined independently of bark beetles by physical processes of fungal spore dispersal, such as wind or rain splash, or by growth of endophytic fungi already present in the logs. Both abundant bark beetle species were accidentally introduced to New Zealand (Brockerhoff et al. 2003, Brockerhoff et al. 2006a), and both are known to be vectors of sapstain fungi that attack *P. radiata* timber in New Zealand (Reay et al. 2001, Reay et al. 2005, 2006a, Romon et al. 2007, Kim et al. 2011).

3.2 Materials and methods

3.2.1 Site selection and experimental setup

To test the role of bark beetle attack in sapstain development in cut logs, 10 un-caged and 10 caged logs (ca 0.5 m in length) were placed at each of two sites in the Nelson region of the South Island, New Zealand. The 40 logs (plus an additional 6 logs of the same size that were used to monitor sapstain development at regular intervals) were cut from four 7 year-old *Pinus radiata* trees on the day the experiment was established, on the 25th of January 2011. Healthy trees of a suitable size were selected, felled with a chainsaw, de-limbed and cut into lengths. The 46 logs were checked to ensure there was no inadvertent damage to the bark, and rapidly placed under cover to prevent insect attack prior to caging. The period of time between cutting the logs and setting up the experiment did not exceed one day, and care was taken not to damage the logs in transit.

The two sites were selected in second-rotation *P. radiata* forests that had been harvested within the six months leading up to January 2011. Recently-harvested sites were selected to ensure sufficient bark beetle activity was present to provide high colonisation rates of un-caged logs. Both sites were flat, un-shaded, and not flood-prone so that pooling of water did not affect the progression of beetle colonisation and stain within the logs.

Caged logs were protected from bark beetle attack with 1.8 × 1.4 mm aluminium mesh that was small enough to exclude the common pine-infesting bark beetles that are present in New Zealand. It is known that there are many smaller insects, other than bark beetles, that colonise logs, and whose larvae feed within and under the bark layer, but excluding them in this study was unrealistic as mesh small enough to create a barrier to them would probably have altered the microclimate within the cage. This was not thought to be an issue in terms of fungal colonisation in this case as I was investigating the effect of bark beetles as vectors of fungi, and the successional colonisation of timber by saproxylic invertebrates is known to occur, independent of the presence of bark beetles. However, it is possible that feeding and tunnelling

activities of other insects might affect fungal growth and distribution within the log, following colonisation. Therefore, evidence of attack by insects other than bark beetles was recorded and considered in the analyses.

Care was taken to ensure that no part of the upper surfaces of the caged logs was in contact with the mesh. As *H. ater* and *H. ligniperda* are known to preferentially colonise logs that are in contact with the ground (Mausel et al. 2007), the full length of every log was in full contact with the ground along its entire length. At each site, the 10 un-caged and 10 caged logs were placed at random points in a 4.5 x 6 m grid pattern, all facing north, separated by an equal distance of 1.5 m from any other log. In addition, three of the sapstain monitoring logs were placed 1.5 m away from the experimental logs at each of the two sites. One of these three logs was cut open every two weeks, at each site, to monitor beetle colonisation and sapstain growth, so that the most suitable timing of sampling of the experimental logs could be estimated.

3.2.2 Log processing

The logs were left out for a total of 34 days during the warmest months of the Southern Hemisphere summer, when the prevalence of fungal staining is at its height (Zeff 1999). The logs were collected when approximately half of each cut face in the monitoring logs was covered in stain. Each experimental log was removed from its enclosure, where necessary, and placed in sealed plastic bags, with the upper surface of the log precisely marked so that it was known which surface was in contact with the ground. To limit post-collection stain growth, logs were kept as cool as possible until processing, which was completed within four days of collection.

Measurements were taken of log length, and circumference and bark thickness at the ends and middle of the log. Each log was delineated into eight sections along the length of the log, and within each section, six radial segments were delineated, as illustrated in Figure 3.1. The two end sections (1 and 8) were thinner than other sections in order to measure the amount of stain and beetle attack in the immediate vicinity of the exposed cut ends. The remaining six sections were divided equally into the remaining length of the log. As each log was around 0.5

m long, sections 2 – 7 were typically about 75 mm thick, but due to slight inconsistencies in log length and saw-cut width, section thickness varied by up to 5 mm. Within each section, the radial segments closest to the ground had finer divisions to increase the resolution of sapstain and beetle attack measurements where colonisation was expected to be greatest.

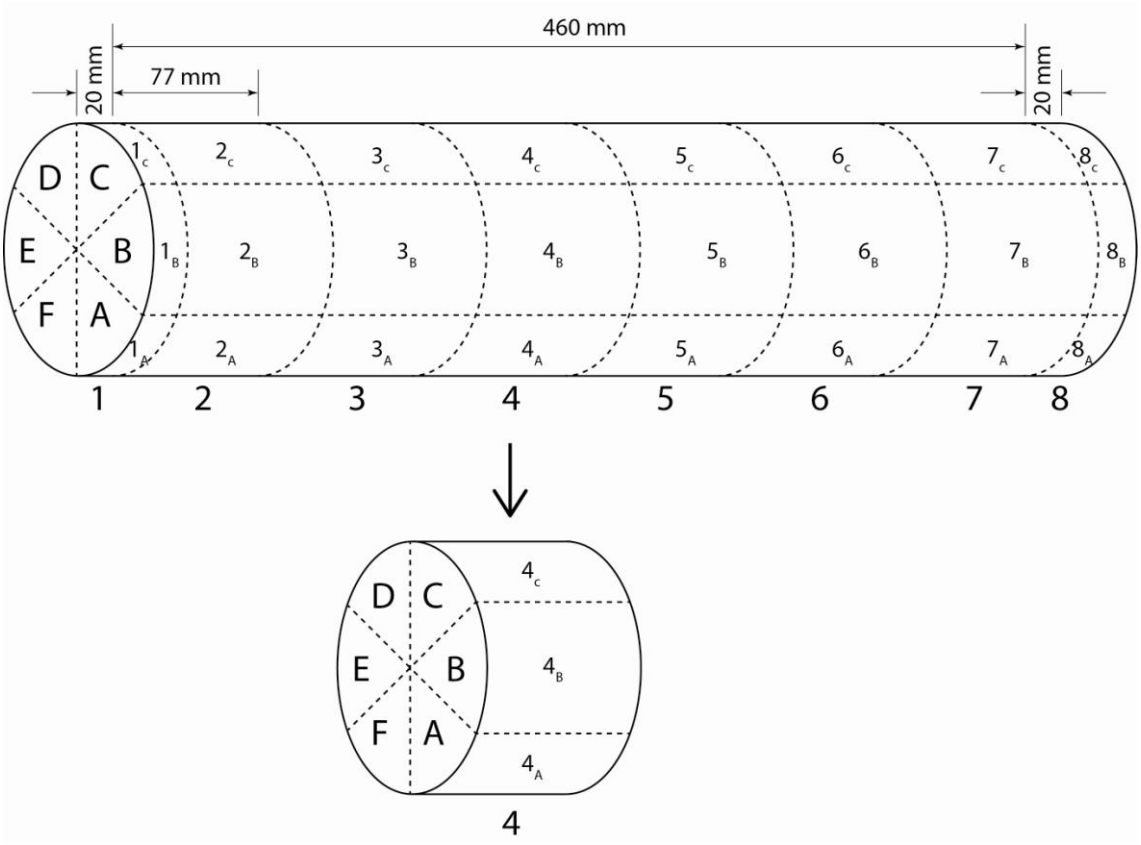


Figure 3.1. Schematic illustration of sampling areas on the experimental logs. Numbers 1 to 8 indicate log sections (cuts are the dashed lines dividing the sections), and letters A to F indicate log segments within each section. The two outer sections, where stain penetration was expected to be greatest, were cut 20 mm thick, and the remaining length of log was divided into six equal-sized sections (ideally 77 mm, but varying from 75 – 79 mm, depending on variability between individual logs and individual section-cuts). The entire outer surface of the log was divided into discrete areas (1_A to 8_F) where insect attack was measured.

Logs were processed by first making a visual inspection of external insect attack in the external sectors indicated in Figure 1. To do this, the bark was carefully stripped away and bark beetles, their larvae, and their galleries were identified and counted within each sector. Insect larvae and galleries that were not those of bark beetles were also counted, but not identified further. Following this, the logs were cut into the eight delineated sections, and a photograph was taken of each cut face. Care was taken to ensure that the segments were aligned in their correct vertical orientation in the photo, allowing accurate placement of a digital grid over each image when making stain measurements. Note, that as there were eight sections this results in seven surfaces where stain was measured, these were between sections 1 & 2, 2 & 3, and so on, through to the cut face of segments 7 & 8. Only one image was required for each cut from one of the cut faces as the amount of stain on each face is identical to the other. The measurement points were then referred to as 'section cuts', each with one section on either side.

In order to measure the proportion stain in each segment, the entire image was imported into Adobe Photoshop® (CS4 Extended, Version 11.0) and a radial grid was overlaid on the image, as illustrated in Figure 2. Each segment was extracted and saved as a single-coloured (black) bitmap image (one image file per segment) to determine total segment area. This process was then repeated for each segment, but this time drawing around stain-covered areas only. Image J (version 1.43u) was then used to calculate the number of pixels in the stain-covered area versus the total segment area (Figure 2).

3.2.3 Data analysis

The effect of bark beetle exclusion on variation in proportion sapstain cover was tested using a generalised linear mixed effects model (GLMM) with fixed categorical factors for the caging treatment, and log segment nested within log section, as well as random factors specified for site replicate and log replicate. The sapstain model was tested as a GLMM with binomial errors using the lme4 package in the R programming environment (Bates et al. 2011). If overdispersion was evident in the fitting of the GLMM, then this was controlled for using a

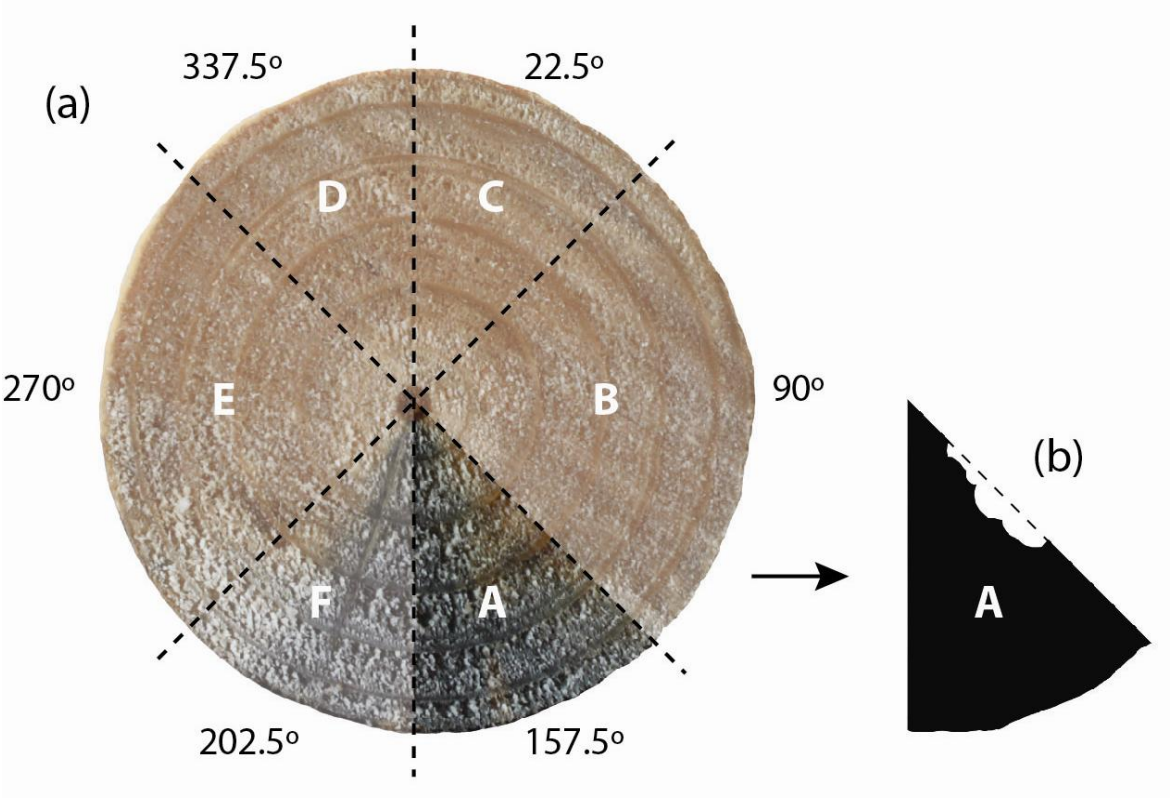


Figure 3.2. An example of the stain percentage calculation, showing (a) the entire face of one log section divided into six segments (A – F), and (b) a single segment extracted and digitally converted to a bitmap of relative colour intensity, in order to determine percent stain coverage (in this case, 94.4% of a 214151.04 pixel segment).

model with Poisson lognormal error structure (Elston et al. 2001). Model simplification was performed using an information theoretic approach with AICc (Akaike Information Criterion corrected for small sample size) and akaike weight (W_m) to rank and subsequently select the best model describing the data, as recommended by Burnham & Anderson (2002).

To assess the effect of the caging treatment, evidence of insect attack was separated into a binary 'beetle evidence' dataset (evidence = 1, no evidence = 0), where bark beetle evidence constitutes the presence of adults, larvae, or galleries, and a binary 'other insect evidence' dataset, where the evidence of insects other than bark beetles constitutes the presence of the larvae themselves or their galleries. For each sapstain measurement, corresponding measures of beetle evidence and other insect evidence were recorded from the two adjacent segments (combined) on either side of the cut-face (e.g. 2_B and 3_B, Figure 3.1).

Finally, to test the relative contribution of bark beetle attack and other-insect attack in explaining the distribution of sapstain within caged and un-caged logs, the sapstain GLMM was repeated with 'beetle evidence' and 'other insect evidence' entered as covariate predictors ahead of the fixed caging, section and segment variables in the model. Models were compared and selected using AICc and W_m values, as above. If the spatial distribution of insect attack was sufficient in its own right to explain the spatial distribution of sapstain then the covariate would subsume all the variance attributable to the fixed factors in the model. All statistical analyses were performed using R 2.13.1 software (R Development Core Team, 2011).

3.3 Results

3.3.1 Spatial distribution of sapstain

The amount of sapstain cover varied from as low as 0% in the central section cuts and upper segments of the log, to as high as 100% in the terminal cuts and lower segments of the log nearest to the ground (Figure 3.3a,b). Evidence of sapstain fungal colonisation was evident to some degree in all logs in the study, whether they were un-caged or caged. However, on average the un-caged logs had a noticeably greater occurrence of stain along the lower segments of the log which were in contact with the ground (Figure 3.3a), whereas caged logs typically only had intense staining near the terminal (cut) ends of these logs (Figure 3.3b). Surprisingly in the Poisson lognormal GLMM analyses, this apparent difference in total stain between caging treatments appeared not to be significant ($P = 0.08$), and instead the best-supported model showed significant effects of caging and segment location (i.e. the distribution of stain within the log, depending on whether it was caged or not), as well as their associated interaction, on the proportion cover of stain per segment (Table 3.1a). The caging by segment interaction effect was driven by higher levels of stain at the bottom of the un-caged logs than that of the caged logs (Figure 3.4a,b).

3.3.2 Spatial distribution of insect colonisation

Insect attack was evident among both caged and un-caged logs, at both sites. While most insect attack on caged logs was by species other than bark beetles (only 2 out of 20 caged logs had no evidence of insect attack at all), bark beetles did penetrate some cages. The total number of bark beetles recorded in all of the caged logs was 26, in comparison to 420 in the un-caged logs, suggesting a 93.8% exclusion of colonising bark beetles. In the GLMM analysis, there was a significantly greater level of bark beetle attack in the un-caged logs than in the caged logs, and this attack was concentrated in the area of the logs in contact with the ground (Figure 3.3c,d, Table 3.2b). The attack on the caged logs, from the few beetles that did penetrate, was sporadic

Table 3.1. Results of model selection assessing all combinations of fixed factors using AICc values of the GLMMs. (a) stain as a response variable GLMMs with a Poisson lognormal error structure to account for overdispersion in the binomial stain model, (b) evidence of beetle attack as a response in GLMMs with binomial error structure, and (c) GLMMs testing whether the fixed covariate effects of beetle evidence and other-insect evidence explain the distribution of stain within and among logs, with Poisson lognormal error structure. All models included site replicate and log replicate as random factors. Models within 2 ΔAICc units of the best model (ΔAICc = 0) were considered to have equivalent explanatory power, as indicated in bold. All models also included the random effects of site replicate and log replicate.

	Model	AICc	ΔAICc	W_m	Rank
(a) Stain	**cage * segment**	6973.9	0.0	1.0	1
– Poisson lognormal	Segment	7110.9	137.0	0.0	2
	cage	7671.4	697.5	0.0	3
	cage + section	7671.4	697.5	0.0	4
	section	7474.0	700.1	0.0	5
	cage * section	7676.0	702.1	0.0	6
	cage * section/segment	8257.3	1283.4	0.0	7
	section/segment	8334.6	1360.7	0.0	8
	cage + section/segment	8341.8	1367.9	0.0	9
	cage + segment	40937.9	33960.4	0.0	10
(b) Beetle	**cage * segment**	861.7	0.0	1.0	1
– binomial	cage + segment	912.2	50.5	0.0	2
	segment	946.5	84.8	0.0	3
	cage + section/segment	1047.6	185.8	0.0	4
	section/segment	1080.7	219.0	0.0	5
	cage * section/segment	1449.5	587.8	0.0	6
	cage	1496.9	635.2	0.0	7
	cage + section	1499.7	638.0	0.0	8
	cage * section	1501.4	639.7	0.0	9
	section	1531.0	669.3	0.0	10
(c) Stain	**beetle + insect + cage * segment**	6973.5	0.0	0.6	1
(with beetle predictor)	**beetle + insect + segment**	6974.2	0.7	0.4	2
– Poisson lognormal	beetle + insect + cage + segment	6979.4	5.9	0.0	3
	beetle + cage + segment	7054.6	81.1	0.0	4
	beetle + cage * segment	7057.8	84.3	0.0	5

insect	7183.7	210.1	0.0	6
beetle + insect	7215.1	241.6	0.0	7
beetle + insect + cage	7254.5	281.0	0.0	8
beetle	7356.3	382.8	0.0	9
beetle * cage	7377.1	403.6	0.0	10
beetle * segment	122846.0	115872.5	0.0	11
beetle + cage	207836.0	200862.5	0.0	12

but generally also concentrated to the lower segments of logs (Figure 3.3d). In the un-caged logs there was a much greater intensity of beetle colonisation on the lower surfaces, than on the upper, whereas in the caged logs the colonisation rates were similar on the bottom and the sides – probably due to the small number of beetles that got in (Table 3.2b, Figure 3.4b)

3.3.3 Insect attack driving sapstain distribution

Spatial patterns of log attack by bark beetles and other insects corresponded strongly with sapstain distribution and intensity (Figure 3.3). A GLMM analysis of stain distribution showed that when attack by beetles and attack by other insects were entered first into the model, ahead of caging and segment effects, there was still a highly significant effect of segment position (i.e., the radial angle of segments) on stain intensity, but only weak and equivocal remaining influence of the caging effect (Figure 3.3, Table 3.1c, Table 3.2c,d). This suggests that the caging effect on stain intensity is predominantly driven by reduction in beetle and other insect attack (despite the fact that some beetles did penetrate cages), but that the beetle and other insect evidence is not sufficient in its own right to explain variation in stain intensity among segments within logs (Table 3.1c).

Table 3.2. Co-efficients output from selected models showing significance values for all factors. (a) stain as a response variable in a GLMM with a Poisson lognormal error structure, (b) evidence of bark beetles as a response in a GLMM with binomial error structure, (c) stain as a response variable in a GLMM with beetle, insect, cage, segment, and a cage * segment interaction as factors with a Poisson lognormal error structure, and (d) stain as a response variable in a GLMM with beetle, insect and segment as factors with a Poisson lognormal error structure. Significance codes indicate *P*: < 0.001 "***", 0.001 – 0.01 "**", 0.01 – 0.05 "*", 0.05 - 0.1 "+"

	Fixed factors	Estimate	Standard error	Z value	*P*	Significance
(a) Stain – Poisson lognormal	intercept	0.885	0.449	1.97	0.049	*
	Cage	0.683	0.394	-1.73	0.083	+
	segmentB	-2.420	0.032	-74.54	< 0.001	***
	segmentC	-3.931	0.041	-96.57	< 0.001	***
	segmentD	-3.820	0.040	-96.09	< 0.001	***
	segmentE	-1.921	0.031	-61.45	< 0.001	***
	segmentF	0.046	0.031	1.47	0.141	
	cage:segmentB	1.208	0.043	27.99	< 0.001	***
	cage:segmentC	1.794	0.051	34.86	< 0.001	***
	cage:segmentD	2.319	0.049	47.05	< 0.001	***
	cage:segmentE	1.440	0.042	34.56	< 0.001	***
	cage:segmentF	0.435	0.042	10.40	< 0.001	***
(b) Beetle – binomial	intercept	2.899	0.619	4.68	< 0.001	***
	cage	-6.568	0.767	-8.55	< 0.001	***
	segmentB	-5.389	0.484	-11.14	< 0.001	***
	segmentC	-6.338	0.567	-11.17	< 0.001	***
	segmentD	-5.941	0.525	-11.33	< 0.001	***
	segmentE	-4.759	0.454	-10.48	< 0.001	***
	segmentF	-0.833	0.428	-1.94	0.051	+
	cage:segmentB	3.738	0.789	4.74	< 0.001	***
	cage:segmentC	-11.222	1391.715	-0.01	0.994	
	cage:segmentD	4.292	0.814	5.27	< 0.001	***
	cage:segmentE	4.443	0.646	6.87	< 0.001	***
	cage:segmentF	1.962	0.594	3.29	< 0.001	***
(c) Stain (beetle & insect predictors)	intercept	-0.0956	0.874	-0.11	0.913	
– Poisson lognormal	beetle	0.83	0.422	1.97	0.049	*
	insect	1.9341	0.343	5.64	< 0.001	***
	cage	-0.3364	0.827	-0.41	0.684	

	segmentB	-1.4707	0.572	-2.58	0.010	**
	segmentC	-3.5386	0.627	-5.65	< 0.001	***
	segmentD	-3.1295	0.610	-5.13	< 0.001	***
	segmentE	-0.7504	0.546	-1.38	0.169	
	segmentF	0.816	0.479	1.70	0.088	+
	cage:segmentB	0.49	0.725	0.68	0.499	
	cage:segmentC	0.686	0.778	0.88	0.378	
	cage:segmentD	1.3868	0.759	1.87	0.068	+
	cage:segmentE	0.8619	0.714	1.21	0.227	
	cage:segmentF	0.4642	0.677	0.69	0.493	
(d) Stain (beetle & insect predictors)	intercept	-0.3212	0.737	-0.44	0.663	
– Poisson lognormal	beetle	0.8858	0.379	2.34	0.019	*
	insect	1.9104	0.347	5.51	< 0.001	***
	segmentB	-1.2379	0.393	-3.15	0.002	**
	segmentC	-3.2982	0.434	-7.60	< 0.001	***
	segmentD	-2.4861	0.420	-5.92	< 0.001	***
	segmentE	-0.3488	0.369	-0.95	0.367	
	segmentF	0.8377	0.345	2.43	0.015	*

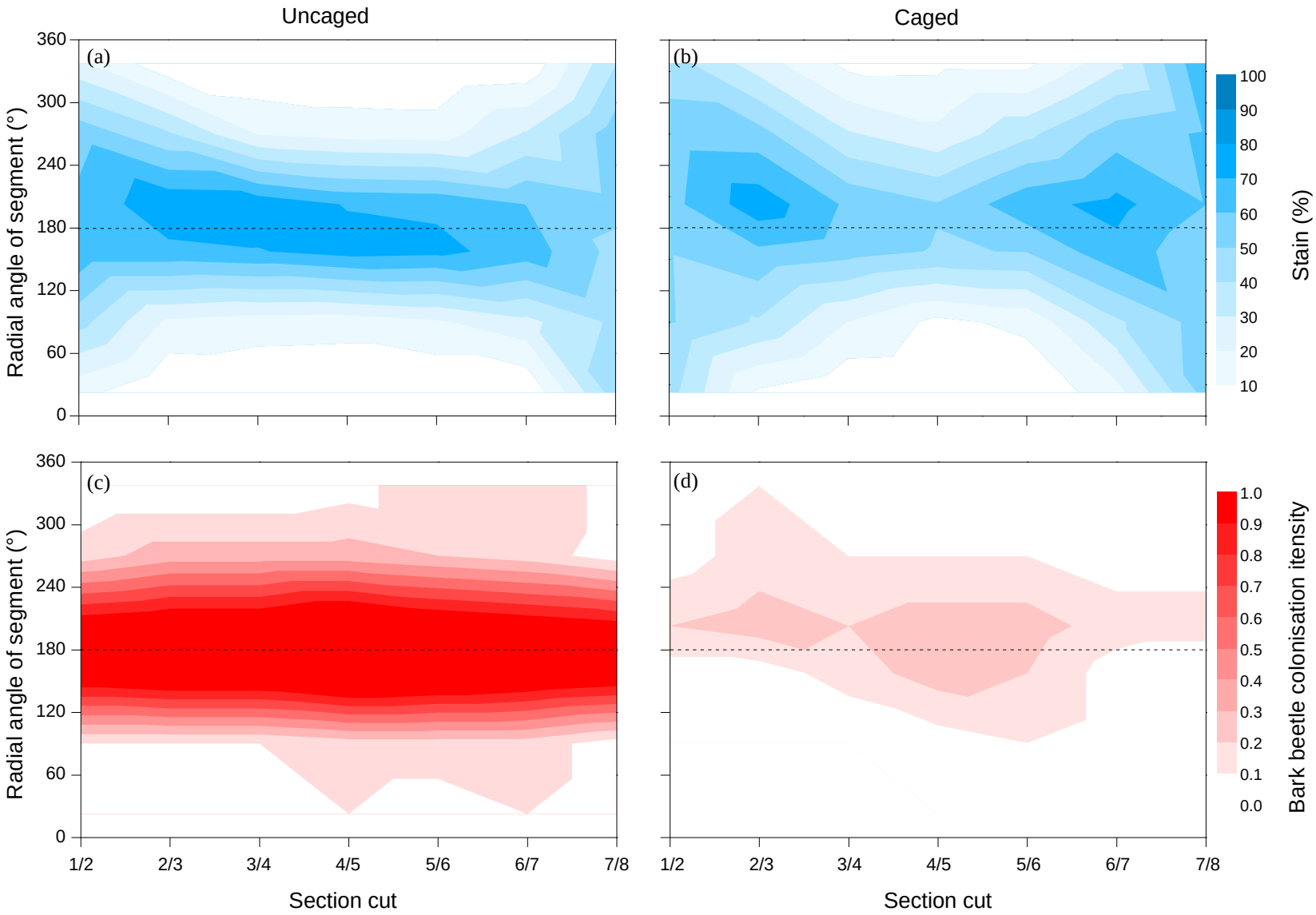


Figure 3.3. Comparison of average stain intensity for (a) un-caged and (b) caged logs, as well as the corresponding bark beetle attack intensity for the same (c) un-caged and (d) caged logs (average of n = 20 experimental logs in each case). The radial angle of the segment indicates the spatial orientation of the log, with 180° representing the point of ground contact, as indicated by the dashed line. Bark beetle attack intensity is measured as the frequency of attack across logs.

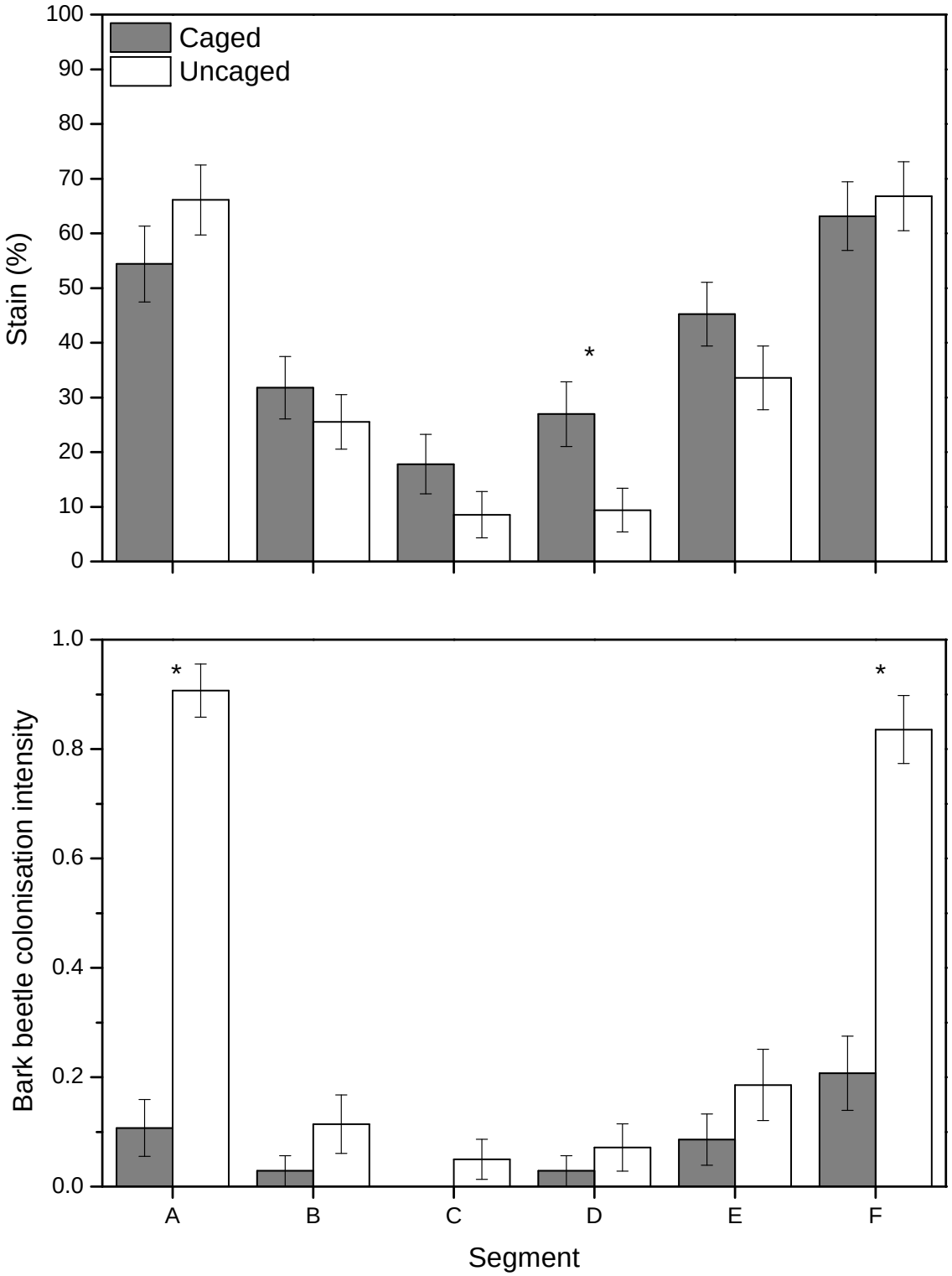


Figure 3.4. Mean (a) stain percentage and (b) beetle colonisation intensity for all segments within caged and un-caged logs. Error bars are +/- 95% confidence intervals around the mean.

3.4 Discussion

Sapstain was present in all logs in this study, however beetle attack was an important factor in the colonisation of logs by fungi. My experimental design used a caging treatment to exclude beetle colonisation while keeping other site-level and seasonal environmental factors constant. Although the cages were not entirely effective in preventing beetle attack, there was a strong correspondence between the spatial distributions of bark beetle attack and areas of high sapstain intensity within and between logs. This clearly indicates that bark beetle attack is an important factor influencing sapstain colonisation. However, it is important to note that sapstain was found on all experimental cut logs, regardless of whether they were un-caged or caged, indicating that sapstain colonisation can occur without insect vectors, although the intensity of sapstain was clearly more pronounced in the central section of un-caged cut logs that were accessible to bark beetles and other insects.

This confirms that vectors have a quantitative contributing effect on sapstain distribution and intensity, but that they are not essential for the occurrence of sapstain colonisation. Furthermore, the feeding and tunnelling activity of other saproxylic insects and their larvae also appeared to contribute to the onset and extent of stain within timber (see also Suckling et al. 1999, Uzunović et al. 1999a). Consequently, although bark beetles do appear to be instrumental in the colonisation and spread of sapstain to damaged timber (Reay et al. 2006a, Romon et al. 2007), it seems that endophytic or wind blown fungal spores and the distribution of insect activity within fallen timber, also play an important role in sapstain. I discuss the implications of these findings for the forest industry to aid in the protection of both logs already harvested, and for the effective salvage-harvest of trees damaged by stochastic events such as fire and storm damage.

3.4.1 Stain and beetle attack

There was a low level of stain in the upper segments of all logs, especially toward the middle of the log where the sapwood is protected by the bark, and beetles hadn't attacked. If left in the elements for longer, these sections would presumably have also become stained as the 'clean'

timber succumbs to stain fungi growing from the sites of beetle attack, and from the exposed ends over time. In the un-caged logs, the greater intensity of staining was along the lower surface of the log, where the log was in contact with the ground (Figure 3.3). This is mirrored almost exactly by the pattern of bark beetle attack, in this case by *Hylastes ater* and *Hylurgus ligniperda*. As these species are known to feed preferentially on roots, and other parts of trees that are in ground contact, they are behaviourally adapted to preferentially attack logs where they are in contact with the ground (Mausel et al. 2007). There was evidence of staining in the upper sections of logs, but only at the cut ends where the sapwood was exposed allowing a faster rate of desiccation and exposure to wind-blown spores (Figure 3.3).

While the cages were not entirely effective the spatial distribution of stain within a caged log was significantly different to that of an un-caged log. The stain within a caged log is concentrated at the exposed ends where wind and rain-splashed sources of inoculum can enter the unprotected sapwood, and where the sapwood also dries faster. This stain was dramatically reduced where protection from the bark layer along the middle of the logs length prevents the available points of entry for fungal spores (Figure 3.3). The reduction of stain intensity through the lower sections of the middle of the log is explained by the reduction of bark beetle colonisation in this treatment.

Bark beetles were an important factor in predicting and influencing the distribution of stain in this study. Of the stain fungi vectored by bark beetles, ophiostomatoid fungi are the primary concern to foresters world-wide for both forest health (pathogenic) and economic (stain) reasons (Gibbs and Inman 1991, Paine et al. 1997, Farrell et al. 2001, Harrington 2005). Although bark beetles play an important role in vectoring sapstain fungi to its host, it is not exclusively a beetle-mediated phenomenon. The stain fungus and plant pathogen *Diplodia pinea* is considered the most common stain fungus in New Zealand (Farrell et al. 1997, Thwaites et al. 2005, McCarthy et al. 2010). This species has large spores upwards of 30 µm in length (de Wet et al. 2000), much larger than those of ophiostomatoid fungi which are generally around 5 µm (Jacobs and Wingfield 2001). The relatively small spore size of ophiostomatoid fungi makes insect

dispersal more successful as these are produced in abundance and adhere to an insect exoskeleton with ease, whereas larger spores such as that of *D. pinea* require more resources to produce and won't be picked up as easily by chance on a passing insect. Insect dispersal is documented for *D. pinea* (Wingfield and Knox-Davies 1980, Romon et al. 2007, Whitehill et al. 2007), however this is generally in low abundance and may be an opportunistic association where spores may adhere to a beetle by chance, without a mutualistic relationship or co-evolutionary history. A survey of staining fungi present on *H.* ater and *H. ligniperda*, the same bark beetle species as in this study, in New Zealand found no evidence of a *D. pinea* association (Reay et al. 2006a). A recent survey of stained trees in the same geographic region showed *D. pinea* as the dominant stain species, along with some isolates of ophiostomatoid fungi (*Ophiostoma piceae*, and *Grosmannia huntii*) (McCarthy et al. 2010). The trees in this study were damaged by windthrow, where branches and other fallen trees held most of the fallen tree from the ground, which probably prevented colonisation by bark beetles. In contrast to a windthrow situation when harvested trees are stacked for storage, all of the logs are in at least partial contact with another creating a similar environment around the logs as when there is contact with the ground, providing habitats ideal for bark beetle colonisation.

It is possible that *D. pinea* is a latent endophyte in healthy *Pinus* trees (unlike ophiostomatoid fungi), and often only show signs of infestation once a tree becomes damaged or stressed (Smith et al. 1996, Flowers et al. 2001, Reay et al. 2006b, Bihon et al. 2011). Therefore, it is likely that the fungal inoculum was already present in the experimental logs and grew from the ends as the timber dried allowing aerobic fungal growth, or conversely, the spores may have been carried to the exposed ends by wind. Further studies could be implemented using sterile pine logs, or logs protected from wind and rain dispersed fungi, in order to test the effect of this potentially endophytic species. Either way, there is an indication that non-insect-mediated stain fungi do affect damaged pines.

3.4.2 Stain and 'other-insect' attack

Curiously, unidentified insect larvae also had a significant influence of stain, and were

commonly found in both the caged and un-caged logs. The distribution of these larvae also significantly explained the stain variation. These larvae were likely to have entered the cages through the mesh directly, or burrowed below the log and oviposited through. The ability of flies, mites, beetles, and their larvae, to vector sapstain fungi is documented (Powell et al. 1995, Suckling et al. 1999, Uzunović et al. 1999a), and is therefore not a surprise. The possibility of other insects as vectors of of sapstain is not commonly considered – further research is needed to examine and identify the species involved, and quantify their respective importance as vectors of sapstain fungi.

3.4.3 Caveats on interpretation of experimental findings

Although the cages were not completely effective at protecting their enclosed logs from bark beetle colonisation, bark beetles and other insects were still the most important predictors of stain distribution (Table 3.2c,d). Due to the bark beetles penetrating this treatment logs could not be tested by the caging factor alone as a surrogate for "no insect attack". Moreover, analysis could not be restricted to those logs without insect colonisation, as sample sizes would have been too small to allow this. Significant interactions between stain and bark beetles, and stain and other insects, show that both are important predictors of stain, over and above the caging effect (Table 3.2c,d). It is nearly impossible to predict the distribution of stain in the total absence of bark beetles and other insects, however, the results show that there would have been slower colonisation and growth of sapstain without insects.

3.4.3 Applied management implications

Risk of timber discolouration caused by sapstain colonisation is greatest during harvesting, storage and transport of logs, and following storm events that may warrant salvage-harvesting. Bark beetles play an important role in the transport of sapstain fungi to susceptible logs, and the protection of these logs from bark beetles should be attempted. This study shows that if logs are not protected from bark beetles, sapstain will progress rapidly following their colonisation, acting either synergistically or additively with wind blown spores to stain the timber.

In the case of storm damaged stands where salvage harvest may be warranted, New Zealand is in the situation where the two major bark beetles species, *Hylastes ater* and *Hylurgus ligniperda*, are behaviourally adapted to be feed preferentially on roots, and other parts of the tree that are in ground contact, and therefore colonise logs along the surface in contact with the ground (Mausel et al. 2007). The lack of ground contact following such events limits the amount of the log likely to be colonised. Colonisation at ground level was demonstrated in this study also, where the overwhelming majority of bark beetle attack were in the lower segments of the experimental logs (Figure 3.3c, 3.4b). In a recent study investigating the onset of sapstain following windthrow, only 28 of 480 (5.8%) discs sampled systematically from fallen trees had any form of contact with the ground or another tree. This was primarily due branches propping the tree up and ensuring the stem remains elevated (McCarthy et al., unpublished data). Although these trees will still sustain some beetle attack, and they are susceptible to staining by wind dispersed and endophytic fungi (McCarthy et al. 2010), the extent of the damage will be lessened without extensive beetle attack. Mausel et al. (2007) found that logs stacked in a manner that reduces contact with the ground, and other logs, greatly reduces attack numbers of both *H. ater* and *H. ligniperda*. This is likely to be the case for other bark beetles with similar attack strategies.

There are, however, many species of bark beetle that do attack standing trees and stems stored above ground level such as the mountain pine beetle (*Dendroctonus ponderosae*). The mountain pine beetle is one of the most destructive forest pests, and the current outbreak in British Columbia is an order of magnitude larger in severity and area than all previous outbreaks, due to climate driven range shifts and the susceptibility of naive tree populations (Kurz et al. 2008, Cudmore et al. 2010). Countries containing species with similar attack strategies need to implement rigorous barrier or insecticide methods to protect harvested or damaged trees; and countries without need to ensure such species do not become established there (Brockerhoff et al. 2006b). Many species of aggressive bark beetles, including *D. ponderosae*, have been intercepted at the New Zealand border, and have probably entered the

country also (Brockerhoff et al. 2003). The current climate of New Zealand is likely to be unsuitable for the establishment of *D. ponderosae*, or indeed many other aggressive bark beetle species, but under predicted global change scenarios and associated expansion of species ranges this is unlikely to remain the case (Parmesan and Yohe 2003, Parmesan 2006).

3.4.4 General conclusions

This study shows that colonisation of trees by the bark beetles *Hylastes ater* and *Hylurgus ligniperda* increases the amount of stain within the colonised logs. The effective protection of susceptible logs, whether they be harvested or storm damaged, will require examination of the bark beetle species most likely to colonise, and their attack strategies. In countries such as New Zealand, where species predominantly attack logs in contact with the ground or other hard surfaces, log stacking strategies in order to reduce the amount of contact between them, such as simple methods of log stacking to reduce this contact could be implemented such as the use of small "spacers" in order to reduce points of contact between stacked logs. In areas with more aggressive bark beetles that attack logs under all conditions, more intensive methods of insect and fungal management will be required.

Chapter 4 – Bark Beetle-Fungus Interactions

4.1 Introduction

Bark beetles (Coleoptera: Curculionidae, Scolytinae) share an intimate relationship with ophiostomatoid blue-staining wood fungi that dates back as far as the late cretaceous, over 70 million years before the present (Sequeira and Farrell 2001). These relationships were first postulated by von Schrenk (1903), and confirmed by Leach et al. (1934) who observed that the bark beetles *Ips pini* and *I. grandicollis* alone were responsible for the introduction of ophiostomatoid fungi to freshly cut logs of Norway pine (*Pinus resinosa*). The blue staining of timber is of great concern to the forestry industry world-wide, as the value of infected timber is greatly reduced due to the adverse cosmetic effect of darkly pigmented fungal hyphae growing through the tissues of infected timber (Gibbs 1993, Seifert 1993).

At present, New Zealand has two prominent species of introduced bark beetles naturalised within *Pinus radiata* D. Don plantations, *Hylastes ater* (Paykull) and *Hylurgus ligniperda* (Fabricius) (Brockerhoff et al. 2003). Both species were accidentally introduced to New Zealand and breed prolifically in the abundance of woody debris available following harvesting of production forest stands (Reay and Walsh 2001, Brockerhoff et al. 2003). *Hylastes ater* was first detected in the South Island of New Zealand in 1929 (Clark 1932) and is now found wherever pine forests occur. It is native to Europe, and in addition to New Zealand now occurs in Australia, South Africa and Chile (Ciesla 1988, Brockerhoff et al. 2003). *Hylurgus ligniperda* is also of European origin and was first discovered in the North Island of New Zealand in 1974 (Bain 1977a), and is now widely established among pine plantations reaching as far south as Dunedin (Kerr 2010). In addition to New Zealand, *H. ligniperda* is now established in North America, Australia, Japan, Sri Lanka, South America, Algeria and South Africa (Ciesla 1988, Brockerhoff et al. 2003, Haack 2006). Of the approximately 19 native scolytines in New Zealand (Ciesla 1988, Brockerhoff et al. 2003, Haack 2006), only *Pachycotes peregrinus* commonly attacks pine, and other softwoods (Bain 1977b). This species has not been the focus

of many studies, and its relationship with ophiostomatoid fungi is unknown.

Both *H. ater* and *H. ligniperda* feed on the inner bark of the stumps and logs of dead pine trees, limiting the economic impact of these species in comparison with those that colonise and kill dead trees. There is, however, evidence of *H. ater* attacking the root collar of young pine seedlings, often resulting in seedling death (Ciesla 1988, Reay and Walsh 2002, Reay et al. 2002, Kerr 2010). These two species are known to vector ophiostomatoid fungi to seedlings (Reay et al. 2005, 2006a). However, an area that has not been studied in detail in New Zealand is the importance of these species as vectors of sapstain fungi to commercial timber. In New Zealand there are at least 16 different species of fungi that cause sapstain, 15 ophiostomatoid fungi and *Diplodia pinea* (syn. *Sphaeropsis sapinea*) (Thwaites et al. 2005). Ophiostomatoid fungi are known to be major causes of sapstain world-wide (Seifert 1993), however, in New Zealand there is evidence that the dominant staining fungus is *D. pinea* (Farrell et al. 1997, McCarthy et al. 2010). Unlike ophiostomatoid fungi, *Diplodia pinea* can be a latent endophyte in healthy *Pinus* trees, often becoming obvious by displaying disease symptoms such as leader dieback, crown wilt and whorl canker when the tree becomes damaged or stressed (Chow 1984, Smith et al. 1996, Flowers et al. 2001, Reay et al. 2006b). Short-distance dispersal of this species is generally attributed to wind and rain splash (Thwaites et al. 2005), with methods of long distance dispersal not known in any great detail (Brookhouser and Peterson 1971, Palmer et al. 1988). Although there is some evidence suggesting dispersal by bark beetles (Wingfield and Knox-Davies 1980, Romon et al. 2007, Whitehill et al. 2007), there was no evidence suggesting this species was present on any wood inhabiting Coleoptera or Diptera in a recent New Zealand survey (Suckling et al. 1999).

Here, I test the differences in ophiostomatoid fungi vectored by bark beetles in New Zealand by classical methods, and compare these with ophiostomatoid fungi extracted from windthrown timber. In addition, a novel rapid screening technique using high resolution melt curve technology to identify fungal associates of bark beetles is developed. The traditional approach to isolating fungi from bark beetles is to place beetles in a malt extract agar medium, and

identify those species that grow from the beetle inoculum (Reay et al. 2001, Reay et al. 2005, 2006a, Romon et al. 2007, Masuya et al. 2009) or to vortex the beetles in water, which is then used to inoculate malt agar plates (Erbilgin et al. 2005, Erbilgin et al. 2009). These methods, while effective, are time-consuming and there is the potential for one fungal species, whichever grows fastest, to quickly surround the beetle in the medium, not allowing other, slower growing species to grow at all, precluding their detection.

Recent advances in the development of real-time, quantitative polymerase chain reaction (PCR) allows rapid detection, quantification, and possible identification to species level from DNA sequences (VanGuilder et al. 2008). This technology uses a florescent dye that intercalates with double-stranded DNA (dsDNA) resulting in a change in florescence when dsDNA unwinds ('melts') (Reed et al. 2007) resulting in a highly specific peak that is specific to a particular species when used with species-specific, or even universal primers (Winder et al. 2011). Yvon et al. (2009) differentiated between viral variants, and showed that high resolution melt (HRM) results were identical for multiple samples. Standard and high resolution melt curve analysis has been used to identify mammalian carnivores (Berry and Sarre 2007), algae (Kavanagh et al. 2010, Andree et al. 2011), bacteria (Skow et al. 2005, Winchell et al. 2010), insect quarantine pests (Barcenas et al. 2005), and fungi (Hsu et al. 2003).

Comparisons between ophiostomatoid fungi found in wood and on insects are made in this study, and the presence of *D. pinea* on bark beetles is investigated also, all using the standard technique of isolating from beetles directly on agar plates. In addition, a novel application of HRM analysis was investigated where fungal DNA is amplified from bark beetles, and compared to pre-selected fungal "standards" using primers of the internal transcribed spacer (ITS) region of the ribosomal RNA genes, which allows direct amplification of fungal DNA from substrates containing DNA from multiple sources such as plant or soil tissue (Peay et al. 2008). This technique will provide an economical and efficient method to search for, and identify species of interest without the need for traditional culturing techniques.

4.2 Materials and methods

4.2.1 Bark beetle capture

Three beetle species were collected for isolation and identification of associated ophiostomatoid fungi. These were the exotic, but naturalised, bark beetles *Hylurgus ligniperda* (F.), and *Hylastes ater* (Paykull), and the native wood-boring scolytine *Pachycotes peregrinus* (Chapuis) (Coleoptera: Curculionidae: Scolytinae). All beetles were captured aseptically using forceps that had been sterilised in the field with a hand-held butane torch, and then specimens were deposited into sterile, twice autoclaved, 1.5 mL microcentrifuge tubes. Due to large differences in the abundance of each species, different methods had to be utilised for the capture of each of the three species.

Hylurgus ligniperda was the easiest of the three species to capture, being abundant in recently-harvested sites during the summer months (Reay and Walsh 2001). Twenty lures of host volatiles, ten each containing 150 mL of α-pinene, and150 mL of ethanol (Brockerhoff et al. 2006a) , were distributed among one area of recently-harvested *Pinus radiata* plantation forest in the Nelson region. The lures were made using a 400 mm length of 150 µm flat plastic tubing, with felt strips sealed inside with the host volatiles to assist in an even release of volatiles. At 10:30 am, the peak flight time for this species of (Kerr 2010), beetles that had been attracted to the host volatiles were aseptically picked from the lures themselves and from surrounding logs

Hylastes ater is not as common as *H. ligniperda* (Reay and Walsh 2001, Kerr 2010), and therefore more difficult to capture. Specimens were collected from Bottle Lake Forest in Christchurch, New Zealand, from the stumps remaining in the ground following recent harvesting of *P. radiata* trees. The bark was carefully removed from the portion of the stumps above ground level, whereby care was taken not to dislodge the beetles. Adult beetles were then picked aseptically from the stump and inner surface of the removed bark.

Pachycotes peregrinus is a closed-canopy dwelling species (Alwin Sky (University of

Canterbury), unpublished data), and therefore uncommon in harvested stands. During the summer months in Nelson where this species is known to be active, specimens were captured by locating logs from thinning operations with evidence of colonisation (McCarthy et al. 2010). Attack by *P. peregrinus* was recognised by the exudation of white or cream-coloured frass (typical of xylophagous beetles), as opposed to the brown frass of bark beetles (which are phloeophagous). These logs were cut into small sections and placed in emergence buckets with mesh sections to allow air-flow. These emergence chambers were checked daily, with emerged beetles collected aseptically as per the methods used for *H. ligniperda* and *H. ater* (above).

4.2.2 Isolation of fungi from beetles

Fungal isolations were attempted from a total of 48 specimens of each beetle species to identify potential ophiostomatoid and sapstain fungi that may be associated with them. All isolations were attempted on two agar mediums, one general malt extract medium for the isolation of most fungi present, and one selective medium with the addition of the eukaryotic antibiotic cyclohexamide, which ophiostomatoid fungi are tolerant of (Harrington 1981, Jacobs and Wingfield 2001). The standard media consisted of malt agar (3% malt extract, and 2% agar) with 100 µg/ml streptomycin to inhibit bacterial growth. The selective media consisted of malt agar and streptomycin as above, as well as 400 µg/ml cyclohexamide, to inhibit growth of non-ophiostomatoid fungi.

Four beetles of each species were either pressed directly into the agar, allowed to walk freely on the agar for ten minutes before being removed, or squashed between two sterile microscope slides with the gut content spread on the surface of an agar plate, using an inoculation loop. This was repeated on each medium, and half of all beetles within each treatment were surface sterilised by soaking in 4.8% hypochlorite solution for 2 minutes in order to kill spores on the outer surface of the beetle allowing just gut flora to grow, then rinsed twice using sterilised distilled water (as in Reay et al. 2005). After incubation periods of up to 10 weeks, emerging fungal isolates were subcultured in tubes of malt agar (2% malt extract, and 2% agar). Bacterial colonies were recorded, but not isolated or identified further. As I was interested in the fungal

species present on each species, fungal species data was pooled for each species for interpretation.

4.2.3 Isolation of fungi from wood

Ophiostomatoid fungal isolations from wood was shared between Chapters 1 and 3. Methods are repeated below:

Wood discs approximately 50 mm thick were collected from trees felled in both the Nelson region of the northern South Island of New Zealand, and Bottle Lake forest in Christchurch, also in the South Island of New Zealand. There were four sites of *Pinus radiata* plantation forest selected in each region, with trees 10-11 years old. Trees were felled in a systematic manner during each season (spring, summer, autumn, and winter in Nelson, summer and winter in Christchurch), then left in the field for varying lengths of time to account for fungal succession and growth over periods of time following the felling of the tree (see Table 2.2). In Nelson, 12 trees per site were felled per season. Of these 12 trees, three were harvested after three, six, nine and 12 months, respectively, in order to take seasonal and temporal scales into account when sampling fungal growth.

Each tree had a disc cut from the bottom of the cut stem, discarding the first 5 cm, and a disc taken from 4.5 m up the length of the tree. After cutting, discs were immediately labelled, sealed in plastic bags, and stored at 4°C within 24 hours in order to prevent moisture loss and prevent excess fungal growth before sampling. Isolations to identify ophiostomatoid fungi were undertaken within 10 days of sampling from both discs of every tree. An arbitrary wedge was cut from each disc using a small axe. This was bisected aseptically along the radial longitudinal plane using an axe and mallet to initiate the split, and then separated manually, avoiding any external contact of the newly exposed surfaces. Five small chips were excised with a sterile scalpel from along a radial line on one of the freshly exposed surfaces (as in McCarthy et al. 2010), the first and fifth of which were directly below the cambium, and above the pith, respectively. The remaining three were taken at equidistant lengths between the 1st and 5th chips. This was repeated twice per wedge, once inoculating the standard media, and once

inoculating the ophiostomatoid selective media (as in 4.2.2). After incubation periods of up to 10 weeks, emerging fungal isolates were subcultured in tubes of malt agar (2% malt extract and 2% agar). Occasionally, isolations were taken directly from insect holes in the wood. Bacterial colonies were recorded but not isolated or identified further.

4.2.4 Fungal identification

Emerging isolates were organised into groups, and identified where possible from their vegetative morphology and fruiting structures. All isolates believed to be ophiostomatoid were resolved using sequence analysis of the internal transcribed spacer (ITS) using the primers ITS1F (Bruns and Gardes 1993), and β-tubulin regions using the primers Bt2a and Bt2b (Glass and Donaldson 1995).

Isolates were sub-cultured on sterile GelAir cellophane (BIO-RAD Laboratories, Hercules CA, USA) that was spread over 1.5% malt agar plates. Mycelium was scraped directly from the cellophane and placed into a sterile 1.5 mL microcentrifuge tube for DNA extraction, which was processed using the REDExtract-N-Amp™ plant PCR kit (Sigma, St Louis, Missouri, USA), following the manufacturer's instructions. If this DNA failed to amplify, it was re-extracted using the ChargeSwitch® gDNA plant PCR kit (Invitrogen, Carlsbad, California, USA), following the manufacturer's instructions. DNA was amplified using the 5 × HOT FIREPol® Blend Master Mix, Ready to Load (dnature, Gisborne, New Zealand). Amplifications were performed in an Eppendorf Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany) under the following conditions for ITS and β-tubulin regions: (1) ITS - initial denaturation 95°C for 15 min (to activate the HOT FIRE polymerase), followed by 35 cycles of 95°C for 30 sec, 60°C annealing for 40 sec and 72°C extension for 40 sec, followed by a final extension period of 72°C for 5 min, (2) β-tubulin - initial denaturation 94°C for 15 min, followed by 30 cycles of 94°C for 60 sec, 58°C annealing for 60 sec and 72°C extension for 60 sec, followed by a final extension period of 72°C for 10 min.

PCR products were visualised by electrophoresis and purified using the ExoSAP method (Exonuclease I, *Escherichia coli* and Shrimp Alkaline Phosphatase, SAP) according to the

supplier's instructions (Fermantas Life Sciences, Lithuania). Sequence analysis was carried out at Macrogen Inc., Korea. Sequences were edited and aligned using Sequencher version 5.0 (GeneCodes Corp., Ann Arbor, Michigan, USA) and identities were determined by Genbank BLASTn search (Altschul et al. 1990). For identification, a minimum of 95% sequence identity to an ITS sequence of at least 450 bp from a known specimen in the database was required. For β-tubulin sequences, the required sequence length was 350 bp. Those samples with 97-100% identity match to a known species were considered a match and named to the species level, provided the morphological characteristics of the isolate matched that of the name derived from sequence data.

4.2.5 High resolution melt (HRM) analysis

A search of the literature resulted in 10 ophiostomatoid fungi commonly associated with the bark beetles *Hylastes ater* and *Hylurgus ligniperda* (Reay et al. 2005, Thwaites et al. 2005, Romon et al. 2007). Seven fungal species were selected as standards, depending on availability of samples, and relevance to the insect species. The fungal species selected were: *Ophiostoma stenoceras*, *O. quercus*, *O. piceae*, *O. pluriannulatum*, *Grosmannia huntii*, and *Sporothrix inflata.* All samples were obtained from the archived NZ Forest Research fungal culture collection (Rotorua, New Zealand).

All fungal samples were removed from storage and sub-cultured on sterile GelAir cellophane (BIO-RAD Laboratories, Hercules CA, USA) over 1.5% malt agar plates. Mycelium was scraped off the cellophane directly with a flame sterilised blade and placed into a sterile 1.5 mL microcentrifuge tube. The DNA was then extracted using the Extract-N-Amp™ Plant PCR kit (Sigma, St. Louis, Missouri, USA) following the manufacturer's instructions. The ITS2/ 4 was amplified using the following primers ITS2F (5'GGCATGCCTGTYCGAGCGTCATTWC3') and ITS2R (5'CCGCTGAACTTAAGCATATCAATAAGCGGAGGA3') under the conditions: initial denaturation at 94°C for 2 min, followed by 25 cycles of 94°C for 30 sec, 55°C annealing for 30 sec and 72°C extension for 45 sec, followed by a final extension period of 72°C for 1 min (see Appendix B for primer descriptions). In brief, the crude fungal DNA (2 µl) was used to

amplify the ITS 2/4 region using: 0.5 µl forward, and 0.5 µl reverse primers, 8 µl H_2O, 10 µl *Taq* polymerase (Kapa Biosciences, South Africa). Amplifications were performed in an Eppendorf Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany).

PCR products were resolved by agarose gel electrophoresis. The amplicons were excised and purified using the GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The amplicons were cloned into pGEM-T easy vectors and sequenced at Macrogen Inc, South Korea. Sequences were edited and aligned using MEGA 5 (Tamura et al. 2011) and confirmation of their identities were determined by Genbank BLAST search (Altschul et al. 1990).

HRM analysis was undertaken using real time PCR methods using a Rotor-gene thermal cycler (Quigen N.V., Venio, Netherlands). All of the cloned DNA samples were diluted to 100 pg/µl and run in triplicate as 10 µl reactions consisting of: 5 µl KAPA qPCR Kit (Kapa Biosystems, South Africa), 0.25 µl each of both forward and reverse primers, 3.5 µl H_2O, and 1 µl DNA. First, DNA was amplified under the following conditions: initial denaturation 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec, 60°C annealing for 15 sec and 72°C extension for 20 sec, followed by a final extension period of 72°C for 5 min. This was followed by step-wise denaturation from 72°C to 99°C with 0.1°C increments and 2 sec hold. During this protocol, fluorescence data were converted to melt profile charts using Rotor-Gene Q – Pure Detection software which plotted the negative derivative of fluorescence against temperature (dF/dT vs temperature). Mean melt peak temperatures, among replicates, were determined manually using the software output listing the melt peak temperatures. Combinations of cloned DNA were then assessed to determine whether multiple peaks within a single sample could be resolved. Due to the possibility of small differences between runs of the machine, and the extreme sensitivity of the technology, all standards were included in every run in order to be able to accurately compare outputs.

Fungi were extracted from beetles using a modification of a crude extraction method described by Cenis (1992). A 2 mL microcentrifuge tube was filled with 1 mL sterile malt

extract broth, a culture was then started by adding a beetle to the tube and left for 96 hours at 20°C in an orbital shaker. The beetle was then removed with a sterile pipette tip, and the mycelial mat was pelletted by centrifugation for 5 min at 13000 rpm. The remaining broth was decanted and the pellet washed with 500 µl TE buffer and paletted again. The TE buffer was decanted, and 300 µl of extraction buffer (220 mM Tris HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS) was added. The mycelium was then crushed with a small, sterile eppendorf pestle fitting exactly in the tube until a uniform solution was achieved. Following this, 150 µl of 3 M sodium acetate pH 5.2 was added. The tubes were then left at -20°C for at least 10 mins. Tubes were then centrifuged, and the supernatant was transferred to another tube. An equal amount of isopropanol was added, and left for 5 mins at room temperature, then precipitated DNA was paletted by centrifugation. The paletted DNA was then washed with 70% ethanol, and then dried until all ethanol had evaporated. The pellet was then re-suspended in 50 µl of TE buffer. Samples were diluted to 100 pg/µl before being analysed by HRM. Beetle DNA extractions were run in triplicate, along with all fungal standards (also run in triplicate), with 10 µl reactions using the HRM protocol above. Molecular grade water samples were also used as negative controls. Fungal DNA melt peaks could then be averaged, and compared with those of the standards. This was repeated with four beetles, in order to test whether the method provides repeatable results.

A full field trial is planned as an application of this method over the summer to be conducted by myself, where beetles collected from the field and assessed for their fungal communities. The aim of this study is to describe the differences in fungal communities on beetle species at both inter- and intra-specific levels to find out if species, or even populations, have unique fungal compositions.

4.3 Results

4.3.1 Beetle isolations

In total 134 isolations were attempted from bark beetles in this study, with seven species of ophiostomatoid fungi isolated. *Diplodia pinea* was not present on any bark beetles in this study. ophiostomatoid fungi found to be associated with *Hylastes ater* included *O. piceae* (non-sterilised, in media), *O. radiaticola* (non-sterilised, walking on media, also squashed), and *Grosmannia huntii* (both sterilised and non-sterilised, walking on media). ophiostomatoid fungi found on *H. ligniperda* were *G. huntii* (sterilised, in media and squashed) and *O. nigrocarpum* (sterilised, from gut content). There was one species, *G. huntii* that was found on both *H. ater* and *H. ligniperda*.

This is the first study to attempt to isolate ophiostomatoid fungi from the native scolytine wood borer *Pachycotes perigrinus*. Ophiostomatoid fungi isolated included *O. breviusculum*-like (non-sterilised, walking on media), *O. pallidulum* (non-sterilised, walking on media), *Pesotum fragrans* (non-sterilised, walking on media). *Diplodia pinea* was not isolated from any beetle species in this study. All of the ophiostomatoid species found on beetles in the study are summarised in Table 4.1, and complied with associations from the literature in Table 4.2.

4.3.2 Wood isolations

In total, 5520 isolations were taken during the course of this study from wood discs, 3600 from Nelson and 1920 from Christchurch. Of these isolates, only a total of 21 (0.58%) from Nelson, and 7 (0.36%) from Christchurch, were ophiostomatoid fungi. Nine species of ophiostomatoid fungi were isolated from wood discs collected in this study, all nine of which were found at the Nelson site, whereas only two of these were found at the Christchurch site. The eight species found in Nelson were: *Ophiostoma nigrocaropum* (0.39% of the total isolates from selective media), *Ceratocystiopsis collifera*-like (0.33%), *O. pallidulum* (0.22%), *O. nigrocarpum*

Table 4.1. Ophiostomatoid fungal isolations from the bark beetles *Hylastes ater* and *Hylurgus ligniperda* and the wood borer *Pachycotes peregrinus*.

	Hylastes ater						*Hylurgus ligniperda*						*Pachycotes peregrinus*					
	Sterilised			Non-sterilised			Sterilised			Non-sterilised			Sterilised			Non-sterilised		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Grosmannia huntii		✓		✓	✓	✓	✓	✓				✓						
Ophiostoma breviusculum-like																		✓
Ophiostoma pallidulum																		✓
Ophiostoma nigrocarpum							✓											
Ophiostoma piceae					✓													
Ophiostoma radiaticola				✓		✓												✓
Petosum fragrans																		✓

A – squashed, B – in media, C – on surface walking

[a]Isolated from wood in McCarthy et al 2010

Table 4.2. Comparison of ophiostomatoid fungi on bark beetle species found in New Zealand, *Hylastes ater* and *Hylurgus ligniperda* from this study, and the literature. Species marked with an asterisk (*) were found in this study, species found for the first time on a beetle species from this study are bolded.

Bark beetle	Fungus	Reference
Hylastes ater	*Ophiostoma floccosum*	(Reay et al. 2005)
	Ophiostoma galerforme	(Reay et al. 2001, Zhou et al. 2004c, Reay et al. 2005)
	Leptographium guttulatum	(Wingfield and Gibbs 1991, Romon et al. 2007)
	**Grosmannia huntii*	(Reay et al. 2001, Zhou et al. 2004c, Reay et al. 2005)
	Ophiostoma ips	(Reay et al. 2001, Reay et al. 2002, Reay et al. 2005, Romon et al. 2007)
	Leptographium lundbergii	(Jacobs and Wingfield 2001)
	Ophiostoma minus	(Gibbs 1993)
	Grosmannia olivacea	(Romon et al. 2007)
	Ophiostoma penicillatum	(Mathiesen 1950)
	Ophiostoma piceae	
	Ophiostoma piliferum-like	(Romon et al. 2007)
	Ophiostoma pluriannulatum	(Reay et al. 2001, Romon et al. 2007)
	Leptographium procerum	(Jacobs and Wingfield 2001, Reay et al. 2001, Reay et al. 2005)
	Ophiostoma quercus	(Reay et al. 2001, Zhou et al. 2004c, Reay et al. 2005, Romon et al. 2007)
	Ophiostoma radiaticola	
	Ophiostoma rectangulosporium-like	(Romon et al. 2007)
	Leptographium serpens	(Wingfield and Gibbs 1991)
	Ophiostoma setosum	(Reay et al. 2001, Reay et al. 2005)
	Ophiostoma stenoceras	(Reay et al. 2005, Romon et al. 2007)
	Leptographium wingfieldii	(Romon et al. 2007)
	Leptographium truncatum	(Wingfield and Gibbs 1991, Reay et al. 2001, Reay et al. 2005, Romon et al. 2007)
Hylurgus ligniperda	*Ophiostoma floccosum*	(Reay et al. 2006a, Kim et al. 2011)
	Opgiostoma galeiforme	(Zhou et al. 2001, Zhou et al. 2004c, Reay et al. 2006a, Kim et al. 2011)
	Leptographium guttulatum	(Romon et al. 2007)
	**Grosmannia huntii*	(Reay et al. 2006a, Kim et al. 2011)
	Ophiostoma ips	(Zhou et al. 2001, Zhou et al. 2004a, Zhou et al. 2004c, Reay et al. 2006a, Kim et al. 2011)
	Leptographium lundbergii	(Jacobs and Wingfield 2001, Zhou et al. 2001)
	Ceratocystiopsis minuta	(Zhou et al. 2001, Zhou et al. 2004c)
	Ophiostoma nigrocarpum	
	Ophiostoma penicillatum	(Jacobs and Wingfield 2001)
	Ophiostoma piceae	(Zhou et al. 2001, Kim et al. 2011)
	Ophiostoma pluriannulatum	(Zhou et al. 2001)
	Leptographium procerum	(Jacobs and Wingfield 2001, Reay et al. 2006a)
	Ophiostoma quercus	(Reay et al. 2006a, Kim et al. 2011)
	Leptographium serpens	(Zhou et al. 2001, Kim et al. 2011)
	Ophiostoma setosum	(Reay et al. 2006a)
	Ophiostoma stenoceras	(Zhou et al. 2001, Kim et al. 2011)
	Leptographium tereforme	(Kim et al. 2011)
	Leptographium truncatum	(Reay et al. 2006a)

(0.10%), *Sporothrix inflata* (0.06%), *O. radiatacolata* (0.06%), *O. setosum* (0.06%), *O. sparsiannulatum* (0.06%) and *O. breviusculum*-like (0.06%). The two species found in Christchurch were *Sporothrix inflata* (0.10%) and *O. piliferum* (0.06%). In addition, five isolates of *O. nigrocarpum* (0.52%) was isolated from the standard media in Christchurch wood samples (Table 4.3). Some additional ad hoc isolations were taken from wood surrounding insect holes in Nelson, resulting in two isolations for *O. nigrocarpum* and one isolation of *O. pallidulum*.

4.3.3 HRM analysis

Individual plasmid DNA samples from known fungal isolates formed smooth peaks that were consistent between each triplicate (Figure 4a,b). Mixed samples of more than one DNA isolate also resulted in a double peak, such as those depicted in Figure 4b, where *Grosmannia huntii* and *S. inflata* form two easily distinguishable peaks at 90.07°C and 91.76°C, respectively, in comparison with the single isolate standards of 90.35°C and 91.85°C. The height of the peaks is a quantitative measure of DNA abundance, and was not relevant to this study. The method was also successful at determining the identity of ophiostomatoid fungal DNA grown from bark beetle inoculated malt extract broth media. With four *H. ligniperda* specimens, I was able to identify ophiostomatoid fungi from two of them - *S. inflata* from one, and *G. huntii* from the other (Figure 4.1c,d).

Table 4.3. Comparison between ophiostomatoid fungal species found living in cut logs and being carried on beetle species from Nelson and Christchurch. Bolded species were found in both wood and on beetles.

Ophiostomatoid species	Wood	Scolytid beetles		
	Pinus radiata	*Hylastes ater*	*Hylurgus ligniperda*	*Pachycotes peregrinus*
Ceratocystiopsis collifera-like	✓			
Grosmannia huntii	✓[a]	✓	✓	
Ophiostoma breviusculum-like	✓			✓
Ophiostoma nigrocarpum	✓		✓	
Ophiostoma pallidulum	✓			✓
Ophiostoma piceae		✓		
Ophiostoma piliferum	✓			
Ophiostoma radiaticola	✓	✓		✓
Ophiostoma setosum	✓			
Ophiostoma sparsiannulatum	✓			
Pesotum fragrans				✓
Sporothrix inflata	✓			

[a] In McCarthy et al (2010)

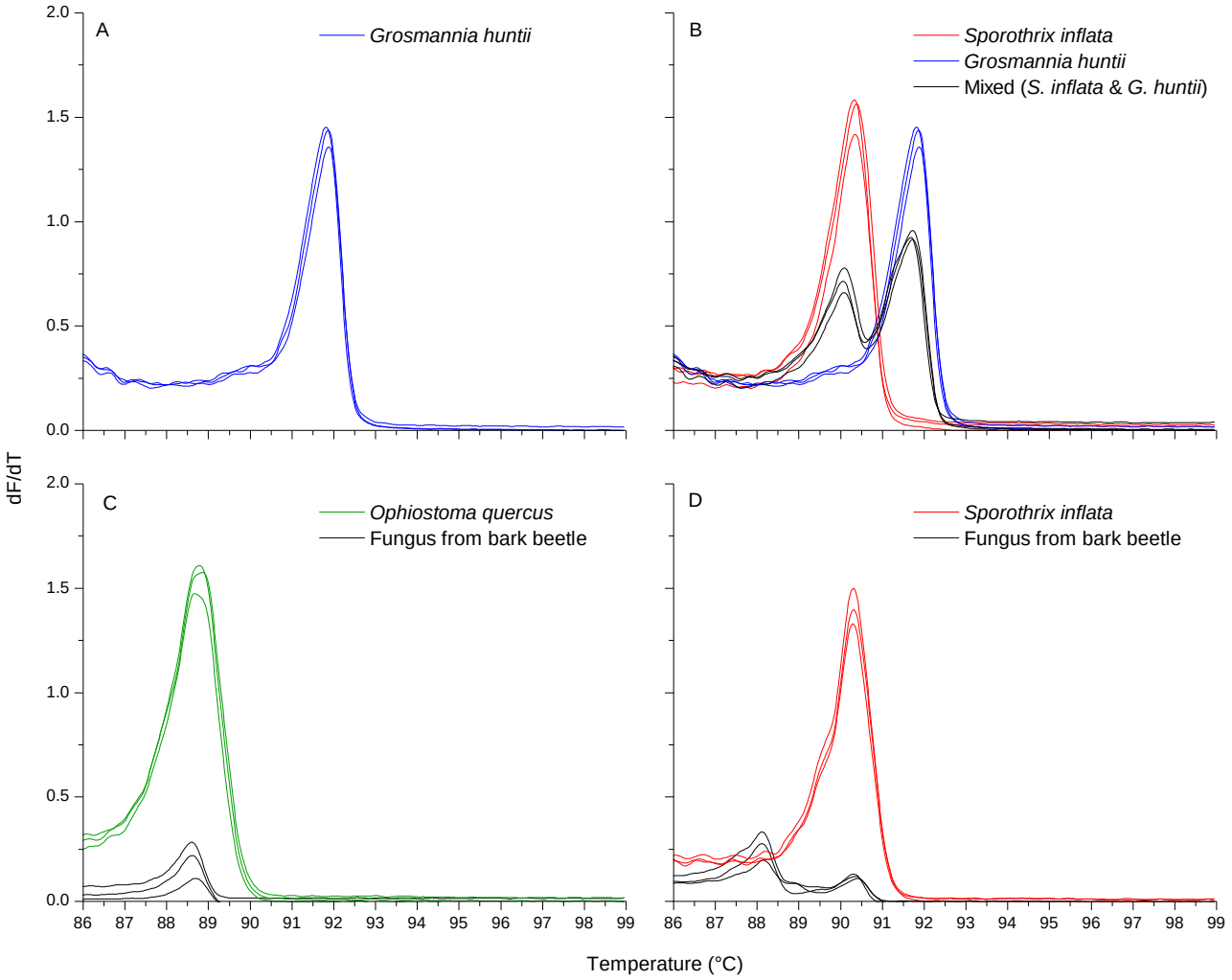


Figure 4.1. High Resolution Melt (HRM) peaks of fungal DNA for identifying unknown fungal samples, from known standards. All samples were run in triplicate. (A) *Grosmannia huntii* melt peak (av. 91.85°C) showing repeatability of triplicate runs. (B) Mixed samples can be differentiated: *Sporothrix inflata* (av. 90.35°C) and *G. huntii* (av. 91.85°C) pure DNA isolates, compared with a single sample with both DNA samples mixed together (av.'s 90.07°C and 91.71°C). (C) *Ophiostoma quercus* melt peak (av. 88.63°C) matches that of an unknown fungus grown from a bark beetle, *Hylurgus ligniperda* (88.77°C). The height of the curves is a quantitative measure of abundance (not investigated further), but the melt peak average is indicative of the DNA make-up of a specific species. (D) *S. inflata* melt peak (av. 90.31) matches that of an unknown fungus grown from a bark beetle, *H. ligniperda* (av. 90.36°C). In (D) there is also a peak at 88.13°C (av.) of an unknown fungus that was not one of the standards, and therefore represents an unidentified species.

4.4 Discussion

As a result of a co-evolutionary history, bark beetles are important vectors of ophiostomatoid fungi that cause sapstain in timber. In this study, 12 species of ophiostomatoid fungi, including *Ceratocystiopsis*, *Grosmannia*, *Ophiostoma*, *Pesotum*, and *Sporothrix* spp., were isolated from the bark beetles *Hylastes ater* and *Hylurgus ligniperda*, the wood borer *Pachycotes peregrinus*, and samples of *Pinus radiata*. There were five species of ophiostomatoid fungi that were found on both, beetles and wood: *Ophiostoma nigrocarpum* was found on both *P. radiata* and *H. ligniperda*; *O. radiaticola*, *O. pallidulum* and *O. breviusculum*-like were found on both *P. radiata* and *P. peregrinus*; *Grosmannia huntii* and *O. radiaticola* were found on both *P. radiata* and *H. ater*; and *G. huntii* was found on *H. ligniperda* after being isolated from *P. radiata* by McCarthy et al. (2010) from the same region, with a similar experimental design . Among these are associations new to science, and this is the first time any ophiostomatoid fungi have been isolated from *P. peregrinus*. Finding ophiostomatoid fungi that infect pine on this native borer may have consequences on our native forest tree species if this can lead to an introduction of exotic tree pathogens to native trees.

The high resolution melt curve (HRM) species identification tool developed here allows mass screening of samples at unprecedented temporal and economic scales. Classical techniques of identifying fungi from beetles and wood involves inoculating agar with the sample, sub-culturing a pure isolate, and morphological and possibly molecular identification. Growing periods alone for the fungi can be multiple weeks before morphological features are distinguishable. This application of the HRM technique allows a beetle-inoculated fungal broth, from which DNA can be extracted and identified by matching DNA denaturing temperatures. One broth culture costs approximately NZ 50c (at time of publication of this thesis), and results are available just 2 hours following DNA extraction. This technique has potential applications in the rapid identification of quarantine samples of both pathogens and pests (Winder et al. 2011).

4.4.1 Ophiostomatoid fungi from wood and beetles

There were six species that were isolated from one source only. *Ceratocystiopsis collifera*-like, *O. setosum*, *O. piliferum*, *O. sparsiannulatum*, and *Sporothrix inflata* were found on *P. radiata* samples only. *Ophiostoma piceae*, was found on *H. ater* only, and *Pesotum fragrans* was found on *P. peregrinus* only. There were no fungal species found on *H. ligniperda* alone, as all were also found on wood. This is the first survey of the fungi associated with *P. peregrinus*, and finding four species of ophiostomatoid fungi associated with this insect is important to the forest industry as it commonly inhabits timber damaged by storms that lie in its preferred habitat of closed canopy forests (Alwin Sky (University of Canterbury), unpublished data).

Aside from the new associations with *P. peregrinus*, I believe two of the associations listed above to be new to science. This is the first reported association between both *O.* nigrocarpum and *G. huntii* with *H. ligniperda*, and between *O. piceae* and *H. ater*. *O. nigrocarpum* was commonly found on wood in this study, and has been intercepted previously on logs being exported from New Zealand to Japan, and had also not previously been reported there (Kim et al. 2005a). It is distributed in pines in Austria, Canada, Japan, New Zealand, South Africa, and the United States (de Beer et al. 2003a) . *G. huntii* is a known root pathogen, potentially able to kill pine saplings and cause sapstain in infected trees (Matusick and Eckhardt 2010). It has been previously discovered on *H. ater* (Reay et al. 2001, Zhou et al. 2004a, Reay et al. 2005) which, along with *H. ligniperda*, prefers to attack wood in ground contact (Ciesla 1988). This shared target of root attack explains the common association with both of these beetle species. *phiostoma piceae* is closely related to the highly pathogenic *O. ulmi* and *O. novo-ulmi* which both caused Dutch elm disease pandemics across Europe and North America (de Beer et al. 2003b). *Ophiostoma piceae* is not considered a pathogen, but is associated with the bluestain of conifer timber (Schirp et al. 2003a). This species is known to be associated with *H. ligniperda* (Zhou et al. 2001, Kim et al. 2011), and occurs almost exclusively on coniferous hosts (Kim et al. 1999, de Beer et al. 2003b).

Ceratocystiopsis collifera-like was found on wood in our study, and has been reported to be

associated with the bark beetle *Dendroctonus valens* (Owen et al. 1987, Marmolejo and Butin 1990), though it was not found to be associated with any of the beetles here. *Ophiostoma breviusculum*-like is a recently described species that forms part of the *O. piceae*-complex, and is morphologically similar to *O. piceae* and *O. quercus*, all of which cause sapstain to pines (Chung et al. 2006), and it was found to be associated with both wood and the wood-borer *P. peregrinus* in this study. *Ophiostoma pallidulum* was found on *P. peregrinus* for the first time. This species is a known stain-causing fungus from pines, and has been isolated from the bark beetle *Hylastes brunneus* in Finland (Linnakoski et al. 2010). This species seems to be present on multiple beetle species world-wide, including one closely related to *H. ater*, indicating the potential for it to be spread by this species also. The last species to be associated with *P. peregrinus* is *P. fragrans*. *Pesotum fragrans* is a stain fungus that has been associated with many coniferous hosts and weevil species in China (Paciura et al. 2010), Korea (Kim et al. 2007), Poland (Jankowiak and Kolařík 2010), South Africa (Zhou et al. 2006), Australia, North America, and New Zealand (Harrington et al. 2001, Jacobs et al. 2003, Jacobs and Seifert 2004). It was not isolated from wood in this study, but it is likely that it does inhabit *P. radiata* logs in New Zealand.

Ophiostoma radiaticola was only found in wood in this study. It is a stain fungus that has been found on New Zealand *P. radiata* previously, both within New Zealand and in trees exported to Korea (Kim et al. 2005a, Kim et al. 2005b), and is a member of the *O. galeiformis*-complex (Zhou et al. 2004b). *Ophiostoma setosum* was also found in wood in this study, and along with *O. breviusculum* is a member of the *O. piceae*-complex and is found primarily on conifers (Uzunović et al. 2000, Harrington et al. 2001). It is a relatively aggressive sapstain fungus (Fleet et al. 2001), and it is already known to be present among logs and stumps harvested in New Zealand (Reay et al. 2005, Thwaites et al. 2005). It is another species found in logs exported from New Zealand to Korea (Kim et al. 2005a), and has been found on bark beetles in China (Paciura et al. 2010), and on *H. ater* in New Zealand (Reay et al. 2005). *Ophiostoma sparsiannulatum* is another species to be found only on wood in this study. It is a

recently-described species and a member of the *O. pluriannulatum*-complex, and until now was only known from the roots of *Pinus taeda* damaged by various root-infesting insects (Zanzot et al. 2010). It is not thought to be a pathogenic fungus, and it is not known for certain to be distributed by insects, however Zanzot et al. (2010) suggested further research should be undertaken to confirm this. The final species to be isolated in this study was *S. inflata*. This is a common soil borne fungus with a world-wide distribution (de Hoog 1993), and is known to attack Douglas-fir (*Pseudotsuga menziesii*), multiple oak (*Quercus*) species, and has been isolated from bark beetle *Dryocetes autographus* (Halmschlager and Kowalski 2003). This is possibly the first time this species has officially been isolated from *P. radiata*.

There were no discernible patterns between species isolated from beetles walking on the surface of the media, placed in the agar, or from the gut contents (Table 4.1). Many species were deposited from beetles walking on the surface, indicating that these species "shed spores" in their travels. Also, *G. huntii* and *O. radiaticola* were the only fungi isolated from the gut contents of a beetle (*H. ater*), however these same species were also shed while walking on the agar surface, and grew from beetles placed in agar (Table 4.1).

The lack of occurrence of a fungal species on a particular beetle does not say that there is definitely not an association between them. Traditional culturing techniques are time consuming and cumbersome, and processing the beetle numbers required in order to be sure that all associations were recorded was beyond the scope of this thesis. In order to study this effectively and efficiently, I developed the use of the HRM method for this purpose which can now be implemented to conduct such studies more efficiently.

4.4.2 HRM species identification tool

The melt profile temperature of a region of the ITS domain was sufficient to discriminate fungal species cultured from beetles from fungal standards. Winder et al. (2011) were able to distinguish between three insect (weevil) species, four arachnid (tick) species, and a virus by standard (not high resolution) melt peak analysis. Due to the high degree of inter-relatedness between species in our standards, HRM was required in this case in order to attain more definite

and consistent separation of peaks. This method requires half the time, and a small fraction of the cost, of PCR followed by gel electrophoresis showing the advantages of adopting this technique for species identification.

Currently, isolation and identification of ophiostomatoid fungi from beetles by plating whole beetles, crushed beetles, or beetle parts is the standard method for investigating their associated fungi. Multiple studies, including this one, have employed this technique with some success (Klepzig and Wilkens 1997, Reay et al. 2005, Bleiker et al. 2009, Bueno et al. 2010, Kim et al. 2011), however it is time consuming and subject to a selection bias towards faster growing fungi.

Kim et al (1999) developed a rapid method of detecting the sapstain fungi *O. piceae* and *O. quercus* in stained wood, using PCR and gel electrophoresis alone Their method eliminated the need to grow the organisms in artificial media saving money and reducing chances of cross-contamination. Tsui et al. (2010) designed padlock probes to target species-specific single nucleotide polymorphisms of two ophiostomatoid fungi associated with the bark beetle *Dendroctonus ponderosae*. This approach was able to distinguish the two fungal species from other species on *D. ponderosae*, however, a large number of padlock probes would be required to test for multiple species at once, increasing the cost, and reducing the efficiency of the technique.

Another recent method reported involves the development of DNA probes to identify the major fungal species associated with *D. ponderosae* (Khadempour et al. 2010). They were able to distinguish between fungal species on beetles and on wood. Their technique, however, still requires the use of target-specific primers, and gel electrophoresis. High resolution melting analysis has recently been implemented for the early detection of the sapstain fungus *Diplodia pinea*, and was able to distinguish the species from others closely related to it (Luchi et al. 2011). The technique was validated by screening symptomatic and symptomless pine shoots, showing its effectiveness as an early detection method of the pathogenic fungus.

Other methods of rapid identification and detection of ophiostomatoid fungi have been

investigated over the years, and none have become a standard technique. These techniques cannot efficiently distinguish between large numbers of species (> 5) requiring multiple runs of gel electrophoresis, and also require the design of expensive probes. The time- and cost-efficiency of this method is a major advantage, and with further development and design of target species standards, could be implemented in laboratories for species identification.

4.4.3 General conclusions

In summary, the results described here show that there are many ophiostomatoid sapstain fungi present in New Zealand that infect pines, and many of these species are transmitted on scolytines that colonise susceptible timber. Biosecurity strategies need to be implemented not only to ensure that new bark beetle pests are not introduced, nor any fungi that may be associated with them. Earlier studies have either focussed on on fungi present on beetles, or fungi present on wood – very few have examined both at once. Future studies would benefit from enlarged beetle sample sizes, implementation of rapid screening techniques, and also comparing fungal communities present within beetle species from population and regional sampling scales.

Species identification through HRM curves, in conjunction with specific primers, represents a promising tool to better understand fungal population dynamics on beetles, and screen beetles for potentially pathogenic and stain causing fungal associates. Advantages of this method over the classical techniques include a dramatic reduction in cost, no need to pour gels or use hazardous chemicals (such as ethidium bromide), higher chance of detecting slower growing organisms, and a rapid turnaround time of a matter of hours. With the development, and reduction in costs associated with qPCR equipment, this method could be implemented at points of interception of invasive organisms offering quick and accurate identification aiding in the prevention and response to biosecurity incursions.

Chapter 5 - General Discussion

5.1 Overview

Forest managers world-wide face increasing challenges from escalating biosecurity and forest health issues that threaten the production of timber and the provision of ecosystem goods and services (MEA 2005). Outbreaks of forest pests and diseases and continuing invasions of exotic forest insect pests and pathogens are important drivers of forest health issues world-wide (Brockerhoff et al. 2006b, Aukema et al. 2010). These forest health issues are exacerbated by the increasing frequency and magnitude of storm events in a changing global climate (Easterling et al. 2000, Emanuel 2005). To some extent, these interactions between pest complex, stand structure, and environmental conditions have been one of the primary concerns of foresters since the advent of large scale production, particularly in plantation monocultures that are especially vulnerable to outbreaks (Dinoor and Eshed 1984). However changing climatic conditions are seeing the expansion of historical geographic ranges of many pest species, bringing about rapid changes in the activity, development, phenology, and survival of novel pest species on commercially-important host tree species (Karban and Strauss 2004). For example, the pine processionary moth (*Thaumetopoea pityocampa* Denis & Schifferműller) is an important defoliator of pine trees. A warming climate and milder winters in southern and central Europe have led to a northward expansion of its geographic range, and outbreaks are now occurring in areas that were previously too cold and not affected by this pest (Battisti et al. 2005).

Novel pest and pathogen interactions with trees are also becoming more common as the increasing global movements of people and products transport species across biogeographic barriers. For example, in the USA the spread of Dutch elm disease (*Ophiostoma ulmi*), a pathogenic fungus of Himalayan origin, was facilitated by a novel vector-pathogen association with European and North American bark beetles (Webber 1990). In the converse situation, the establishment of tree crops in novel environments can also lead to unexpected pest or pathogen

problems. For example, *Eucalyptus nitens* plantations established in South Africa have been attacked by the moth *Coryphodema tristis* which is normally a pest of fruit such as grapes or quince, but has unexpectedly switched hosts (Gebeyehu et al. 2005).

Bark beetles are among the most damaging insect pests in plantations, and climate change has led to several outbreaks of these beetles due to warming at higher latitudes and elevations (Williams and Liebhold 2002). This is especially important when it involves pathogen-vectoring tree-killing bark beetles such as the southern pine beetle (*Dendroctonus frontalis*) and the mountain pine beetle (*D. ponderosae*), both of which are spreading across North America as the climate warms (Williams and Liebhold 2002, Kurz et al. 2008). Fortunately, New Zealand does not (yet) have any tree-killing bark beetles, but there is evidence of geographic range expansion in the secondary pests that are present. *Hylastes ater* was detected in New Zealand in 1929 (Clark 1932), and is now present wherever pines occur across the country. *Hylurgus ligniperda*, on the other hand, was first detected in 1974 in the North Island (Bain 1977a), but has only recently been detected as far south as Dunedin (Kerr 2010).

The interactions observed today between species present in New Zealand plantation forests are mostly the result of a small subset of species being drawn from natural pine ecosystems on the other side of the world which have been deposited in a novel biogeographic region, with a wide range of interacting species native to New Zealand. There are interactions between trees and bark beetles, and bark beetles with fungal pathogens from all around the globe resulting in a unique novel assemblage of species together for the first time. Understanding the dynamics of these species in their novel environment is crucial to effectively responding to potential pest threats.

This is the first study in New Zealand quantifying the importance of bark beetles as vectors of sapstain fungi, in comparison to those distributed by wind and rain. The goal of this research was to investigate the role of bark beetles as vectors of sapstain and to investigate the potential synergistic interactions between beetles and sapstain fungi under differing seasonal and environmental conditions that might exacerbate the impact of sapstain on timber

merchantability following increasingly frequent storm damage.

5.2 The effects of beetle colonisation on sapstain development

In this study trees were experimentally felled to simulate windthrow and track the development of sapstain over time, depending on the region and season of the (simulated) storm. Bark beetle colonisation of windthrown logs was relatively uncommon, and where it did occur there was no correlation between insect attack and sapstain development. This was supported by the fungal isolations where 96% and 95% of all sapstain fungi isolations in Nelson and Christchurch, respectively, were identified as *Diplodia pinea* – a species not commonly vectored by bark beetles. The low level of bark beetle attack was consistent with the environment provided by the windthrow simulated trees where the logs are held in the air by branches and other trees, rather than being in contact with the ground. This is not conducive to attack by either *H. ater* or *H. ligniperda*, which generally colonise wood that is in contact with the ground (Mausel et al. 2007).

To elucidate the role of bark beetles further under more controlled conditions, a manipulative field experiment was established where logs, in contact with the ground, were either protected from beetles with cages or left exposed to allow natural levels of beetle colonisation. Although some bark beetles did manage to penetrate the exclusion netting (the cages were ca. 94 % effective), there was a clear correspondence between bark beetles and stain, where the presence of bark beetles (along with larvae of a few other unidentified beetles and flies) increased the amount of stain in the section of wood affected by bark beetle attack. Bark beetle colonisation of the uncaged logs occurred almost exclusively in the parts of logs that were in contact with the ground, with no colonisation of the upper surfaces, and this was matched by the pattern of the stain. The stain of the caged logs, however, was primarily concentrated at the exposed cut faces at the terminal ends. Stain development was reduced near the centre of these logs, where the undamaged bark provides protection to the sapwood from wind-blown spores.

These findings indicate that, in New Zealand, the role of bark beetles in vectoring sapstain fungi is not as important overall as in other countries where other bark beetle species occur that attack the entire log and not just those parts that are in ground contact. This is relevant in windthrow situations where substantial parts of logs are not in ground contact, with a concomitant reduction in the risk of beetle-facilitated sapstain attack. The greater importance in New Zealand of *D. pinea* as a sapstain-causing fungus, compared with ophiostomatoid fungi, also contributes to a diminished role of bark beetles as vectors of sapstain fungi because, unlike many ophiostomatoid fungi, *D. pinea* is not vectored by bark beetles. The fact that bark beetles still play a potentially important role in facilitating sapstain, as seen in the experiment comparing caged and uncaged logs in ground contact, is probably due to two factors: (i) logs in ground contact are clearly at greater risk from attack by bark beetles as well as beetle-vectored sapstain fungi, and (ii) bark damage caused by beetle tunnelling is likely to provide access also to the omnipresent *D. pinea*.

5.3 Sapstain species associated with New Zealand bark beetles

Although bark beetles do not appear to be vectors of the most important stain fungus in New Zealand, *D. pinea*, I still found a rich flora of ophiostomatoid fungi associated with all three species (*H. ater*, *H. ligniperda*, and *Pachycotes peregrinus*). These fungal species did not contribute significantly to stain levels of windthrown timber in this study, but they have been shown to be vectored to *P. radiata* seedlings in New Zealand and even cause seedling death (Reay et al. 2001, Reay et al. 2002, Reay et al. 2005). Although seedling mortality is generally low, large numbers of seedlings can be damaged by the beetle feeding around the root collar. Under certain circumstances, such as drought or sub-lethal damage by bark beetles, the pathogenicity of vectored ophiostomatoid fungi may become an issue and contribute to seedling growth inhibition, or death (Desprez-Loustau et al. 2006).

Pachycotes peregrinus is a native wood borer that attacks all exotic softwoods, as well as native softwoods, such as *Podocarpus ferrugineus* (miro), *Dacrydium cupressinum* (rimu) and

Agathis australis (kauri) among others (Bain 1977b). In this thesis, *P. peregrinus* was found to vector ophiostomatoid fungi for the first time. As *P. peregrinus* is a non host-specific species, there is the potential for this species to transmit both exotic pathogens to native trees, and native pathogens to exotic trees. Both *H. ater* and *H. ligniperda* are not known to colonise native trees (Brockerhoff et al. 2003), so the potential for *P. peregrinus* to be a vector of these exotic (and native) pathogenic fungi could have effects on both native biodiversity, and economic productivity of plantation forests.

Such issues are not commonly considered in plantation forest management within a wider landscape context, and there is an unappreciated wealth of species- and interaction-diversity among these production systems. Methods to identify the presence of such interactions, from biosecurity, pathology, and forestry points of view are crucial in order to effectively protect these important aspects of both the native landscape, and forest economy.

5.4 Rapid screening of bark beetles for ophiostomatoid fungi

A new method to identify fungal species on bark beetles, or indeed any substrate, was developed in this thesis. Extremely precise high resolution melt (HRM) technology was used in order to identify fungal species on a particular beetle, from a pre-defined library of known fungal species. These known species formed a library, whose exact DNA melt temperatures were known, and these were compared with the melt points of unknown fungi grown from beetles in conjunction with specially designed primers.

This new technique is thought to have a broad suite of applications such as biosecurity screening of plant and insect matter, identification of pathogenic fungi from trees, and confirmation of species identification. It has a range of advantages: it only costs approximately 50 c per beetle to process (in NZ dollars, 2011), the procedure is rapid and results can be obtained within just a few hours, and it is safer than usual methods of identification via gel electrophoresis where hazardous substances such as ethidium bromide may be used. A large scale application of the technique to assess the differences of fungal populations on different

beetle species, and different populations of the same beetle species, is under way, however results were not available in time for publication of this thesis.

5.5 Management impacts from varying rates of sapstain fungal colonisation of fallen timber

In the experimental windthrow study there were marked differences in the extent of sapstain development between the two climatically different regions, Nelson and Canterbury, and there were equally marked differences in the rate of sapstain onset depending on the seasonal occurrence of the (simulated) windthrow. Sapstain onset is not simply a time-dependent relationship, but depends on seasonal temperatures that influence the drying rate of the wood. For example, following a storm in the Nelson region during summer, less than three months would be available to salvage-harvest damaged trees before average sapstain cover is greater than 60 %, where its value is reduced considerably. Consequently, it is recommended that salvage harvest operations are commenced immediately, with the most valuable stands harvested first. Conversely, after the same period of time following a summer storm in Canterbury, the average stain cover would only reach 11.3 % [...and what would that mean for economic loss?]. Following storms in winter, stain levels remained very low (~0 %) for at least six months in both regions.

The strong context-dependence in sapstain development might suggest that management guidelines for windthrown timber would be difficult to develop, or would need to be specific to regions and seasons. While this might be true to an extent, there does appear to be a fairly simple deterministic relationship between tree moisture content and sapstain growth that might underlie the observed variation in sapstain development. There was also a moisture content threshold (~100%) above which sapstain did not occur, but once moisture content fell below that, stain developed in response to temperature. Due to the warmer temperatures of summer and spring, windthrown logs dried out faster, and therefore were more heavily and quickly stained than those that fall during winter and autumn. [need an extra sentence saying that both

regions seem to follow a remarkably similar relationship between moisture and sapstain]. There was some uncertainty as to the reasons for faster moisture loss and faster sapstain development in Nelson than Canterbury. The expectation might have been that trees felled in Bottle Lake should have developed stain at a faster rate than those in Golden Downs due to higher temperatures and lower rainfall, but this was not the case. Felled trees in Nelson had consistently faster levels of drying and higher levels of stain, although the factors driving this remain unknown.

Although bark beetles were present at all sites, they did not have a significant effect on stain development in either region. These beetles were predominantly present during the warmer months of spring, summer, and autumn. Actual bark beetle attack of fallen logs was relatively rare. The physical effects of branches holding logs from the ground during windthrow prevent mass-colonisation by bark beetles in New Zealand for this reason, but this is unlikely to be the case in countries with more aggressive species of bark beetle, such as *Dendroctonus ponderosae*. Perhaps because of the limited beetle attack, the main stain species isolated was *D. pinea* – a non insect-vectored fungus, which accounted for 96.4 % and 95.3 % of all stain fungi isolated from Golden Downs and Bottle Lake, respectively. Eight species of ophiostomatoid fungi were also isolated from wood in this study, but they were all rare. This is consistent with other studies of its type in Nelson (McCarthy et al. 2010), and also with the non-significant effect of bark beetles on stain development , except when logs are in direct contact with the ground.

5.6 Overall synthesis, and suggested future research

Bark beetles are vectors of sapstain fungi in New Zealand, however their behaviour and attack strategies reduce their impact as stain vectors affecting windthrown timber. The stain fungi are carried on the beetles exoskeleton, or in the gut contents. There is also the potential that bark damage caused by beetle tunnelling is providing access to *D. pinea* who are the main culprits of sapstain in this context. The attack patterns of *H. ater* and *H. ligniperda* suggest they would be

more problematic to logs in ground contact and those stacked following harvesting once their branches are removed. Salvage harvest of wind damaged stands should be immediate if the damage occurs during summer. If the damage occurs at any other point of time during the year, logs will develop very low levels of stain until the following summer, when stain development will increase rapidly.

Many questions have arisen from this set of initial experiments. These questions provide the basis for some suggestions of future research directions in four key areas, which are listed below:

1. Why are there differences in the rate of sapstain onset between regions? Is it solely a temperature- and nutrient-driven process, or are other environmental factors important such as rainfall or propagule density in the landscape? What are the sources of inoculum for wind and rain dispersed species, and how do they differ regionally?
2. What is the importance of other insects as sapstain vectors, and what groups are contributing? It is possible that there are insect groups that are equally, or even more important than bark beetles as vectors of sapstain fungi, even though this possibility has received little attention so far.
3. What contributions do endophytic fungi make to sapstain development, in comparison to those dispersed by wind or rain?
4. What effect would border-incursion of an aggressive bark beetle species, that does colonise standing trees and fallen trees that aren't in ground contact, have on the rate of sapstain development following windthrow?

References

Aghayeva, D. N., M. J. Wingfield, Z. W. de Beer, and T. Kirisits. 2004. Two new *Ophiostoma* species with *Sporothrix* anamorphs from Austria and Azerbaijan. Mycologia **96**:866-878.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. Journal of Molecular Biology **215**:403-410.

Andree, K. B., M. Fernández-Tejedor, L. M. Elandaloussi, S. Quijano-Scheggia, N. Sampedro, E. Garcés, J. Camp, and J. Diogène. 2011. Quantitative PCR coupled with melt curve analysis for detection of selected *Pseudo-nitzschia* spp. (Bacillariophyceae) from the northwestern Mediterranean Sea. Applied and Environmental Microbiology **77**:1651-1659.

Anon. 2011a. New Zealand Fire Weather. Date accessed: 20/10/2011. http://www.nrfa.org.nz/fire_weather/weather/20080611/HTML/RawData/RawData.asp

Anon. 2011b. New Zealand Forest Industry; Facts and Figures 2010/2011. New Zealand Forest Owners Association and Ministry for Agriculture and Forestry, Wellington.

Aukema, J. E., D. G. McCullough, B. V. Holle, A. M. Liebhold, K. Britton, and S. J. Frankel. 2010. Historical accumulation of nonindigenous forest pests in the continental United States. Bioscience **60**:886-897.

Ayres, M. P., R. T. Wilkens, J. J. Ruel, M. J. Lombardero, and E. Vallery. 2000. Nitrogen budgets of phloem-feeding bark beetles with and without symbiotic fungi. Ecology **81**:2198-2210.

Bain, J. 1977a. *Hylurgus ligniperda* (Fabricius) (Coleoptera: Scolytidae). New Zealand Forest Service, Forest and Timber Insects of New Zealand No. 18.

Bain, J. 1977b. *Pachycotes peregrinus* (Chapuis) (Coleoptera: Scolytidae). New Zealand Forest Service, Forest and Timber Insects in New Zealand No. 19.

Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, J. Farrar, J. E. G. Good, R. Harrington, S. Hartley, T. H. Jones, R. L. Lindroth, M. C. Press, I. Symrnioudis, A. D. Watt, and J. B. Whittaker. 2002. Herbivory in global climate change research: Direct effects of rising temperature on insect herbivores. Global Change Biology **8**:1-16.

Ballard, R. G., M. A. Walsh, and W. E. Cole. 1982. Blue-stain fungi in xylem of lodgepole pine: a light-microscope study on extent of hyphal distribution. Canadian Journal of Botany **60**:2334 - 2341.

Barcenas, N. M., T. R. Unruh, and L. G. Neven. 2005. DNA diagnostics to identify internal feeders (Lepidoptera: Tortricidae) of pome fruits of quarantine importance. Journal of Economic Entomology **98**:299-306.

Bates, D., M. Machler, and B. Bolker. 2011. Linear mixed-effects models using S4 classes. R package version 0.999375-41. http://CRAN.R-project.org/package=lme4.

Batra, L. R. 1963. Ecology of ambrosia fungi and their dissemination by beetles. Transactions of the Kansas Academy of Science (1903-) **66**:213-236.

Battisti, A., M. Stastny, S. Netherer, C. Robinet, A. Schopf, A. Roques, and S. Larsson. 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. Ecological Applications **15**:2084-2096.

Beal, E., J. F. Webber, and R. A. Eaton. 2010. Comparative susceptibility of pine, spruce and larch to sapstain. Forest Pathology **40**:116-128.

Beaver, R. A. 1989. Insect-fungus relationships in the bark and ambrosia beetles. Pages 121-143 *in* N. Wilding, N. M. Collins, P. M. Hammond, and J. F. Webber, editors. Insect-Fungus Interactions. London: Academic.

Berry, O. and S. D. Sarre. 2007. Gel-free species identification using melt-curve analysis. Molecular Ecology Notes **7**:1-4.

Bihon, W., B. Slippers, T. Burgess, M. J. Wingfield, and B. D. Wingfield. 2011. Sources of *Diplodia pinea* endophytic infections in *Pinus patula* and *P. radiata* seedlings in South Africa. Forest Pathology **41**:370-375.

Bleiker, K. P., S. E. Potter, C. R. Lauzon, and D. L. Six. 2009. Transport of fungal symbionts by mountain pine beetles. Canadian Entomologist **141**:503-514.

Brockerhoff, E. G., L. A. Berndt, and H. Jactel. 2005. Role of exotic pine forests in the conservation of the critically endangered New Zealand ground beetle *Holcaspis brevicula* (Coleoptera: Carabidae). New Zealand Journal of Ecology **29**:37-43.

Brockerhoff, E. G., H. Jactel, J. A. Parrotta, C. P. Quine, and J. Sayer. 2008. Plantation forests and biodiversity: oxymoron or opportunity? Biodiversity and Conservation **17**:925-951.

Brockerhoff, E. G., D. C. Jones, M. O. Kimberley, D. M. Suckling, and T. Donaldson. 2006a. Nationwide

survey for invasive wood-boring and bark beetles (Coleoptera) using traps baited with pheromones and kairomones. Forest Ecology and Management **228**:234-240.
Brockerhoff, E. G., M. Knízek, and J. Bain. 2003. Checklist of indigenous and adventive bark and ambrosia beetles (Curculionidae: Scolytinae and Platypodinae) of New Zealand and interceptions of exotic species (1952-2000). New Zealand Entomologist **26**:29-44.
Brockerhoff, E. G., A. M. Liebhold, and H. Jactel. 2006b. The ecology of forest insect invasions and advances in their management. Canadian Journal of Forest Research **36**:263-268.
Brook, B. W., N. S. Sodhl, and P. K. L. Ng. 2003. Catastrophic extinctions follow deforestation in Singapore. Nature **424**:420-423.
Brookhouser, L. W. and G. W. Peterson. 1971. Infection of Austrian Scots, and Ponderosa Pines by *Diplodia pinea*. Phytopathology **61**.
Brooks, T. M., R. A. Mittermeier, C. G. Mittermeier, G. A. B. Da Fonseca, A. B. Rylands, W. R. Konstant, P. Flick, J. Pilgrim, S. Oldfield, G. Magin, and C. Hilton-Taylor. 2002. Habitat loss and extinction in the hotspots of biodiversity. Conservation Biology **16**:909-923.
Bruns, T. D. and M. Gardes. 1993. Molecular tools for the identification of ectomycorrhizal fungi-taxon-specific oligonucleotide probes for suilloid fungi. Molecular Ecology **2**:233-242.
Bueno, A., J. J. Diez, and M. M. Fernandez. 2010. Ophiostomatoid fungi transported by *Ips sexdentatus* (Coleoptera; Scolytidae) in Pinus pinaster in NW Spain. Silva Fennica **44**:387-397.
Burnham, K. P. and D. R. Anderson. 2002. Model selection and multi-model inference: a practical information-theoretic approach. Second edition. Springer, New York.
Cenis, J. L. 1992. Rapid extraction of fungal DNA for PCR amplification. Nucleic Acids Research **20**:2380.
Childs, B. H. 1966. Salvage of a windthrown forest. New Zealand Journal of Forestry **11**:66-81.
Chow, C. K. S. 1984. *Diplodia* leader dieback, *Diplodia* crown wilt, and *Diplodia* whorl canker. New Zealand Forest Research Institute, Forest Pathology in New Zealand No. 7.
Chung, W. H., J. J. Kim, Y. Yamaoka, A. Uzunovic, H. Masuya, and C. Breuil. 2006. *Ophiostoma breviusculum* sp. nov. (Ophiostomatales, Ascomycota) is a new species in the *Ophiostoma piceae* complex associated with bark beetles infesting larch in Japan. Mycologia **98**:801-814.
Ciesla, W. M. 1988. Pine bark beetles: a new pest management challenge for Chilean foresters Journal of Forestry **86**:27-31.
Clark, A. F. 1932. The pine-bark beetle, *Hylastes ater*, in New Zealand. New Zealand Journal of Science and Technology **14**:1-20.
Clifton, N. C. 1978. Sprinkler storage of windblows proves effective and economic. World Wood **19**:26-27.
Colley, R. H. and C. T. Rumbold. 1930. Relation between moisture content of the wood and blue stain in Loblolly pine. Journal of Agricultural Research **41**:389-399.
Cooper, P.c2000. Validation of the sapstain danger index. University of Waikato, Hamilton.
Cramer, V. A., R. J. Hobbs, and R. J. Standish. 2008. What's new about old fields? Land abandonment and ecosystem assembly. Trends in Ecology and Evolution **23**:104-112.
Cudmore, T. J., N. Bjorklund, A. L. Carroll, and B. S. Lindgren. 2010. Climate change and range expansion of an aggressive bark beetle: Evidence of higher beetle reproduction in native host tree populations. Journal of Applied Ecology **47**:1036-1043.
de Beer, Z. W., T. C. Harrington, H. F. Vismer, B. D. Wingfield, and M. J. Wingfield. 2003a. Phylogeny of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia **95**:434-441.
de Beer, Z. W., B. D. Wingfield, and M. J. Wingfield. 2003b. The *Ophiostoma piceae* complex in the Southern Hemisphere: A phylogenetic study. Mycological Research **107**:469-476.
de Hoog, G. S. 1993. *Sporothrix*-like anamorphs of *Ophiostoma* species and other fungi. Pages 53-60 *in* M. J. Wingfield, K. A. Seifert, and J. F. Webber, editors. *Ceratocyctis* and *Ophiostoma*: taxonomy, ecology and pathogenicity. The American Phytopathological Society, St. Paul, Minnesota.
de Wet, J., T. Burgess, B. Slippers, O. Preisig, B. D. Wingfield, and M. J. Wingfield. 2003. Multiple gene genealogies and microsatellite markers reflect relationships between morphotypes of *Sphaeropsis sapinea* and distinguish a new species of *Diplodia*. Mycological Research **107**:557-566.
de Wet, J., M. J. Wingfield, T. Coutinho, and B. D. Wingfield. 2002. Characterisation of the 'C' morphotype of the pine pathogen *Sphaeropsis sapinea*. Forest Ecology and Management **161**:181-188.
de Wet, J., M. J. Wingfield, T. A. Coutinho, and B. D. Wingfield. 2000. Characterization of *Sphaeropsis sapinea* isolates from South Africa, Mexico, and Indonesia. Plant Disease **84**:151-156.
Desprez-Loustau, M. L., B. Marçais, L. M. Nageleisen, D. Piou, and A. Vannini. 2006. Interactive effects of drought and pathogens in forest trees. Annals of Forest Science **63**:597-612.
Dinoor, A. and N. Eshed. 1984. The role and importance of pathogens in natural plant communities.

Annual Review of Phytopathology **22**:443-466.
Eagen, R., A. Brisson, and C. Breuil. 1997. The sap-staining fungus *Ophiostoma piceae* synthesizes different types of melanin in different growth media. Canadian Journal of Microbiology **43**:592-595.
Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate extremes: Observations, modeling, and impacts. Science **289**:2068-2074.
Elston, D. A., R. Moss, T. Boulinier, C. Arrowsmith, and X. Lambin. 2001. Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. Parasitology **122**:563-569.
Emanuel, K. 2005. Increasing destructiveness of tropical cyclones over the past 30 years. Nature **436**:686-688.
Erbilgin, N., T. R. Gordon, D. L. Wood, and A. J. Storer. 2009. Bark beetle-mediated fungal infections of susceptible trees induce resistance to subsequent infections in a dose dependent manner. Agricultural and Forest Entomology **11**:255-263.
Erbilgin, N., A. J. Storer, D. L. Wood, and T. R. Gordon. 2005. Colonization of cut branches of five coniferous hosts of the pitch canker fungus by *Pityophthorus* spp. (Coleoptera: Scolytidae) in central, coastal California. Canadian Entomologist **137**:337-349.
Farrell, B. D., A. S. Sequeira, B. C. O'Meara, B. B. Normark, J. H. Chung, and B. H. Jordal. 2001. The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). Evolution **55**:2011-2027.
Farrell, R. L., S. M. Duncan, A. P. Ram, S. J. Kay, E. Hadar, Y. Hadar, R. A. Blanchette, T. C. Harrington, and D. McNew. 1997. Causes of sapstain in New Zealand.*in* B. Kreber, editor. Strategies for Inproving Protection of Logs and Lumber. New Zealand Forest Research Institute Limited, Rotorua, New Zealand.
Finnigan, J. J. and Y. Brunet. 1995. Turbulent airflow in forests on flat and hilly terrain. Pages 3-40 *in* M. P. Coutts and J. Grace, editors. Wind and Trees. Cambridge University Press, Cambridge.
Flechtmann, C. A. H., M. J. Dalusky, and C. W. Berisford. 1999. Bark and ambrosia beetle (Coleoptera: Scolytidae) responses to volatiles from aging loblolly pine billets. Environmental Entomology **28**:638-348.
Fleet, C., C. Breuil, and A. Uzunovic. 2001. Nutrient consumption and pigmentation of deep and surface colonizing sapstaining fungi in *Pinus contorta*. Holzforschung **55**:340-346.
Flowers, J., E. Nuckles, J. Hartman, and L. Vaillancourt. 2001. Latent infection of Austrian and Scots pine tissues by *Sphaeropsis sapinea*. Plant Disease **85**:1107-1112.
Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science **309**:570-574.
Francke-Grosmann, H. 1967. Ectosymbiosis in Wood-Inhabiting Insects. Pages 141-205 *in* M. Henry, editor. Symbiosis. Academic Press, New York.
Franklin, J. F., T. A. Spies, R. V. Pelt, A. B. Carey, D. A. Thornburgh, D. R. Berg, D. B. Lindenmayer, M. E. Harmon, W. S. Keeton, D. C. Shaw, K. Bible, and J. Chen. 2002. Disturbances and structural development of natural forest ecosystems with silvicultural implications, using Douglas-fir forests as an example. Forest Ecology and Management **155**:399-423.
Gandhi, K. J. K., D. W. Gilmore, S. A. Katovich, W. J. Mattson, J. C. Zasada, and S. J. Seybold. 2008. Catastrophic windstorm and fuel-reduction treatments alter ground beetle (Coleoptera: Carabidae) assemblages in a North American sub-boreal forest. Forest Ecology and Management **256**:1104-1123.
Gebeyehu, S., B. P. Hurley, and M. J. Wingfield. 2005. A new lepidopteran insect pest discovered on commercially grown Eucalyptus nitens in South Africa. South African Journal of Science **101**:26-28.
Gibbs, J. N. 1993. The biology of Ophiostomatoid fungi causing sapstain in trees and freshly cut logs. Pages 153-160 *in* M. J. Wingfield, K. A. Seifert, and J. F. Webber, editors. *Ceratocyctis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. American Phytopathological Society, St. Paul.
Gibbs, J. N. and A. Inman. 1991. The pine shoot beetle *Tomicus piniperda* as a vector of blue stain fungi to windblown pine. Forestry **64**:239-249.
Glass, N. L. and G. C. Donaldson. 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology **61**:1323-1330.
Grayson, A. J. 1989. The 1987 Storm: Impacts and Responses. HMSO, London.
Grulke, N. E. 2011. The nexus of host and pathogen phenology: Understanding the disease triangle with

climate change. New Phytologist **189**:8-11.
Haack, R. A. 2006. Exotic bark- and wood-boring Coleoptera in the United States: Recent establishments and interceptions. Canadian Journal of Forest Research **36**:269-288.
Halmschlager, E. and T. Kowalski. 2003. *Sporothrix inflata*, a root-inhabiting fungus of *Quercus robur* and *Q. petraea*. Mycological Progress **2**:259-266.
Harrington, T. C. 1981. Cycloheximide sensitivity as a taxonomic character in ceratocystis Mycologia **73**:1123-1129.
Harrington, T. C. 1993. Diseases of Conifers Caused by Species of *Ophiostoma* and *Leptographium*. Pages 161-172 *in* M. J. Wingfield, K. A. Seifert, and J. F. Webber, editors. *Ceratocyctis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. The American Phytopathological Society, St. Paul.
Harrington, T. C. 2005. Ecology and Evolution of Mycophagous Bark Beetles and Their Fungal Partners. Pages 257-291 *in* F. E. Vega and M. Blackwell, editors. Insect-fungal Associations: Ecology and Evolution. Oxford University Press, New York.
Harrington, T. C., D. McNew, J. Steimel, D. Hofstra, and R. Farrell. 2001. Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia **93**:111-136.
Hobbs, R. J., S. Arico, J. Aronson, J. S. Baron, P. Bridgewater, V. A. Cramer, P. R. Epstein, J. J. Ewel, C. A. Klink, A. E. Lugo, D. Norton, D. Ojima, D. M. Richardson, E. W. Sanderson, F. Valladares, M. Vilà, R. Zamora, and M. Zobel. 2006. Novel ecosystems: Theoretical and management aspects of the new ecological world order. Global Ecology and Biogeography **15**:1-7.
Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology and Evolution **24**:599-605.
Hood, I. A. 1989. Armillaria root disease in New Zealand forests. New Zealand Journal of Forestry Science **19**:180-197.
Hood, I. A. and M. Ramsden. 1997. Sapstain and decay following fire in stands of *Pinus elliottii* var. *elliottii* near Beerburrum, south east Queensland. Australian Forestry **60**:7-15.
Hosking, G. 1977. Insect survey in the Canterbury windthrow. Pages 1-4 What's New in Forest Research. New Zealand Forest Research Institute, Rotorua, New Zealand.
Hsu, M. C., K. W. Chen, H. J. Lo, Y. C. Chen, M. H. Liao, Y. H. Lin, and S. Y. Li. 2003. Species identification of medically important fungi by use of real-time LightCycler PCR. Journal of Medical Microbiology **52**:1071-1076.
Hulcr, J. and R. R. Dunn. 2011. The sudden emergence of pathogenicity in insect-fungus symbioses threatens naive forest ecosystems. Proceedings of the Royal Society B: Biological Sciences **278**:2866-2873.
Jacobs, K. and K. A. Seifert. 2004. Fungi Canadenses No. 347 *Pesotum fragrans*. Canadian Journal of Plant Pathology **26**:79-80.
Jacobs, K., K. A. Seifert, K. J. Harrison, and T. Kirisits. 2003. Identity and phylogenetic relationships of ophiostomatoid fungi associated with invasive and native *Tetropium* species (Coleoptera: Cerambycidae) in Atlantic Canada. Canadian Journal of Botany **81**:316-329.
Jacobs, K. and M. J. Wingfield. 2001. *Leptographium* Species: Tree Pathogens, Insect Associates, and Agents of Blue-Stain. The American Phytopathological Society, St. Paul.
Jankowiak, R. and M. Kolařík. 2010. Fungi associated with the fir bark beetle *Cryphalus piceae* in Poland. Forest Pathology **40**:133-144.
Karban, R. and S. Y. Strauss. 2004. Physiological tolerance, climate change, and a northward range shift in the spittlebug, *Philaenus spumarius*. Ecological Entomology **29**:251-254.
Kavanagh, S., C. Brennan, L. O'Connor, S. Moran, R. Salas, J. Lyons, J. Silke, and M. Maher. 2010. Real-time PCR detection of *Dinophysis* species in Irish coastal waters. Marine Biotechnology **12**:534-542.
Kerr, J. L.c2010. Olfactory and visual cues in host finding by bark beetles. University of Canterbury, Christchurch.
Khadempour, L., S. Massoumi Alamouti, R. Hamelin, J. Bohlmann, and C. Breuil. 2010. Target-specific PCR primers can detect and differentiate ophiostomatoid fungi from microbial communities associated with the mountain pine beetle *Dendroctonus ponderosae*. Fungal Biology **114**:825-833.
Kim, G. H., J. J. Kim, and C. Brueuil. 2007. Sap-staining fungi from logs and boards of two commercially important pines in Korea. Holzforschung **61**:333-336.
Kim, G. H., J. J. Kim, W. L. Young, and C. Breuil. 2005a. Ophiostomatoid fungi isolated from *Pinus radiata* logs imported from New Zealand to Korea. Canadian Journal of Botany **83**:272-278.
Kim, J. J., Y. W. Lim, K. A. Seifert, S. H. Kim, C. Breuil, and G. H. Kim. 2005b. Taxonomy of *Ophiostoma radiaticola* sp. nov. (Ophiostomatales, Ascomycetes), the teleomorph of *Pesotum pini*, isolated from logs of *Pinus radiata*. Mycotaxon **91**:481-496.

Kim, S., T. C. Harrington, J. C. Lee, and S. J. Seybold. 2011. *Leptographium tereforme* sp. nov. and other Ophiostomatales isolated from the root-feeding bark beetle *Hylurgus ligniperda* in California. Mycologia **103**:152-163.
Kim, S. H., A. Uzunovic, and C. Breuil. 1999. Rapid detection of *Ophiostoma piceae* and *O. quercus* in stained wood by PCR. Applied and Environmental Microbiology **65**:287-290.
Kimberley, M. O., I. A. Hood, and J. F. Gardner. 2002. Armillaria root disease of Pinus radiata in New Zealand. 6: Growth loss. New Zealand Journal of Forestry Science **32**:148-162.
Klepzig, K. D. and R. T. Wilkens. 1997. Competitive interactions among symbiotic fungi of the southern pine beetle. Applied and Environmental Microbiology **63**:621-627.
Komonen, A., L. M. Schroeder, and J. Weslien. 2011. *Ips typographus* population development after a severe storm in a nature reserve in southern Sweden. Journal of Applied Entomology **135**:132-141.
Kramer, M. G., A. J. Hansen, M. L. Taper, and E. J. Kissinger. 2001. Abiotic controls on long-term windthrow disturbance and temperate rain forest dynamics in southeast Alaska. Ecology **82**:2749-2768.
Kurz, W. A., C. C. Dymond, G. Stinson, G. J. Rampley, E. T. Neilson, A. L. Carroll, T. Ebata, and L. Safranyik. 2008. Mountain pine beetle and forest carbon feedback to climate change. Nature **452**:987-990.
Kuuluvainen, T. 1994. Gap disturbance, ground microtopography, and the regeneration dynamics of boreal coniferous forests in Finland: a review. Annales Zoologici Fennici **31**:35-51.
Lagerberg, T., G. Lundberg, and E. Melin. 1927. Biological and practical researches into blueing in pine and spruce. Svenska skogsvardsfforeningens tidskrift (Journal of the Swedish Forestry Society) **25**:561-739.
Lanfranco, D., S. Ide, and H. Peredo. 2004. An analysis of health risk reduction in Chilean primary forest products for export. Forestry **77**:193-203.
Lang, K. D., L. A. Schulte, and G. R. Guntenspergen. 2009. Windthrow and salvage logging in an old-growth hemlock-northern hardwoods forest. Forest Ecology and Management **259**:56-64.
Leach, J. G., L. W. Orr, and C. Christensen. 1934. The inter-relationships of bark beetles and bluestaining fungi on felled Norway pine timber. Journal of Agricultural Research **49**:315-342.
Leahy, M. J. A., T. H. Oliver, and S. R. Leather. 2007. Feeding behaviour of the black pine beetle, *Hylastes ater* (Coleoptera: Scolytidae). Agricultural and Forest Entomology **9**:115-124.
Leather, S. R., K. R. Day, and A. N. Salisbury. 1999. The biology and ecology of the large pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): A problem of dispersal? Bulletin of Entomological Research **89**:3-16.
Lee, K. and J. N. Gibbs. 1996. An investigation of the influence of harvesting practice on the development of blue-stain in Corsican pine logs. Forestry **69**:137-141.
Liese, W. 1984. Wet storage of windblown conifers in Germany. New Zealand Journal of Forestry **29**:119-135.
Lindenmayer, D. B., D. R. Foster, J. F. Franklin, M. L. Hunter, R. F. Noss, F. A. Schmiegelow, and D. Perry. 2004. Salvage Harvesting Policies after Natural Disturbance. Science **303**:1303.
Lindenmayer, D. B. and R. F. Noss. 2006. Salvage logging, ecosystem processes, and biodiversity conservation. Conservation Biology **20**:949-958.
Lindenmayer, D. B. and K. Ough. 2006. Salvage logging in the montane ash eucalypt forests of the Central Highlands of Victoria and its potential impacts on biodiversity. Conservation Biology **20**:1005-1015.
Linnakoski, R., Z. W. De Beer, J. Ahtiainen, E. Sidorov, P. Niemela, A. Pappinen, and M. J. Wingfield. 2010. *Ophiostoma* spp. associated with pine- and spruce-infesting bark beetles in Finland and Russia. Persoonia - Molecular Phylogeny and Evolution of Fungi **25**:72-93.
Littlejohn, R. N. 1984. Extreme winds and forest deforestation resulting from Cyclone "Bernie". Weather and Climate **4**:47-52.
Lu, M., M. J. Wingfield, N. E. Gillette, S. R. Mori, and J. H. Sun. 2010. Complex interactions among host pines and fungi vectored by an invasive bark beetle. New Phytologist **187**:859-866.
Luchi, N., N. Pratesi, L. Simi, M. Pazzagli, P. Capretti, A. Scala, B. Slippers, and P. Pinzani. 2011. High-Resolution Melting Analysis: a new molecular approach for the early detection of *Diplodia pinea* in Austrian pine. Fungal Biology **115**:715-723.
Malloch, D. and M. Blackwell. 1993. Dispersal Biology of the Ophiostomatoid Fungi. Pages 195-206 *in* M. J. Wingfield, K. A. Seifert, and J. F. Webber, editors. *Ceratocyctis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. American Phytopathological Society, St. Paul.
Marmolejo, J. G. and H. Butin. 1990. New conifer-inhabiting species of *Ophiostoma* and *Ceratocystiopsis* (Ascomycetes, Microascales) from Mexico. Sydowia **42**:193-199.
Martin, T. J. and J. Ogden. 2006. Wind damage and response in New Zealand forests: A review. New

Zealand Journal of Ecology **30**:295-310.
Masuya, H., Y. Yamaoka, S. Kaneko, and Y. Yamaura. 2009. Ophiostomatoid fungi isolated from Japanese red pine and their relationships with bark beetles. Mycoscience **50**:212-223.
Mathiesen, A. 1950. Über einige mit Borkenkäfern assoziierte Bläuepilze in Schweden. Oikos **2**:275-308.
Matusick, G. and L. G. Eckhardt. 2010. The pathogenicity and virulence of four Ophiostomatoid fungi on young Longleaf pine trees. Canadian Journal of Plant Pathology **32**:170-176.
Mausel, D. L., R. I. Gara, D. Lanfranco, C. Ruiz, S. Ide, and R. Azat. 2007. The introduced bark beetles *Hylurgus ligniperda* and *Hylastes ater* (Coleoptera: Scolytidae) in Chile: Seasonal flight and effect of *Pinus radiata* log placement on colonization. Canadian Journal of Forest Research **37**:156-169.
McCarthy, J. K., I. A. Hood, E. G. Brockerhoff, C. A. Carlson, S. M. Pawson, M. Forward, K. Walbert, and J. F. Gardner. 2010. Predicting sapstain and degrade in fallen trees following storm damage in a *Pinus radiata* forest. Forest Ecology and Management **260**:1456-1466.
MEA. 2005. Millennium Ecosystem Assessment. Island Press, Washington, DC.
Milligan, R. H. 1978. *Hylastes ater* (Paykull) (Coleoptera: Scolytidae). New Zealand Forest Service, Forest and Timber Insects on New Zealand No. 29.
Mitchell, S. J. 1995. A synopsis of wind throw in Britich Colombia: occurrence, implications, assessment and management.*in* M. P. Coutts and J. Grace, editors. Wind and Trees. Cambridge University Press, Cambridge.
Nieuwenhuis, M. and P. J. Fitzpatrick. 2002. An assessment of stem breakage and the reduction in timber volume and value recovery resulting from a catastrophic storm: An irish case study. Forestry **75**:513-523.
Nieuwenhuis, M. and E. O'Connor. 2001. Financial impact evaluation of catastrophic storm damage in Irish forestry: A case study. I. Stumpage losses. Forestry **74**:369-381.
Örlander, G., U. Nilsson, and G. Nordlander. 1997. Pine weevil abundance on clear-cuttings of different ages: A 6-year study using pitfall traps. Scandinavian Journal of Forest Research **12**:225-240.
Owen, D. R., K. Q. Lindahl, D. L. Wood, and J. R. Parmeter. 1987. Pathogenicity of fungi isolated from *Dendroctonus valens*, *D. brevicomisa*, and *D. ponderosae* to pine seedlings. Phytopathology **77**:631-636.
Paciura, D., X. D. Zhou, Z. W. De Beer, K. Jacobs, H. Ye, and M. J. Wingfield. 2010. Characterisation of synnematous bark beetle-associated fungi from China, including *Graphium carbonarium* sp. nov. Fungal Diversity:1-14.
Paine, R. T., M. J. Tegner, and E. A. Johnson. 1998. Compounded Perturbations Yield Ecological Surprises. Ecosystems **1**:535-545.
Paine, T. D., K. F. Raffa, and T. C. Harrington. 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annual Review of Entomology **42**:179-206.
Palmer, M. A., R. E. McRoberts, and T. H. Nicholls. 1988. Sources of inoculum of *Sphaerosis sapinea* in forest tree nurseries. Phytopathology **78**.
Panayotov, M., D. Kulakowski, L. Laranjeiro Dos Santos, and P. Bebi. 2011. Wind disturbances shape old Norway spruce-dominated forest in Bulgaria. Forest Ecology and Management **262**:470-481.
Parker, I. M. and G. S. Gilbert. 2004. The evolutionary ecology of novel plant-pathogen interactions. Pages 675-700.
Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Pages 637-669.
Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature **421**:37-42.
Pawson, S. M., E. G. Brockerhoff, E. D. Meenken, and R. K. Didham. 2008. Non-native plantation forests as alternative habitat for native forest beetles in a heavily modified landscape. Biodiversity and Conservation **17**:1127-1148.
Pawson, S. M. and M. S. Watt. 2009. An experimental test of a visual-based push-pull strategy for control of wood boring phytosanitary pests. Agricultural and Forest Entomology **11**:239-245.
Pawson, S. M., M. S. Watt, and E. G. Brockerhoff. 2009. Using differential responses to light spectra as a monitoring and control tool for *Arhopalus ferus* (Coleoptera: Cerambycidae) and other exotic wood-boring pests. Journal of Economic Entomology **102**:79-85.
Paynter, Q. E., O. Anderbrant, and F. Schlyter. 1990. Behavior of male and female spruce bark beetles, *Ips typographus*, on the bark of host trees during mass attack. Journal of Insect Behavior **3**:529-543.
Peay, K. G., P. G. Kennedy, and T. D. Bruns. 2008. Fungal community ecology: A hybrid beast with a molecular master. Bioscience **58**:799-810.
Powell, M. A., R. A. Eaton, and J. F. Webber. 1995. The role of micro-arthropods in the defacement of sawn lumberby sapstain and mould fungi. Canadian Journal of Forest Research **25**:1148-1156.
Raffa, K. F., T. W. Phillips, and S. M. Salom. 1993. Strategies and mechanisms of host colonization by

bark beetles.*in* T. D. Schowalter and G. M. Filip, editors. Beetle-Pathogen Interactions in Conifer Forests. Academic Press, San Diego.
Reay, S. D., J. M. Thwaites, and R. L. Farrell. 2005. A survey of Ophiostoma species vectored by *Hylastes ater* to pine seedlings in New Zealand. Forest Pathology **35**:105-113.
Reay, S. D., J. M. Thwaites, and R. L. Farrell. 2006a. Survey of Ophiostomataceae associated with *Hylurgus ligniperda* (Curculionidae: Scolytinae) in New Zealand. New Zealand Entomologist **29**:21-26.
Reay, S. D., J. M. Thwaites, R. L. Farrell, and T. R. Glare. 2006b. The lack of persistence of Ophiostomataceae fungi in *Pinus radiata* 3 years after damage by the bark beetle *Hylastes ater*, and the subsequent colonisation by *Sphareopsis sapinea*. Forest Ecology and Management **233**:149-152.
Reay, S. D., J. M. Thwaites, R. L. Farrell, and P. J. Walsh. 2001. The role of the bark beetle, *Hylastes ater* (Coleoptera: Scolytidae), as a sapstain fungi vector to *Pinus radiata* seedlings: A crisis for the New Zealand forestry industry? Integrated Pest Management Reviews **6**:283-291.
Reay, S. D. and P. J. Walsh. 2001. Observations of the flight activity of *Hylastes ater* and *Hylurgus ligniperda* (Curculionidae: Scolytinae) in *Pinus radiata* forests in the central North Island, New Zealand. New Zealand Entomologist **214**:79-85.
Reay, S. D. and P. J. Walsh. 2002. The incidence of seedling attack and mortality by *Hylastes ater* (Coleoptera: Scolytidae) in second rotation *Pinus radiata* forests in the Central North Island, New Zealand. New Zealand Journal of Forestry **47**:19-23.
Reay, S. D., P. J. Walsh, A. Ram, and R. L. Farrell. 2002. The invasion of *Pinus radiata* seedlings by sapstain fungi, following attack by the Black Pine Bark Beetle, *Hylastes ater* (Coleoptera: Scolytidae). Forest Ecology and Management **165**:47-56.
Reed, G. H., J. O. Kent, and C. T. Wittwer. 2007. High-resolution DNA melting analysis for simple and efficient molecular diagnostics. Pharmacogenomics **8**:597-608.
Romon, P., X. Zhou, J. C. Iturrondobeitia, M. J. Wingfield, and A. Goldarazena. 2007. *Ophiostoma* species (Ascomycetes: Ophiostomatales) associated with bark beetles (Coleoptera: Scolytinae) colonizing *Pinus radiata* in northern Spain. Canadian Journal of Microbiology **53**:756-767.
SAS. 2008. SAS Software Version 9.2 for Windows. SAS Institute Inc., Cary, NC.
Schirp, A., R. L. Farrell, and B. Kreber. 2003a. Effects of New Zealand sapstaining fungi on structural integrity of unseasoned radiata pine. Holz als Roh- und Werkstoff **61**:369-376.
Schirp, A., R. L. Farrell, B. Kreber, and A. P. Singh. 2003b. Advances in understanding the ability of sapstaining fungi to produce cell wall-degrading enzymes. Wood and Fiber Science **35**:434-444.
Schroeder, L. M. 2001. Tree mortality by the bark beetle *Ips typographus* (L.) in storm-disturbed stands. Integrated Pest Management Reviews **6**:169-175.
Seifert, K. A. 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis*. Pages 141-151 *in* M. J. Wingfield, K. A. Seifert, and J. F. Webber, editors. *Ceratocyctis* and *Ophiostoma*: Taxonomy, Ecology, and Pathogenicity. American Phytopathological Society, St. Paul.
Sequeira, A. S. and B. D. Farrell. 2001. Evolutionary origins of Gondwanan interactions: How old are *Araucaria* beetle herbivores? Biological Journal of the Linnean Society **74**:459-474.
Six, D. L. 2005. Population Genetics of Bark Beetles and Their Associated Blue-stain Fungi with the Use of Molecular Markers. Pages 31-39 *in* J. E. Lundquist and R. C. Hamelin, editors. Forest Pathology: From Genes to Landscapes. The American Phytopathological Society, St. Paul.
Skow, Á., K. A. Mangold, M. Tajuddin, A. Huntington, B. Fritz, R. B. Thomson Jr, and K. L. Kaul. 2005. Species-level identification of staphylococcal isolates by real-time PCR and melt curve analysis. Journal of Clinical Microbiology **43**:2876-2880.
Smith, H., M. J. Wingfield, P. W. Crous, and I. A. Coutinho. 1996. *Sphaeropsis sapinea* and *Botryosphaeria dothidea* endophytic in *Pinus* spp. and *Eucalyptus* spp. in South Africa. South African Journal of Botany **62**:86-88.
Suckling, D. M., A. R. Gibb, S. Kay, F. Parry, and H. Rohitha. 1999. Are insects vectors of sapstain fungi in New Zealand? Pages 117-121 *in* B. Kreber, editor. The 2nd New Zealand Sapstain Symposium. New Zealand Forest Research Institute Limited, Rotorua, New Zealand.
Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution **28**:2731-2739.
Taylor, S. W., A. L. Carroll, R. I. Alfaro, and L. Safranyik. 2006. Forest, Climate and Mountain Pine Beetle Outbreak Dynamics in Western Canada.*in* L. Safranyik and B. Wilson, editors. The Mountain Pine Beetle: A Synthesis of Biology, Management, and Impacts on Lodgepole Pine. Canadian Forest Service, Victoria.
Thomas, C. D., E. J. Bodsworth, R. J. Wilson, A. D. Simmons, Z. G. Davies, M. Musche, and L. Conradt.

2001. Ecological and evolutionary processes at expanding range margins. Nature **411**:577-581.
Thwaites, J. M., R. L. Farrell, S. M. Duncan, S. D. Reay, R. A. Blanchette, E. Hadar, Y. Hadar, T. C. Harrington, and D. McNew. 2005. Survey of potential sapstain fungi on *Pinus radiata* in New Zealand. New Zealand Journal of Botany **43**:653-663.
Tsui, C. K. M., B. Wang, L. Khadempour, S. M. Alamouti, J. Bohlmann, B. W. Murray, and R. C. Hamelin. 2010. Rapid identification and detection of pine pathogenic fungi associated with mountain pine beetles by padlock probes. Journal of Microbiological Methods **83**:26-33.
Ulanova, N. G. 2000. The effects of windthrow on forests at different spatial scales: A review. Forest Ecology and Management **135**:155-167.
Usbeck, T., T. Wohlgemuth, C. Pfister, R. Volz, M. Beniston, and M. Dobbertin. 2010. Wind speed measurements and forest damage in Canton Zurich (Central Europe) from 1891 to winter 2007. International Journal of Climatology **30**:347-358.
Uzunovic, A., D. O'Callahan, and B. Kreber. 2004. Mechanical tree havesters spread fungal inoculum onto freshly felled Canadian and New Zealand pine logs. Forest Products Journal **54**:34-40.
Uzunović, A., K. A. Seifert, S. Hwan Kim, and C. Breuil. 2000. *Ophiostoma setosum*, a common sapwood staining fungus from western North America, a new species of the *Ophiostoma piceae* complex. Mycological Research **104**:486-494.
Uzunović, A., J. F. Webber, A. J. Peace, and D. J. Dickinson. 1999a. The role of mechanized harvesting in the development of bluestain in pine. Canadian Journal of Forest Research **29**:242-251.
Uzunović, A., D. Q. Yang, P. Gagne, C. Breuil, L. Bernier, A. Byrne, M. Gignac, and S. H. Kim. 1999b. Fungi that cause sapstain in Canadian softwoods. Canadian Journal of Microbiology **45**:914-922.
VanGuilder, H. D., K. E. Vrana, and W. M. Freeman. 2008. Twenty-five years of quantitative PCR for gene expression analysis. BioTechniques **44**:619-626.
Von Schrenk, H. 1903. The 'bluing' and the 'red rot' of the western yellow pine, with special reference to the Black Hills forest reserve. USDA, Bureau of Plant Industry. Bulletin no. 36. 40pp.
Wakeling, R. N. 1997. Issues for successful protection of New Zealand radiata pine logs. Pages 31-38 *in* B. Kreber, editor. The New Zealand Sapstain Symposium - Strategies for Improving Protection of Logs and Lumber. New Zealand Forest Research Institute Limited, Rotorua, New Zealand.
Watt, M. S., R. J. Ganley, D. J. Kriticos, and L. K. Manning. 2011. Dothistroma needle blight and pitch canker: The current and future potential distribution of two important diseases of Pinus species. Canadian Journal of Forest Research **41**:412-424.
Watt, M. S., D. J. Kriticos, S. Alcaraz, A. V. Brown, and A. Leriche. 2009. The hosts and potential geographic range of Dothistroma needle blight. Forest Ecology and Management **257**:1505-1519.
Webber, J. F. 1990. Relative effectiveness of *Scolytus scolytus*, *S. multistriatus* and *S. kirsch*i as vectors of Dutch elm disease. European Journal of Forest Pathology **20**:184-192.
Wermelinger, B. 2004. Ecology and management of the spruce bark beetle *Ips typographus* - A review of recent research. Forest Ecology and Management **202**:67-82.
Whitehill, J. G. A., J. S. Lehman, and P. Bonello. 2007. *Ips pini* (Curculionidae: Scolytinae) is a vector of the fungal pathogen, *Sphaeropsis sapinea* (Coelomycetes), to Austrian Pines, *Pinus nigra* (Pinaceae). Environmental Entomology **36**:114-120.
Wichmann, L. and H. P. Ravn. 2001. The spread of *Ips typographus* (L.) (Coleoptera, Scolytidae) attacks following heavy windthrow in Denmark, analysed using GIS. Forest Ecology and Management **148**:31-39.
Williams, D. W. and A. M. Liebhold. 2002. Climate change and the outbreak ranges of two North American bark beetles. Agricultural and Forest Entomology **4**:87-99.
Winchell, J. M., B. J. Wolff, R. Tiller, M. D. Bowen, and A. R. Hoffmaster. 2010. Rapid identification and discrimination of *Brucella* isolates by use of real-time PCR and high-resolution melt analysis. Journal of Clinical Microbiology **48**:697-702.
Winder, L., C. Phillips, N. Richards, F. Ochoa-Corona, S. Hardwick, C. J. Vink, and S. Goldson. 2011. Evaluation of DNA melting analysis as a tool for species identification. Methods in Ecology and Evolution **2**:312-320.
Wingfield, M. J. and J. N. Gibbs. 1991. *Leptographium* and *Graphium* species associated with pineinfesting bark beetles in England. Safety science **95**:1257.
Wingfield, M. J. and P. S. Knox-Davies. 1980. Observations on diseases in Pine and Eucalyptus plantations in South Africa. Phytophylactica **12**:57-63.
Wylie, F. R., B. Peters, M. DeBaar, J. King, and C. Fitzgerald. 1999. Managing attack by bark and ambrosia beetles (Coleoptera: Scolytidae) in fire-damaged *Pinus* plantations and salvaged logs in Queensland, Australia. Australian Forestry **62**:148-153.
Yang, D. Q. and R. Beauregard. 2001. Sapstain development on jack pine logs in Eastern Canada. Wood and Fiber Science **33**:412-424.

Yvon, M., B. Monsion, J. F. Martin, S. Gutiérrez, and S. Blanc. 2009. PCR-based amplification and analysis of specific viral sequences from individual plant cells. Journal of Virological Methods **159**:303-307.

Zanzot, J. W., Z. W. de Beer, L. G. Eckhardt, and M. J. Wingfield. 2010. A new *Ophiostoma* species from loblolly pine roots in the southeastern United States. Mycological Progress **9**:447-457.

Zeff, C. 1999. Forestry practises to minimise sapstain. Pages 31-34 *in* Proceedings of the 2nd New Zealand Sapstain Symposium. New Zealand Forest Research Institute Limited.

Zhang, Z., C. W. van Epenhuijsen, D. W. Brash, and G. P. Hosking. 2004. Phosphine as a fumigant to control *Hylastes ater* and *Arhopalus ferus*, pests of export logs. New Zealand Plant Protection **57**:257-259.

Zhou, X., Z. W. de Beer, D. Cibrian, B. D. Wingfield, and M. J. Wingfield. 2004a. Characterisation of *Ophiostoma* species associated with pine bark beetles from Mexico, including *O. pulvinisporum* sp. nov. Mycological Research **108**:690-698.

Zhou, X., Z. W. de Beer, T. C. Harrington, D. McNew, T. Kirisits, and M. J. Wingfield. 2004b. Epitypification of *Ophiostoma galeiforme* and phylogeny of species in the *O. galeiforme* complex. Mycologia **96**:1306-1315.

Zhou, X., Z. W. de Beer, and M. J. Wingfield. 2006. DNA sequence comparisons of *Ophiostoma* spp., including *Ophiostoma aurorae* sp. nov., associated with pine bark beetles in South Africa. Studies in Mycology **55**:269-277.

Zhou, X. D., Z. W. de Beer, R. Ahumada, B. D. Wingfield, and M. J. Wingfield. 2004c. *Ophiostoma* and *Ceratocystiopsis* spp. associated with two pine-infesting bark beetles in Chile. Fungal Diversity **15**:261-274.

Zhou, X. D., Z. W. de Beer, B. D. Wingfield, and M. J. Wingfield. 2001. Ophiostomatoid fungi associated with three pine-infesting bark beetles in South Africa. Sydowia **53**:290-300.

Zimmerman, W. C., R. A. Blanchette, T. A. Burnes, and R. L. Farrell. 1995. Melanin and perithecial development in *Ophiostoma piliferum*. Mycologia **87**:857-863.

Zipfel, R. D., Z. W. de Beer, K. Jacobs, B. D. Wingfield, and M. J. Wingfield. 2006. Multi-gene phylogenies define *Ceratocystiopsis* and *Grosmannia* distinct from *Ophiostoma*. Studies in Mycology **55**:75-97.

Appendix A.

ELSEVIER

Forest Ecology and Management

Volume 260, Issue 9, 30 September 2010, Pages 1456–1466

Predicting sapstain and degrade in fallen trees following storm damage in a *Pinus radiata* forest

- J.K. McCarthy[a, b],
- I.A. Hood[c],
- E.G. Brockerhoff[b],
- C.A. Carlson[b],
- S.M. Pawson[b],
- M. Forward[d],
- K. Walbert[c],
- J.F. Gardner[c]

- [a] School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
- [b] Scion (New Zealand Forest Research Institute), PO Box 29237, Fendalton, Christchurch 8540, New Zealand
- [c] Scion (New Zealand Forest Research Institute), Private Bag 3020, Rotorua 3046, New Zealand
- [d] Nelson Forests Limited, Private Bag 5, Richmond, Nelson 7050, New Zealand

- Received 3 June 2010. Revised 29 July 2010. Accepted 29 July 2010. Available online 21 August 2010.

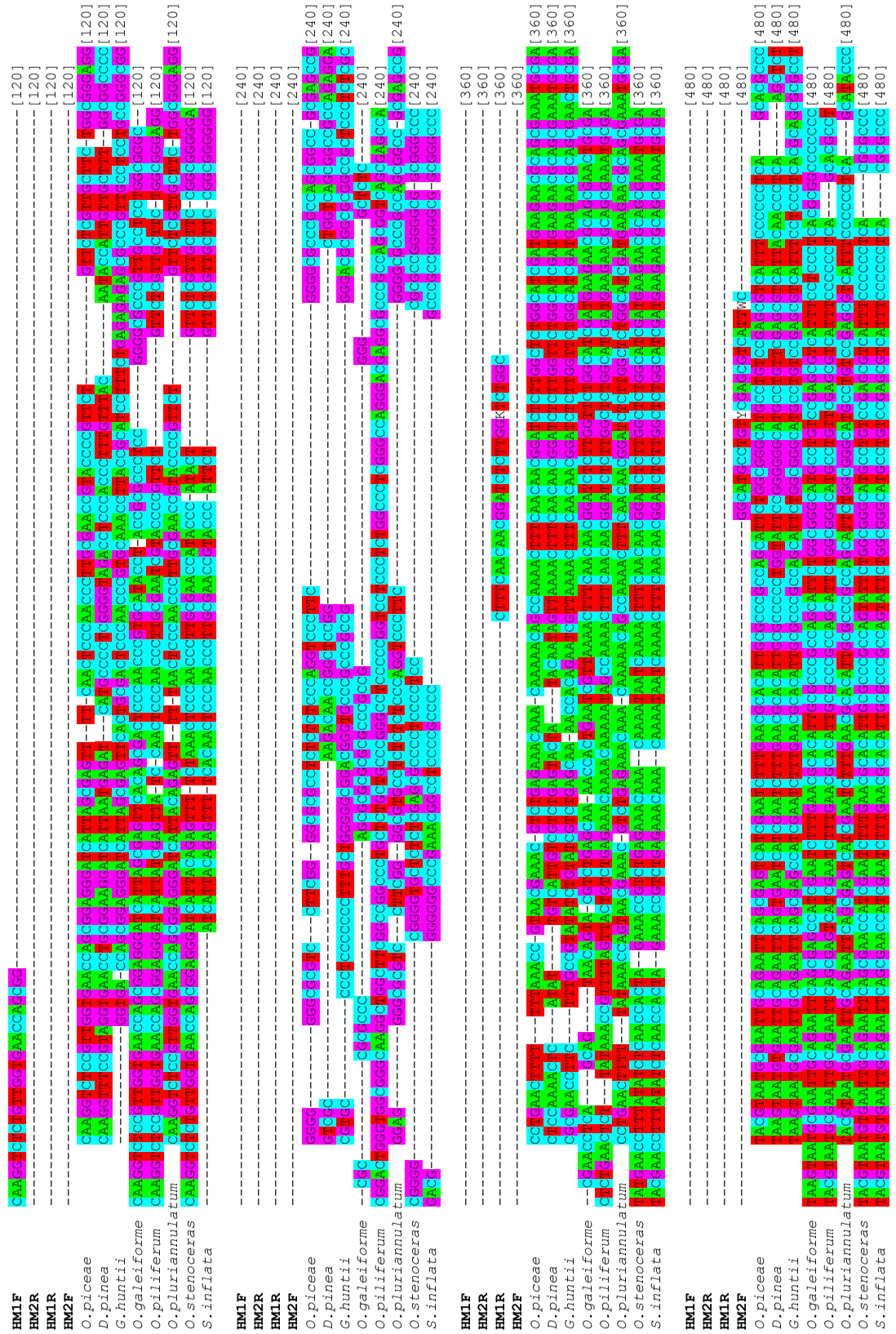
- http://dx.doi.org/10.1016/j.foreco.2010.07.044, How to Cite or Link Using DOI

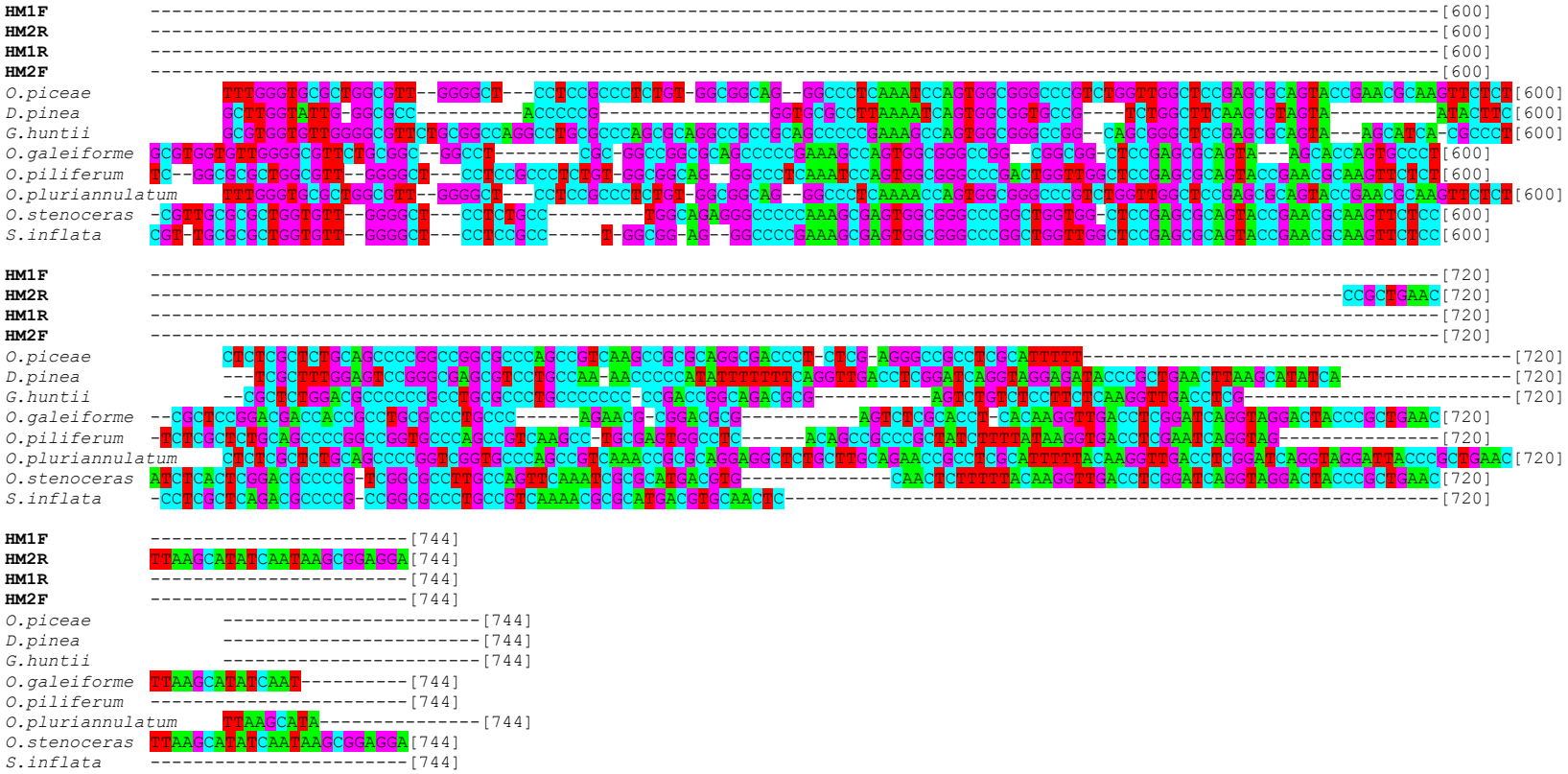
- Permissions & Reprints

Abstract

Storm damage in production forests constitutes a major source of economic loss world wide, yet the retrieval of salvageable timber remains problematic. In particular, an inability to anticipate when sapstain and degrade will appear hampers the planning of log recovery operations. A study was conducted to monitor the deterioration of fallen trees following two winter storms causing wind and snow damage in a *Pinus radiata* plantation forest in the upper South Island of New Zealand. Percentage sapstain, incidence of basidiomycete decay fungi, and frequency of bark beetle infestation increased, while percentage sapwood moisture content decreased, over a period of 1 year. These changes proceeded more rapidly in fallen trees that were severed at stump height, to simulate breakage, than in those that were left partially rooted. There was little beetle activity at the time of the storms, but *Arhopalus ferus* (Coleoptera: Cerambycidae), and *Hylastes ater*, *Hylurgus ligniperda* and *Pachycotes peregrinus* (Coleoptera: Curculionidae: Scolytinae), were collected in flight traps during the following spring and summer. The predominant fungal species associated with sapstain was *Diplodia pinea*, while *Ophiostoma piceae* and *Grosmannia huntii* were isolated near the end of the period. The main decay fungi obtained were *Phlebiopsis gigantea*, *Stereum sanguinolentum*, and *Schizophyllum commune*. A generalized linear mixed model was constructed to predict the development of sapstain in fallen trees for conditions prevailing during the study after a storm at the same time of year. According to the model, a 10 m long butt log of 22 cm mid length diameter will have minimal stain (<10% of the cross sectional area affected) when cut from severed stems up to 4 months after the storm; if taken from still-rooted trees this period will extend to 1 year. However, because of large between-tree variation, economically productive log recovery will also depend on the proportion of trees that lie below an acceptable sapstain threshold. Further research is needed to determine regional and seasonal influences on the development of sapstain in fallen trees.

Appendix B


HM1F
HM2R
HM1R
HM2F
O.piceae
D.pinea
G.huntii
O.galeiforme
O.piliferum
O.pluriannulatum
O.stenoceras
S.inflata


HM1F
HM2R
HM1R
HM2F
O.piceae
D.pinea
G.huntii
O.galeiforme
O.piliferum
O.pluriannulatum
O.stenoceras
S.inflata