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2 May 2011

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Scion report:

Date: 2 May 2011

Contract No: FHRC 2009-03

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EXECUTIVE SUMMARY

Recent wind and snow storm events in New Zealand damaging plantation forests of *Pinus radiata* have raised questions regarding the colonisation of fallen trees by sapstain fungi and how these affect the ability to salvage useable timber. 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 the 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. Following wind and snow storms in the Nelson region in the winter of 2008, a study was set up at sites affected by these storms to measure sapstain accumulation over time and monitor the abundance of bark beetles (Coleoptera: Scolytinae) which are known vectors of some sapstain fungi. We found that snapped trees accumulate sapstain and insect attack faster than trees that topple but remain rooted, and that the most common stain fungus affecting these trees was *Diplodia pinea*. The detailed results of this study have been published and are appended to this report.

Subsequently, more detailed manipulative experiments have been established (as part of an MSc project) to examine seasonal and regional variation in sapstain attack, and the importance of bark beetles as vectors. Trees are being felled at regular intervals to simulate windthrow at different times of the year, to assess seasonal effects. This study will be completed in November 2011 when assessments over 12 months following simulated windthrows in all four seasons have been completed. The results to date indicate that both regional and seasonal differences occur in the onset of sapstain following simulated storms, with stain appearing at a faster rate with warmer temperatures.

To examine the potential role of beetles as sapstain vectors in more detail, experimental billet logs have been caged to exclude beetles and subsequently analyse fungal attack in comparison with identical logs left exposed to beetles. Assessments of individual beetles are being carried out to determine what fungal species are associated with the different beetle species we encountered. This is being done through a variety of methods including Scanning electron microscopy (SEM), inoculating agar media with beetles directly, and the development of specific fungal DNA probes. These aspects of the study are ongoing. The results will become available over the coming months, and these, along with the final results and analyses of all experiments, will be reported with the MSc thesis once this is completed towards the end of 2011.

TABLE OF CONTENTS

1. Introduction 2
2. Methods 4
3. Results 6
4. Discussion 9
Acknowledgements..... 11
References 12
Appendix..... 14

1. Introduction

Severe wind and snow storm events in July and August of 2008 caused significant damage to several production forest estates near Nelson (South Island). These storms raised questions about the time available for the salvage harvest of damaged trees before extensive damage by insects, sapstain (Figure 1), and decay fungi reduce the value of affected timber. Radiata pine (*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 structurally and cosmetically (Childs, 1966). The development of sapstain in windthrown timber reduces the window of opportunity for salvage harvesting before its merchantability is affected, or eliminated entirely (Childs, 1966). In the 1990s, the effects of sapstain caused an estimated revenue loss of NZ\$100 million per year in New Zealand (Wakeling, 1997), a significant loss of revenue for the forestry sector.



Figure 1. *Pinus radiata* log section with blue sapstain visible on both the cut faces, and under the bark (Photo: James McCarthy).

Following the severe storm events in July and August 2008, 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 were assessed for sapstain over a 12 month period at intervals of 1 to 3 months (McCarthy *et al.*, 2010, see Appendix 1). The findings indicate 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 the development of sapstain progresses more slowly than during warmer times of the year.

Bark beetles and wood borers generally play an important role as vectors of fungal spores (Paine *et al.*, 1997; Suckling *et al.*, 1999; Reay *et al.*, 2005, 2006; Romon *et al.*, 2007), although the importance of this relationship to the colonisation of sapstain fungi in trees has not been thoroughly studied in New Zealand. In addition to the damage caused by the vectored fungi, these beetles also damage timber directly by tunnelling into the phloem and sapwood of susceptible trees (Paynter *et al.*, 1990; Mausel *et al.*, 2007) and they are major quarantine pests of export logs from New Zealand (Lanfranco *et al.*, 2004; Zhang *et al.*, 2004). It is important to note, however, that some fungal species have a somewhat ubiquitous distribution such as the major staining fungus *Diplodia pinea* which was commonly found in the aforementioned study of windthrown timber (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). Depending on the climatic conditions it could potentially take just a few weeks until the logs are discoloured to a point where they are no longer merchantable. This has been demonstrated with harvested logs, indicating that in the warmer months sapstain can develop in less than two weeks in some regions (Zeff, 1999). However, our study in Nelson indicated that the colouration timber by sapstain can take a much longer period of time (McCarthy *et al.*, 2010). This may be due to a lower incidence of bark damage compared to harvested logs (bark provides a protective layer to exclude spores), and 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 is available indicating the probability of sapstain onset across seasons (for harvested wood, Zeff, 1999), which is based on an empirical study of seasonal nutrient level, midday temperature, and wood moisture content, information on the phenology of insect and fungal attack at different times of the year is still limited. 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.

As previously mentioned, the bark beetles *Hylastes ater* and *Hylurgus ligniperda* and the burnt pine longhorn beetle *Arhopalus ferus* are among the most significant quarantine pests of export pine logs and timber in New Zealand (Brockhoff *et al.*, 2006). These beetle species are not native to New Zealand and have thrived in this novel environment with an abundance of host material, but lacking their natural enemies. As an extension of the initial study mentioned above, we are determining how important these beetles are in terms of facilitating the colonisation of damaged/windthrown trees by sapstain fungi, ultimately resulting in a decrease of wood quality. Furthermore, we are exploring how the strength of this interaction varies through time. This seasonal variation is thought to be the case because temperature, humidity, and time since felling or storm damage, all affect the strength and prevalence of these ecological interactions. For example, bark beetle activity is strongly seasonal and tends to peak during the warmer months, which is likely to influence the rate of attack and spore distribution to vulnerable wood. In addition, as temperature increases, wood moisture content decreases more rapidly, increasing available oxygen allowing an increase in fungal growth.

2. Methods

The main objectives of this study are (1) to determine how sapstain progresses when windthrow events occur at different times of the year, (2) to assess the influence of climatic variation throughout New Zealand on sapstain development, (3) to identify the fungal species responsible for sapstain, and (4) to investigate the role and relative importance of bark beetles and wood borers as vectors of sapstain fungi. The results of the study will provide data to underpin the development of a 'tool' to help assess the required timeframes of salvage operations, in relation to the seasonality of the occurrence of storms.

The field study is being conducted over 21 months in *Pinus radiata* stands across two sites in the South Island (Table 1). Study sites were established in December 2010 (Nelson – Golden Downs) and January 2011 (Canterbury – Bottle Lake Forest). The project is using experimentally simulated windthrow events, created in a controlled fashion by manually felling trees at each site (Figure 2). In Nelson, windthrow has been simulated in all four seasons and monitoring will continue for 12 months following each simulation to determine the onset and progression of sapstain fungi and beetle attack. In Canterbury, simulated windthrow events are limited to a summer and winter initiation, with repeated measurements taking place thereafter. Sapstain is being monitored by cutting wood disc samples along the length of the fallen trees. It is noted whether or not the discs were in contact with the ground or foliage as this can affect the moisture content of timber and the likelihood of insect attack (Mausel et al., 2007). All of these discs are being assessed for percentage cover of sapstain and moisture content, and a subset of these are being assessed for the presence and identity of fungal species. A photograph is also taken from every disc. Sapstain cover is measured by creating two greyscale images per disc using Adobe Photoshop, from images taken. One of these images is of the entire disc face, the other is just the stained section. The number of pixels in each of these images is determined using "imageJ", these pixel numbers can then be used to calculate the percentage of stain covering a disc face. Moisture content of the inner and outer sapwood was determined (dry weight basis) by cutting two small blocks from each disc, one from the outer half and the other from the inner half along the same radius. Blocks are weighed at room temperature to constant mass before and after drying at 80°C in a ventilated oven. Fungal isolations are being taken from two of the five discs cut per tree, and cultured in a selective agar media for identification. Results from these isolations will then be related to the presence of beetles, moisture content, and time since 'windthrow'. In addition, black Lindgren-type beetle funnel traps baited with α -pinene and ethanol have been set up at each South Island site to monitor beetle presence and abundance (as in Brockerhoff et al., 2006).

Table 1. Characteristics of each study location (m.a.s.l. = metres above sea level)

Name	Mean min. temp (°C)	Mean max. temp (°C)	Rainfall (mm)	Elevation (m.a.s.l.)
Golden Downs	4.8	16.8	1385	ca 550
Bottle Lake	7.3	17.6	524	ca 3



Figure 2. One tree severed at the base, felled for the winter windthrow simulation in Christchurch (Photo: James McCarthy).

To determine if a relationship exists between bark beetles and associated sapstain fungi, several approaches are being used. Mesh has been applied to fresh pine logs to exclude insects to assess whether this will reduce sapstain compared with control logs (without mesh) where bark beetles have unrestricted access and can potentially transmit fungal spores. Bark beetles caught emerging from logs are being used to inoculate agar plates to isolate fungal species transported by the beetles. Whole beetles and different parts of beetles such as the elytra - where fungal spores are thought to be harboured in pits (Francke-Grosmann, 1967; Beaver, 1989; Haberkern *et al.*, 2002) - are being compared. A combination of morphological and molecular techniques is being used to identify fungal species for all components of this project.

In addition, preparations are under way to use a novel molecular technique - high resolution melt curve analysis (Ririe *et al.*, 1997) to search for pre-defined fungal species on beetles. Primers for pre-selected fungal species will be developed, and their exact DNA melting point will be determined. Following this, a bark beetle with any fungal material it carries can then be 'melted' using a PCR system to determine the identity of associated fungal species, using the output melt curves in comparison with those of the pre-selected fungal species. This has the potential to give a quantitative value for the amount of these species that are present on a bark beetle. If successful, this technique could be used to compare fungal communities between beetle populations, communities, and species. Also, other things such as wood and insects intercepted at the New Zealand border could be assessed for fungi present.

3. Results

The results to date indicate that the accumulation of visible sapstain happens at a faster rate in the warmer months of the year (especially summer) than in the cooler months (Figure 3). Furthermore, the accumulation of sapstain increases at a faster rate in the warmer Nelson region than in Christchurch (Table 1, Figure 3). The amount of sapstain will increase over the next six months (from April 2011) as more samples are assessed from earlier initiations of windthrows, and as samples become ready to collect from the field.

The main stain fungus isolated thus far from both regions is *Diplodia pinea*. Other stain fungi isolated from wood are *Sporothrix inflata*, *Ophiostoma abietinum*, *O. pallidulum* and some other species yet to be formally identified (Table 2). Stain fungi isolated from insects include *O. nigrocarpum*, *O. galeiforme*, and *Grosmannia huntii* (Table 2). The number of species of sapstain fungi are likely to increase considerably as identifications of fungal isolates continue. Insects found to be attacking this wood include the exotic bark beetles *Hylastes ater*, and *Hylurgus ligniperda*, the native wood borer *Pachycotes peregrinus*, and the exotic wood borer *Arhopalus fesus* (the burnt pine longhorn beetle).

Table 2. Sapstain fungi species isolated from pine wood and beetles

Fungal species	Isolated from wood	Isolated from insects	Notes
<i>Diplodia pinea</i>	✓		Most common stain fungus.
<i>Sporothrix inflata</i>	✓		
<i>Ophiostoma abietinum</i>	✓		Isolated from insect hole
<i>Ophiostoma pallidulum</i>	✓		Isolated from insect hole
<i>Ophiostoma nigrocarpum</i>		✓	
<i>Ophiostoma galeiforme</i>		✓	
<i>Grosmannia huntii</i>	✓	✓	Isolated from wood in previous study only (McCarthy <i>et al.</i> , 2010)

Several aspects of this project are currently still in progress and the results are not yet available until the study is completed by November 2011. This includes the simulated windthrow study. At the time of writing this report (April 2011), two more sampling harvests were to be completed in Nelson, and one more in Christchurch. All the detailed data analyses are also yet to be completed, especially regarding the relationships of sapstain development with moisture content and temperature. Other aspects of the project that are still in progress are the High Resolution Melt Curve analysis, as well as the collection of fungal and insect samples. All fungal isolations to be used in the original search library are to be sequenced so primers can be developed. Some insect specimens have been aseptically collected in Nelson and placed in individual sterile vials, although more are still to be collected from Christchurch.

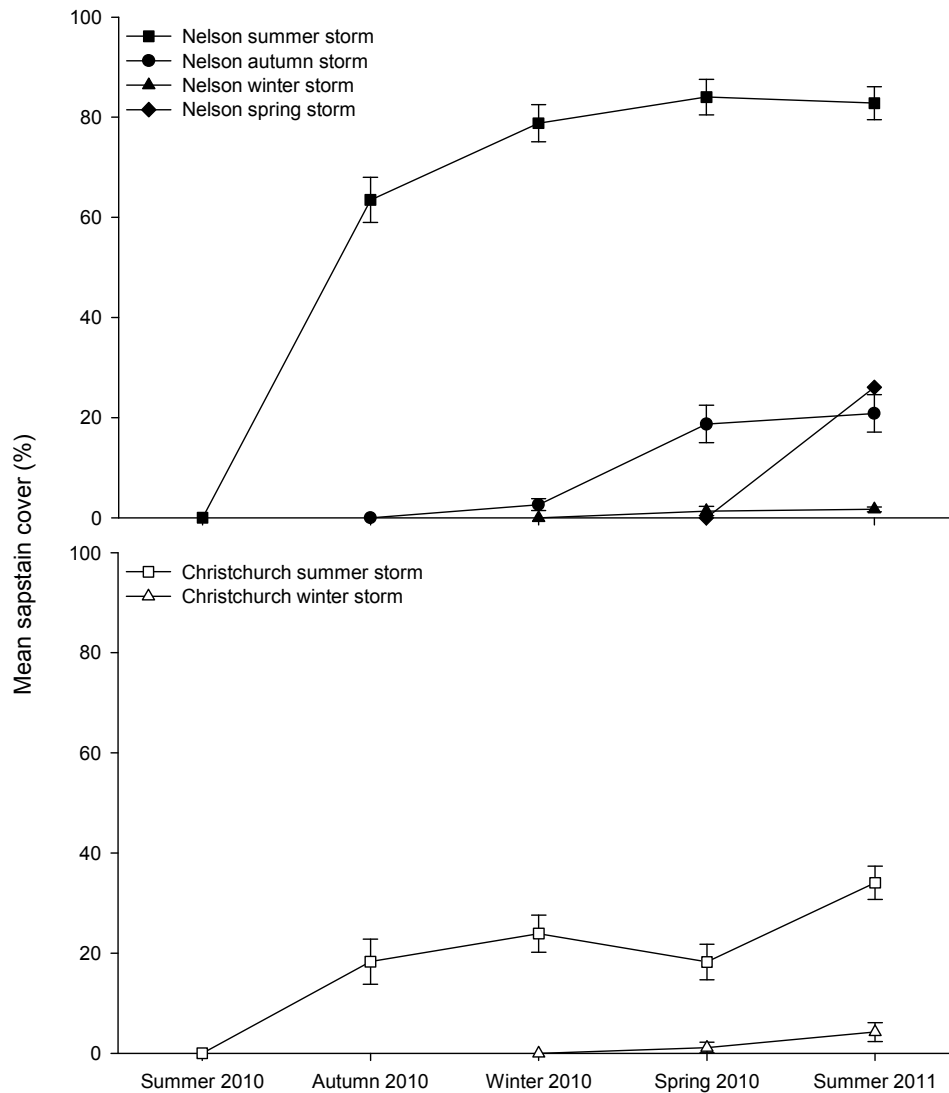


Figure 3. Mean sapstain percentage cover of wood discs from Nelson (filled symbols) and Christchurch (hollow symbols). Note that due to logistical and time constraints, Christchurch storms are only simulated only for summer and winter storm events.

In addition, the caged and un-caged billet logs have been collected from the field and processed (Figure 4A,B,C). Image analysis is currently underway to determine the relationship between insects and the onset of sapstain fungi by analysing the amount of sapstain, and pattern of sapstain onset at seven points down each log in relation to identified insect attack (Figure 4D).



Figure 4. A: Caged and un-caged billet logs set out in a Latin Square design, all logs are facing north. B: A log in its insect exclusion cage with one in the background un-caged. C: Log processing, orientation of the log in the field is recorded and the bark is removed while recording all insects present. D: Stain is measured as a percentage on six segments per cut face (seven cut faces per log).

4. Discussion

At the time of writing this report, the study is still uncompleted and most of the final results are not yet available. Over the coming months these results and analyses of all experiments, will be become available and reported with the MSc thesis once this is completed towards the end of 2011.

However, the results to date already indicate that there is a significant difference in the accumulation of stain fungi depending on both the time of year, and the location of the storm. After a summer storm in Nelson, forest managers would have less than three months to salvage harvest damaged stems before discs would have an average of ~60% sapstain cover (Figure 1). This rate of increase in stain cover then decreases with a drop in temperatures towards winter. A summer storm at the same time of year in the Canterbury region only yields ~20% sapstain cover after the same length of time. Moisture content data are still to be analysed in regards to explaining this difference, but it is likely to be due to the moisture content of the wood as reported in previous studies whereby high wood moisture content inhibits fungal growth (McCarthy *et al.*, 2010). Christchurch is generally cooler and drier than the warmer Nelson region. This could have effects on the speed of drying undertaken by the wood, and therefore allowing fungi to colonise earlier in warmer climates (Hood and Ramsden, 1997).

Conversely, storms occurring in the cooler months of autumn (measured in Nelson, only) and winter (measured in Canterbury and Nelson) result in a much slower rate of increase in stain extent (Figure 3). This decrease is consistent among both regions. This is especially true in winter when the increase is barely noticeable, and as a result there is no significant difference between the regions. In addition to this, there is a reduction in the speed in increase of stain when the temperature cools. This is consistent with results found in the 2008 Nelson storms (which occurred in winter) where stems remained stain free for three months or more (McCarthy *et al.*, 2010).

Although we are still in the preliminary stages of identifying fungal species present in the wood, and on insects, there are some trends beginning to appear. *Diplodia pinea* is the most common stain fungus in both Nelson and Christchurch. This is a fungus that is not dispersed by insects, but has more of a ubiquitous distribution, generally believed to be spread by wind and rain splash. Generally, many ophiostomatoid fungal spores are sticky/oily and spread by contact with insects. This group consists primarily of the teleomorph (sexual stage) genera *Ophiostoma* and *Grosmannia*, and the anamorph (asexual stage) genera *Pesotum*, *Leptographium*, *Hyalorhinocladiella*, and *Sporothrix*. *Sporothrix inflata*, *Ophiostoma abietinum* and *O. pallidulum* have been isolated from wood, and have the strong potential to be vectored by insects due to them being part of the ophiostomatoid group. The ophiostomatoid fungi *O. galeiforme*, *O. nigrocarpum*, and *Grosmannia huntii* have been isolated from the bark beetle *Hylurgus ligniperda*. *G. huntii* was also isolated from wood in the initial Nelson study (McCarthy *et al.*, 2010). This is some evidence for the vectoring of these fungal spores by bark beetles in this system. However, the relatively low isolation frequency of these organisms suggest that insects do not play a major role in the accumulation of sapstain in damaged timber, in New Zealand.

From an applied point of view, this research will provide a thorough documentation of the colonisation and development of sapstain and decay fungi in windthrown timber. This will assist with prioritising harvest and stand recovery after extreme

weather events, which may be increasingly important as climate change is predicted to increase the frequency of extreme weather events (Goldenberg *et al.*, 2001; Emanuel, 2005). By increasing our knowledge of the interactions between the key components of the host-vector-pathogen relationships, the industry as a whole will have an enhanced ability to extract value from storm-affected trees, and prioritise these stands for salvage. The information from this study will complement available sapstain indices (such as Zeff, 1999), and, most importantly, give an indication of the time available to reduce loss sustained by extreme weather events across a seasonal gradient, and across differing climates in New Zealand.

Acknowledgements

The authors would like to thank the numerous forestry companies that have allowed trial sites to be established in their forests. Special thanks go to Mark Forward (Nelson Forests Ltd), and also Marcel van Leeuwen (Selwyn Plantation Board Ltd), Sam Middlemass (Rayonier Asia Pacific) and Nigel Heron (Timberlands Ltd.). We gratefully acknowledge the assistance by William O'Connor (SCION) and Rex Mitchell in setting up the Nelson sites, and Kane Fleet (SCION), Malcom Wano, and Revel Snowden in setting up the North Island sites. This work was funded by the New Zealand Foundation for Research, Science and Technology through contract C04X0302 ("Forest Biosecurity and Protection") and a Tertiary Education Commission Enterprise scholarship to JKM in conjunction with the Forest Health Research Collaborative, with additional assistance from Nelson Forests Ltd.

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Appendix

Earlier work that was undertaken at the beginning of this study has already been published:

McCarthy, J.K., Hood, I.A., Brockerhoff, E.G., Carlson, C.A., Pawson, S.M., Forward, M., Walbert, K., Gardner, J.F., 2010. Predicting sapstain and degrade in fallen trees following storm damage in a *Pinus radiata* forest. *Forest Ecology and Management* 260, 1456-1466.

This is appended here (see file attached)