Chapter 1.
Introduction

1.1. Background

Australia’s forests, both native and plantation, are a highly valuable natural asset (National Forest Inventory, 2005), providing wood and paper products, recreational opportunities and habitat for a diverse range of animals and plants. Traditionally, native forests were the primary source of hardwood timber in Australia (National Forest Inventory, 2005), providing products such as fuel wood, pulp, veneer, and timber. However, the area of native forest available for harvesting is decreasing due to expanding conservation reserves. This has resulted in a rapid expansion of hardwood plantations Australia wide.

A plantation forest is ‘an intensely managed stand of trees of either native or exotic species, created by regular placement of seedlings or seeds’ (National Forest Inventory, 2005). Plantations allow selected species to be grown on short rotation lengths, thereby increasing the volume/ha of desirable products. Though the initial outlay for establishment and ongoing maintenance is high, specific rotation periods for particular products make plantation forestry economically viable. With respect to eucalypt plantations, economic benefits can be seen in as little as 10-15 years for pulpwood, 20-30 years minimum for saw logs, and 40 plus years for large diameter logs (National Forest Inventory, 2005).
Plantation forestry began in Australia in the 1870’s (National Forest Inventory, 2005). In 2005, there were 1.72 million ha of plantation (hardwood and softwood) in Australia (National Forest Inventory, 2005). Of this, 715,531 ha was hardwood and an estimated 95% was planted with *Eucalyptus* species (National Forest Inventory, 2005).

The most commonly planted species, in temperate regions, are *Eucalyptus globulus* Labill. and *Eucalyptus nitens* (Deane and Maiden) Maiden, due to their rapid growth rates, favorable pulp yield, and wood density (Farrington and Hickey, 1989). The majority of hardwood plantations are located in southern Australia, with the largest estates being in Western Australia (270,813 ha), Victoria (164,724 ha), and Tasmania (155,500 ha) (National Forest Inventory, 2005). It is estimated that by 2020 Australia’s hardwood industry will reach 3.3 million ha (National Forest Inventory, 2005).

The rapid expansion of single species monoculture plantations increases the need for constant improvement of management strategies and decision support systems to mitigate biotic and abiotic agents that may adversely affect productivity. Plantation management includes low disease risk site selection and management strategies that accelerate and maximize tree growth rates and biomass production through careful selection of specific genetic material and the appropriate application of fertilizers (Smethurst et al., 2003; Whittock et al., 2004). However, silvicultural practices and the selection for particular traits resulting in accelerated growth rates can make trees less tolerant to abiotic stresses such as drought, frost, water logging and nutrient deficiencies and more susceptible to attack from biotic agents, such as insects,
browsing mammals, and fungal pathogens (O'Reilly-Wapstra et al., 2002; Lou and Baldwin, 2004; Milgate et al., 2005; O'Reilly-Wapstra et al., 2005; Prudic et al., 2005; Rapley, 2005). For example, work by Close et al., (2004) has demonstrated that *E. globulus* and *E. nitens* seedlings with a high nutrient status are more susceptible to browsing damage than seedlings with a low nutrient status, and more succulent seedlings take longer to recover from frost than hardened seedlings (Davidson et al., 2004). There is a need to select for maximal growth rates and biomass, but to limit the effects of damaging agents by selection for true resistance.

### 1.2 *Eucalyptus* plantation pests and their damage symptoms

Endemic agents can be present at low levels in Australian native vegetation, but can increase to epidemic proportions if close to a susceptible host or source of food, such as plantations. The most significant damaging agents present in Australian native eucalypt forests that have caused problems in newly established plantations are the a) chrysomelid leaf beetles (Ohmart, 1990; Neumann, 1993), b) vertebrate pests including the red-bellied pademelon (*Thylogale billardierii*), the common brushtail possum (*Trichosurus vulpecula*), the introduced European rabbit (*Oryctolagus cuniculus*), (Bulinski, 2000; Pietrzykowski et al., 2003b), and c) *Mycosphaerella* species (Carnegie and Keane, 1994; Stone et al., 1998; Park et al., 2000; Milgate et al., 2001; Mohammed et al., 2003; Hunter et al., 2004; Smith, 2006) which cause *Mycosphaerella* Leaf Disease (MLD).

Pests and diseases in eucalypt plantations cause a variety of crown damage symptoms. Vertebrate browsers continue to browse re-growing shoots resulting in stunted and deformed tree growth (Pietrzykowski et al., 2003b). The eucalypt leaf beetles
(Chrysophtharta spp.), scarab beetles (Heteronyx spp), gum-leaf skeletonizer (Uraba lugens) and autumn gum moth (Mnesampela privata) reduce leaf area and defoliate by chewing and scalloping leaves and shoots; and the leaf blister saw fly larvae (Phylacteophaga spp) and psyllids (Cardiaspina spp) cause discoloration and necrosis of leaf tissue (Elliot and de Little, 1984; Loch and Floyd, 2001).

Foliar pathogens of eucalypts e.g. Aulographina (Carnegie and Keane, 2003), Phaeophleospora (Hood et al., 2002a), and the previously mentioned MLD, also cause a number of symptoms such as defoliation, necrosis and discoloration of leaves. These pathogens predominantly infect young, juvenile, and newly expanding foliage during spring and summer (Wall and Keane, 1984; Yuan, 1999; Carnegie, 2000), a period which coincides with the vegetative growth phase of the host species. Phaeophleospora eucalypti has been observed causing damage in E. nitens in New Zealand (Dick, 1982) and Aulographina eucalypti in E. globulus in Victoria, Australia (Carnegie, 1991; Carnegie and Keane, 2003). However in recent years, the most threatening disease of concern to plantation managers in the temperate regions of Australia is caused by species of Mycosphaerella (Mohammed et al., 2003). The symptoms of MLD have become so severe that the disease has become an economic threat to the eucalyptus plantation industry (Park and Keane, 1984; Carnegie and Ades, 2002b; Smith, 2006). The most obvious symptom of MLD infection is “leaf spots”. In severe cases, leaf/shoot blighting, branch cankers, defoliation and premature branch death are observed, resulting in the occasional mortality.
1.3 Mycosphaerella Leaf Disease

Outbreaks of MLD have been reported from South Africa (Hunter, 2002; Hunter et al., 2004), Ethiopia (Gezahgne et al., 2006), New Zealand (Ganpathi, 1979; Dick, 1982; Hood et al., 2002b) and Chile (Wingfield et al., 1995; Ahumada et al., 2003). In Australia significant damage from MLD has occurred in plantations in Western Australia, South Australia, Victoria, and Tasmania (Mohammed et al., 2003). There have also been sporadic MLD defoliation events in *E. globulus* plantations in southeast New South Wales (Stone et al., 1998; Carnegie, 2000). This disease is also present in Queensland, but currently does not cause severe damage, as the hardwood plantation estate is still small and expanding (Mohammed et al., 2003). Severe sporadic epidemics of MLD have been occurring over the past 30 years in *E. globulus* plantations in Tasmania, especially in the northwestern region of the island, which is most likely related to the wet and warm conditions that prevail in this region (Wardlaw, 2001). Recent epidemics have been so significant that Forestry Tasmania has ceased planting *E. globulus* and has replaced it with *E. nitens*, as this species is considered more resistant to infection by MLD (Wardlaw, Forestry Tasmania, pers. comm).

More than 60 *Mycosphaerella* species have been recorded from various eucalypt species around the world, although not all are associated with MLD (Carnegie et al., 1998; Crous, 1998; Carnegie, 2000; Milgate et al., 2001; Maxwell et al., 2003; Crous et al., 2004; Gezahgne et al., 2006; Hunter et al., 2006). *M. nubilosa* (Cooke & Hansf.) Hansf. and *M. cryptica* (Cooke & Hansf.) Hansf. are the two species most commonly associated with MLD in temperate eucalypt plantations in Australia.
It is necessary to understand the impact of the initial damage and how the degree of recovery after infection by MLD influences final productivity. It is therefore vital to assess both-long or short-term growth effects. Snowdon (2002) identified two types of tree growth responses to damage. Firstly, a short term reduction in growth, with the growth potential returning to that of non-affected trees (Type 1), and secondly a long-term growth reduction where the changed growth trajectory is permanent and growth does not return to that exhibited by non-affected trees (Type 2). The management implications of this is that trees following a Type 1 growth response recover to the same growth rates as healthy trees, but with a time lag compared to undamaged trees (Smith, 2006) so that a management option to maintain profitability is to extend the rotation. Trees exhibiting a Type 2 response will not recover without management intervention, and may require a reappraisal of anticipated end products (Wardlaw et al., 2006).

Investigations by Smith (2006) on the impact of MLD on the growth of E. globulus in a long term trial suggest MLD damage of up to 20% (defoliation) follows a Type 1 growth response, and trees with greater damage follow a Type 2 growth response. Lundquist & Purnell (1987) with E. nitens, reported similar results, with reduced growth rates occurring when trees exhibited 25% defoliation. Several other researchers have reported effects of MLD damage (defoliation and/or necrosis) on tree growth. Two examples include, Milgate et al. (2005) (6.2% reduction in growth when
10% damage occurred) and Carnegie & Ades (2002b) (up to 10% leaf area with necrosis reduced height by 13% and diameter by 4%). These results, however, were from short-term trials and should not be directly compared with the long-term studies conducted by Smith (2006).

Variation in the reported effects of MLD on tree growth and recovery suggests that genetics and site factors (site water availability and soil nutrition) influence the response to MLD (Carnegie, 1991; Carnegie and Ades, 2001; Carnegie and Ades, 2002b; Wardlaw, 2002; Milgate et al., 2005; Pinkard et al., 2006; Smith, 2006; Smith et al., 2006). There is significant inter and intra specific variation in the response of eucalyptus to infection by MLD. Certain traits such as epicuticular wax coverage and palisade mesophyll density have been associated with Mycosphaerella resistance in juvenile foliage of *E. globulus* (Smith, 2006). Similar traits have also been identified for *E. nitens* in South Africa (Smith et al., 2006b). Resistant genotypes of *E. nitens* in Tasmania have not been selected for planting as *E. nitens* is generally more resistant to MLD than *E. globulus*.

Wardlaw (2002) investigated the growth response to MLD of *E. globulus* planted in windrows* compared to bays. Results demonstrated that trees planted in windrows were taller, had larger stem diameters and exhibited less crown defoliation and necrosis than trees in bays. Wardlaw (2002) concluded that the additional water and nutrition which were available to windrow trees via the mounding of debris and earth provided them with extra resources to resist the effects of infection by MLD. This conclusion is supported by results from other authors. Carnegie and Ades (2002a)

* Windrows are created as a result of site preparation procedures where debris is mounded and burned. The areas among windrows are called bays.
found higher doses of phosphorus reduced the effects of MLD and that trees planted on a second rotation site (with low nutrient status) recorded growth reductions from low levels of MLD. Fertilizer trials completed by Pinkard et al., (2006) found fertilizer increased the growth rate and recovery time of defoliated trees.

Timing of a MLD epidemic also has repercussions on a tree’s recovery after infection. Candy (1999) reported that *E. nitens* subjected to artificial crown defoliation early in the growing season recovered sooner than trees subjected to defoliation late in the season. A similar recovery rate occurred with *E. globulus* trees that experienced near complete defoliation from MLD in the early in the growing season (Smith, 2006). Alternatively, it is suggested that *E. globulus* trees damaged by MLD later in the growing season may exhibit a slower recovery (Smith, 2006), as their growth rates begin to slow down at this time. In addition to the timing of a MLD epidemic, the level of severity also affects the rate of recovery. For example, Rapley (2005) demonstrated that *E. globulus* trees with lower insect damage levels (5-50% leaf area loss) recovered within 12 months, compared to trees with severe damage levels (>70% leaf area loss) which took more than two years to recover.

### 1.5 MLD epidemiology

Three factors are simultaneously required for a disease epidemic to occur successfully. Firstly, a viable and active source of inoculum; secondly, a large number of susceptible hosts that are at a stage of their life cycle that is susceptible to infection; and lastly, the specific environmental conditions that are required by the pathogen to infect a host, and further colonize and produce new dispersal units (spores) (Lucas, 1998).
The risk of a MLD epidemic is greater in a monoculture than in a mixed forest due to the uniformity of plant species growing (Old and Wingfield, 2003). Trees are also planted in a regular pattern and are at a similar stage of development i.e. susceptible juvenile leaf stage will be present at the same time in a large number of trees, also termed a cohort population (Zadoks & Schein, 1979). These risk factors increase the probability of an epidemic, provided susceptible inoculum (pathogen) and environmental conditions favourable for disease development occur concurrently present. Inoculum could be present either naturally in surrounding native vegetation, or be disseminated by wind or water to the hosts. *Mycosphaerella* lesions are always present, albeit at a low level, within a plantation or in surrounding native forest species such as streamside reserves. For example, in the northwest of Tasmania, low levels of damage were observed on trees planted in 1999 and this may have provided a source of inoculum for the neighbouring plantation that was planted in 2000, along with the favorable environmental conditions for disease development, an epidemic resulting in moderate-high (40%-70%) levels of crown damage (Wardlaw, 2001; Wardlaw, 2002).

The environmental variables that most influence pathogen infection and progression through space and time include relative humidity, temperature, leaf wetness duration, wind (speed and direction), solar radiation and plant nutrition (Lucas, 1998). Each of these variables can affect the infection efficiency and subsequent disease development, either directly (e.g. through the development of the pathogen), or indirectly (e.g. performance of the host). The following diagram (Figure 1.1) depicts stages of disease progression.
Drying and wetting cycles are seen as highly important in determining ascospore discharge in ascomycetes. Air at near-saturation (relative humidity) provides enough moisture to trigger ascospore discharge. It has been proposed that discharge occurs when moisture enters the dry ascus through osmosis, causing its walls to expand and burst, thereby forcibly discharging ascospores (Machardy et al., 2001). As low temperatures can alter the osmosis process by affecting membrane permeability and biochemical processes (Machardy et al., 2001), the discharge mechanism can also be affected by temperature (Stensvand et al., 1997). Wind transports ascospores either within the canopy or further afield if turbulence lifts ascospores to higher wind streams (Aylor, 1990).

Both the mechanisms of, and the conditions required for ascospore discharge in *Mycosphaerella* species, including eucalypt *Mycosphaerella* species, have been the subject of several investigations (Appendix 1). As expected, ascospores of
*M. nubilosa* and *M. cryptica* have been shown to be forcibly discharged from asci within pseudothecia by a mechanism triggered by successive wetting and drying cycles (Cheah, 1977; Beresford, 1978; Cheah and Hartill, 1987; Park and Keane, 1987; Park, 1988b). *Mycosphaerella cryptica* produces sexual (ascospores) and asexual spores (Park and Keane, 1984). The latter are transferred within the host canopy by rain splash (Beresford, 1978; Ganapathi, 1979). *Mycosphaerella nubilosa* only produces ascospores (Park, 1984). Ascospores can be discharged up to a distance of 12-15 mm from the pseudothecia (Park, 1988a). Ideal environmental conditions for ascospore discharge in laboratory studies are temperatures 25 °C for *M. nubilosa* and 20 °C *M. cryptica* and relative humidity above 90% (Park and Keane, 1987; Park, 1988a; Crous, 1998).

Once ascospores are discharged, they are disseminated by wind and water (through rain-splash) and conidia are disseminated by water only. Deposition into the lower crown and to neighbouring trees occurs through alloinfection by rain splash and active discharge from the lesions onto neighbouring leaves (autoinfection). Long distance transportation occurs when ascospores enter upper wind streams in the atmosphere.

Conditions that influence ascospore germination, infection, lesion expansion and ascospore maturation are very similar, i.e. all require similar warm temperatures, high humidity, and a specified duration of leaf wetness (Park, 1984). Reduced temperatures will inhibit lesion expansion and spore maturation (Park, 1984). Leaves are most susceptible to infection while juvenile or newly expanding (Park, 1984). Lesions of *M. nubilosa* will actively sporulate from lesions on an attached leaf and in
leaf litter for up to 8 months compared to *M. cryptica*, which sporulates for up to 4 months (Park, 1984).

*M. cryptica* will continue secondary and sporulating under favourable conditions (Park, 1984) and is polycyclic, whereas *M. nubilosa* is mono or bicyclic (just 1 or 2 disease cycles per season). Under favourable conditions the incubation period (defined as the time required for symptom appearance) on young *E. globulus* leaves is approximately 7-24 days for *M. nubilosa* and *M. cryptica* (Park, 1984). Conditions that influence ascospore germination, infection, penetration, incubation period, lesion and expansion are very similar, i.e. all require warm temperatures, high humidity, and periods of leaf wetness (Park, 1984). Reduced temperatures will inhibit sporulation and infection progression (Park, 1984).

1.6 Forecasting and determining the risk of MLD outbreaks

Epidemiological investigations into why an epidemic occurs at a specific location and the ability to forecast future disease risks are crucial to deploying management tactics such as for site selection, for optimal yield and profit. Numerous studies have been successful in developing models to predict disease risk in various pathosystems in agricultural systems (e.g. Hoppmann and Wittich, 1997; Twengstrom et al., 1998; Carrisse, 2000; de Vallavielle-Pope et al., 2000; Gilles et al., 2000; Llorente et al., 2000; Dalla Marta et al., 2005). Most model development in forestry has focussed on tree related issues such as growth and yield prediction (e.g. Battaglia et al., 2004).

Risk models that exist for forestry are based on coarse climatic data layers and depict regions which have conditions favourable to the disease, e.g. guava rust (*Puccinia psidii*) (Booth et al., 2000b), *Cylindrocladium quingeseptatum* Boedijn and Reitsma.
(teleomorph: *Calonectria quinqueseptata* Figueiredo and Namekata) (Booth et al., 2000a), and Dothistroma needle blight (*Dothistroma septosporum*) (Woods et al., 2005). A possible reason for lack of model development at a finer scale to predict disease risk in forest plantations is the logistical difficulties involved in collecting the level of detailed and replicated site data required. There is marked variation that occurs within and among forest plantation sites due to their vast geographical coverage and the wide array of possible interactions among different microclimates, topography’s and soils. Information about MLD epidemiology has been gleaned from laboratory experiments or a very limited number of field studies with the collection of broad-scale data only (Cheah, 1977; Beresford, 1978; Cheah and Hartill, 1987; Park and Keane, 1987; Park, 1988b) (Appendix 1). The only indications of risk provided by previous research is that MLD (caused by the two most common *Mycosphaerella* species) is predisposed by temperatures among 17-25 °C, 90% RH and a rainfall event.

In an attempt to discern the range of environmental variables contributing to the determination of risk, annual weather information from regions where MLD is or has been active were collated. These were input into AUSGRD, a climatic mapping program developed by CSIRO Forestry and Forest Products in Australia. AUSGRD contains mean climatic data arranged in a coarse grid laid over Australia. The program mapped the distribution of regions where the conditions favorable for MLD were present (Figure 1.2) (Pietrzykowski et al., 2003a). The scale of the map gives an indication of ‘regions’ within Australia where plantations may be at risk to MLD, but does not give any information at a fine scale such as the specific plantation or the specific time that disease risk is high. For example, the map fails to identify regions
in central and far northeast NSW where severe defoliation of *E. globulus* occurs by *M. nubilosa* (A. Carnegie, Forest Health Management, NSW Department of Primary Industry, Pers. Comm., 2006). The coarse nature of the map is an example of a prediction which lacks information on site risk factors such as topography, soil type, water sources and nearby vegetation, which all affect inoculum sources and the microclimate (which could affect forecasting the risk of MLD risk at the local scale).

Figure 1.2. The predicted distribution of Mycosphaerella leaf disease in Australia mapped according to ideal weather conditions based on annual weather data from AUSGRD. Scale-1/8\(^{th}\) of a degree for the Australia wide view and 1/25\(^{th}\) for the Tasmania (inset) view (Pietrzykowski et al., 2003a).
Seasonal and site-specific forecasting systems have been established and are being used by farmers to manage potato late blight (*Phytophthora infestans*) in the UK (Taylor et al., 2003), and tomato late blight (*Phytophthora infestans*) in Italy (Bugiani et al., 1995). These systems aid in alerting farmers to start spraying fungicides and can reduce unnecessary fungicide applications (Taylor et al., 2003). As an example, one forecasting system is Brassica\textsuperscript{spot} (Kennedy and Gilles, 2003). Brassica\textsuperscript{spot} predicts infection of *Albugo candida*, by indicating optimal time for controls to be implemented. This program is successfully being used in the UK, to reduce the number of fungicide sprays by 50% (Kennedy and Gilles, 2003). Fungicide application, however, will never be used in eucalypts due to environmental and economical constraints even though their use is being investigated in Victoria, Australia (Ian Smith, University of Melbourne, Australia). An early warning system to alert managers to apply fertilizer would be more applicable to the eucalypt industry.

Limited work has been completed in developing disease forecasting systems for tree species. One rule-based model developed by Meentemeyer et al. (2004) includes mapping the risk and spread of sudden oak death (*Phytophthora ramorum*) (Meentemeyer et al., 2004). This model maps regions at risk of infection by sudden oak death, based on knowledge of the susceptibility of multiple host species, the pathogens reproductive potential and spread, and climate variables that favour the host and pathogen. The model serves to precisely target high-risk areas for the purpose of prioritising the highest risk areas in need of disease eradication and management. Another example involves a model developed for predicting the presence of *Phytophthora cinnamoni* (Wilson et al., 2003). However, this model is not species-specific and only predicts the presence of *P. cinnamoni* in communities of
native sclerophyll vegetation in south-eastern Australia (Wilson et al., 2003). To date, disease risk modelling for the eucalyptus plantation/MLD pathosystem has been conducted only at a coarse scale (Pietrzykowski et al., 2003a) similar to that completed for guava rust (Booth et al., 2000b).

1.7 Assessment methods

Plantation health surveillance programs are an important component of plantation forest management. Data collected is used by forest managers to develop management regimes and by external groups for forest accreditation. Therefore there is a need to use surveillance methods that are repeatable (for monitoring over time), accurate (consistently include small errors) and are inexpensive (Stone et al., 2003b). Methods also require standardisation and acceptance amongst the forest industry which allows for comparisons to be made among organisations.

To date, in Australia, crown health has been assessed using a number of ground and aerial methods that are not standardized among forest agencies and companies. These include seasonal (short term) and permanent plots, drive-by roadside assessments, and aerial surveys from helicopters or small planes (Stone et al., 2003b; Stone and Haywood, 2006). These methods are time consuming, tend to be subjective, are expensive, and data is often qualitative, hence lacking quantitative detail. Moreover, precision is dependent on the observers’ knowledge and experience. For example, aerial sketch mapping is limited because (i) early infection symptoms may be overlooked as they are less visible and/or not clearly obvious from aerial platforms, and (ii) an experienced observer may not always be available (Stone and Haywood, 2006).
Currently in Australia, data is collected on an ‘ad hoc’ basis with only small proportions of the nation’s total forest resource base being assessed (Stone et al., 2003b). Data collected is mainly descriptive, and hence, is limited in its value for comparison with data from other states and countries. Furthermore, forestry companies and agencies in different states have different methods for reporting on forest health, and this data is also limited at the regional scale. A similar issue exists among forestry companies in the U. S. A. and Europe as shown by Hickey et al., (2005) who have reported on the differences that occurred among 22 forestry companies consisting of both public and privately owned, operating in 15 jurisdictions. The study found that companies have a similar level of formal monitoring, but the range of methods, procedures, scales and indicators which they report differ (Hickey et al., 2005). Developing and adopting a more universal prescription for monitoring forest health, international collaborative research would facilitate the acquisition and sharing among countries.

The United States Department of Agriculture, Forest Service (USDA FS), uses a forest health monitoring program to assess the condition of the nations forests (Mangold, 1998). This program is based on a formal, permanent grid-sampling framework that is temporally stratified. It includes a series of tree and plot scale forest health monitoring indicators, which includes assessing the type of damage on a tree, its location and severity (Stone et al., 2003b). The USDA forest health management program is funded by the federal government and has achieved national coordination and standardisation of data gathering, analysis and reporting. This system, therefore, enables comparisons at state, regional and site scales.
Recently, a new standardised assessment method, the Crown Damage Index (referred to as CDI from here in) was developed in Australia to assess crown damage in young (juvenile foliage) eucalypt plantations (Stone et al., 2003a). It includes assessment methodology associated with estimating disease intensity (incidence and severity) at the tree crown scale, the CDI, as well as protocols for assessing and sampling an entire plantation.

The main objectives of the Crown Damage Index assessment method are to provide data on plantation health, evaluate the effectiveness of insect management programs, assess research trials and formulate future yield projections both within and among plantation forests (Stone et al., 2003b). However, errors occur at the tree crown scale due to assessment error by scorers, and at the plantation scale if the sampling protocol is not utilized as recommended. This sampling methodology also provides sampling error estimates (Smith et al., 2005; Smith, 2006). The CDI is a first step towards developing a standardized assessment protocol that can be adopted at a national level and used by all government environmental agencies, research organisations, and forestry companies.

1.8 Remote sensing

Recent advances in remote sensing technology provide the opportunity for developing a standard and rapid crown health surveillance method for plantations. Optical remote sensing measures the amount of electromagnetic energy reflected and/or emitted from vegetation in non-visible wavebands as well as visible wavebands, and it can potentially identify features that the naked eye cannot (Asrar, 1989; Nutter et al.,
It provides a method for collecting large volumes of quantitative and accurate data in a non-destructive manner, which can potentially allow for early detection of health problems at the crown and plantation scale.

Remote sensing has been used around the world for various purposes for many years. Numerous projects have demonstrated the capacity of this technology in plantation forestry and native forests to monitor tree health, identify and assess severity of biotic or abiotic stresses, and identify physiological changes (e.g. Heller and Bega, 1973; Curran, 1982; Ahern, 1988; Leckie et al., 1988; Ekstrand, 1994; Carter et al., 1996; Carter et al., 1998; Coops, 2001; Blackburn, 2002; Moskal and Franklin, 2004; Leckie et al., 2005; Coops et al., 2006). In Australia it has been used successfully in eucalypt forests to identify and assess leaf and canopy attributes such as crown condition (Coops et al., 2004; Stone and Haywood, 2006), stand volume (Coops et al., 1999; Stone and Haywood, 2006), habitat complexity (Coops, 1997; Coops et al., 1998; Coops, 2001), leaf area (Coops, 2004), leaf water and pigment content (Datt, 1998, 1999; Coops et al., 2003b), and insect and fungal damage (Stone et al., 1998; Stone et al., 2001; Stone and Coops, 2004; Stone et al., 2005). Though remote sensing has been used to assess the severity and extent of Dothistroma needle blight in Pinus radiata plantations (Coops et al., 2003a; Stone et al., 2004a; Stone et al., 2004b), it has not been used in Eucalyptus plantations.

For remote sensing to be applied successfully to assess canopy condition, it is essential to have reliable and accurate ground-based information from discrete plots matching or exceeding the scale and resolution of the imagery acquired remotely (Reike and Jones, 2006; Stone and Haywood, 2006). The reflectance data extracted
from the image is used to characterize the various symptoms exhibited by the crowns, which is also matched to ground-based observations. Algorithms are then developed to differentiate among symptom types and severities at the crown scale. The algorithm can then be applied to the whole image to estimate disease intensity for an entire landscape.

While the cost of image acquisition is currently high, remote sensing has substantial advantages over manual forest health assessments. The data is spatially continuous, referenced and digitized, and hence can be integrated with existing Geographical Information System (referred to as GIS from here in) data. It has the capability to produce disease maps and be incorporated into statistical and forecast modeling. This further provides forest managers with highly valuable information for their management and decision support programs.

1.9 Research overview

1.9.1 Scope

As *Mycosphaerella* leaf disease is of international importance, having been observed causing damage in *Eucalyptus* plantations in many countries around the world (Carnegie and Keane, 1994; Milgate et al., 2001; Ahumada et al., 2003; Mohammed et al., 2003; Crous et al., 2004; Milgate et al., 2005; Gezahgne et al., 2006; Smith, 2006) and, it is the prime pathogen to use as a case study for this research. The primary aim of this work is to develop operationally viable, reliable, and accurate disease forecast and surveillance protocols for use by eucalypt plantation managers. To achieve this aim a number of the hypotheses (below) were tested.
1.9.2 Hypotheses

EPIDEMIOLOGY

1. Atmospheric Mycosphaerella ascospore concentration is correlated with meteorological variables.

2. The relationship among Mycosphaerella atmospheric ascospore concentration and meteorological variables can be used to develop an ascospore risk prediction model.

REMOTE SENSING

1. Spectral analysis can be used to quantify the extent to which Mycosphaerella leaf disease causes damage to eucalypt foliage.

2. Models can be developed to identify symptoms of Mycosphaerella leaf disease at the crown scale and further applied to Digital Multi Spectral Imagery to scale up to the plantation scale.

1.9.3 Objectives and summary of work

The main areas of research completed were: quantifying the effects of meteorological variables on atmospheric ascospore density, forecasting atmospheric ascospore concentration of Mycosphaerella Leaf Disease, remote sensing and spectral characterization of Mycosphaerella Leaf Disease on E. globulus foliage, and the development of a crown-based model to predict Mycosphaerella Leaf Disease when using remotely sensed imagery.

Chapter 2 investigates the relationship among meteorological variables and atmospheric ascospore concentrations in a Eucalyptus plantation suffering from an epidemic of MLD. The first objective of the investigation was to identify more
precisely than previous studies, the meteorological variables correlated with increased atmospheric ascospore concentrations; and secondly, to identify and describe any seasonal and diurnal periodicities in atmospheric ascospore concentrations. The third objective was to develop a model that uses meteorological data to predict when ascospore release and dispersal events would occur and the concentration of the ascospores in the atmosphere at those times. The chapter describes how this model could be used to issue a ‘disease risk alert’ to help plantation managers in selecting low disease risk plantation sites and implement other management intervention. It further explains how it can be used at established plantation sites by managers to predict future disease occurrence.

There has been no investigation to date on the effects of Mycosphaerella Leaf Disease on leaf or crown spectral characteristics. The objectives of Chapter 3 were to characterise MLD severity at the leaf scale using spectral reflectance data, and to provide spectral diagnostic information that will be used to produce reflectance indices and algorithms in the further development of models to predict symptom severity at the crown scale.

Scaling up from leaf to crown has proven to be successful in various pathosystems, such as *Dothistroma* needle blight in *Pinus radiata* (Coops et al., 2003a), and to discriminate among tropical rainforest tree species (Clark et al., 2005). Chapter 4 presents the first study demonstrating the use of remote sensing technology to detect and identify symptoms of MLD in *E. globulus* trees at the crown scale. The first objective was to explain how information at the leaf scale can be used as a basis to develop models that predict symptom severity at the crown scale. Secondly, to
present a crown scale model and demonstrate how the model is applied to the plantation scale and the information that can be extracted and used for plantation management.

Lastly, Chapter 5 integrates the results from Chapters 2, 3 and 4 and discusses a framework (Figure 1.3) of how the methods used in the investigations could be included in a Decision Support System (DSS) to enhance the accuracy of current plantation health management. It explains how identifying the risk of a disease outbreak at the plantation scale is an integral first steps in choosing sites for plantation establishment, and how disease forecasting could become part of an ongoing alert system to predict times of high risk once a plantation is established. Additionally this Chapter discusses how remote sensing offers a uniform and robust alternative method for forest health surveillance. Recommendations are offered on how researchers and forest industries can work on further developing these methods to enable them to be operationally viable and integrate the technology into existing management systems.


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Chapter 2.

Atmospheric ascospore density of Mycosphaerella Leaf Disease in a *Eucalyptus globulus* plantation.

Work from this chapter will be published as:

**Pietrzykowski, E.,** Foster, S., Pinkard, L., and Mohammed, C. Factors influencing the periodicity and density of atmospheric ascospores at a plantation site in Tasmania of *Eucalyptus globulus* infected with *Mycosphaerella nubilosa*. Forest Pathology. In prep.

2.1 Introduction

Our understanding of *Mycosphaerella* spp. epidemiology in eucalyptus has been derived from studies conducted in the late 1970’s and early 1980’s in south-eastern Australia and New Zealand on the species *Mycosphaerella nubilosa* and *M. cryptica* (Cheah 1977; Beresford 1978; Ganpathi 1979; Cheah and Hartill 1987; Park and Keane 1987; 1988). These investigations focused on the taxonomy and biology of the pathogen, including the environmental conditions required for lesion development and ascospore maturation, discharge and germination (see Appendix 1 for details on these studies).

From their studies with *E. delegatensis*, Beresford (1978) and Cheah (1977) concluded that ascospore discharge in the field occurred under similar conditions to those occurring in the laboratory. They reported that the presence of rain or moisture in the form of dew or near-saturation relative humidity were sufficient for ascospore discharge to occur (Cheah 1977; Cheah and Hartill 1987). Beresford (1978) found ascospore density peaked when rainfall occurred after a long dry period, which was in agreement with laboratory results (Park and
Keane 1987; Park 1988). Results from MLD field trials are predominantly descriptive, compared with results from laboratory experiments obtained under controlled conditions. This is because detailed and reliable meteorological data were not easily obtained at the time many of the experiments were conducted.

Spore trapping in New Zealand indicated that the highest atmospheric ascospore density of the *Mycosphaerella* species under investigation coincided with summer and autumn, during the host’s maximal vegetative growth period, and reduced with the onset of winter (Cheah 1977; Beresford 1978; Cheah and Hartill 1987). These are similar to observations made with *Kirramyces eucalypti* (Cook & Massee) J. Walker in *E. nitens* in New Zealand (Hood, Chapman et al. 2002) (Appendix 1). From these results it was concluded that the annual disease cycle of *Mycosphaerella* spp. commences in spring if low levels of ascospore inoculum are available. Disease and ascospore build-up occurs during the host’s vegetative growth period when meteorological variables favourable to MLD are present (long periods of rainfall, high relative humidity and/or prolonged leaf wetness) (Park 1984).

To date, studies completed on *M. cryptica* and *M. nubilosa* have been either short-term laboratory experiments under constant environment conditions, or field experiments that were based on ascospore data counted on a weekly time frame (Cheah 1977; Beresford 1978; Park 1984) (Appendix 1). The most detailed field data collected involved counting *Mycosphaerella* spp. ascospores on *E. delegatensis* in New Zealand on a weekly time step over a two year period (Beresford 1978). Therefore, investigations into diurnal spore patterns could not be ascertained from there study. Park (1984) investigated at an hourly scale in the laboratory and noticed a diurnal pattern of spore release, however, is uncertain whether this pattern would be maintained under field conditions.
To aid in planning of control strategies, especially fungicide application, it is important to know if fungi have spore specific release patterns (periodicity and density). These patterns may be seasonal and/or diurnal and influenced by meteorological factors. The timing of fungicide applications to coincide with threshold values of atmospheric spores and meteorological variables is a common disease management practice in agricultural crops, such as apples (e.g. Rossi, Ponti et al. 2001), broccoli (e.g. Kennedy and Gilles 2003), and potatoes (e.g. Taylor, Hardwick et al. 2003). In apple orchards where *Venturia inaequalis* (Cooke) Wint. is a problem, fungicides are applied when ascospores are actively released into the atmosphere (Rossi, Ponti et al. 2001). The spore trap is employed to monitor atmospheric ascospore density and the information they give trigger intervention. Spore release patterns can also be predicted using simulation models based on meteorological parameters (e.g. Burt, Rosenberg et al. 1999; Aylor and Flesch 2001). As stated in Chapter 1, fungicide application is currently not a feasible option in a eucalyptus plantation system. If a suitable “environmentally-friendly”, low costing fungicide becomes available, it will be necessary to understand and predict *Mycosphaerella* ascospore release patterns.

In the following study, hourly *Mycosphaerella* ascospore density field data were used alongside hourly meteorological data to investigate the effect of meteorological variables on atmospheric ascospore density over approximately 28 months. The field studies were completed to extend our knowledge of the ascospore release patterns of *M. nubilosa* infecting *E. globulus* under Tasmanian conditions. Many previous studies of spore release have been conducted in the laboratory and such results cannot always be applied to explain situations in the field.
Specific aims of this work were to (a) investigate the effects of a range of meteorological variables on atmospheric Mycosphaerella ascospore periodicity and density in an *E. globulus* plantation, (b) identify seasonal and/or diurnal patterns in atmospheric ascospore periodicity and density in the field and which meteorological variables most affect these patterns, and (c) describe mathematically the ascospore release patterns and present the first steps towards producing a MLD warning system.

2.2 Methods

2.2.1 Study site

The study was conducted in a *Eucalyptus globulus* plantation (CH033B) located 15 km from Smithton at Christmas Hills, in north-west Tasmania, Australia (S40° 55’ 21”: E144° 59’ 39”) (Figure 2.1). The plantation was 65.8 ha in size and was planted in 2001. Infection from *Mycosphaerella* was identified soon after planting. The plantation was surrounded by native wet sclerophyll forest and a number of other eucalyptus plantations, all of which exhibited symptoms of infection by MLD. Smith et al. (2006) identified a complex of *Mycosphaerella* species infecting leaves; however, the predominant species in the *E. globulus* plantation was *M. nubilosa*. 


Figure 2.1. Location of the automated weather station and spore trap (Quest Volumetric Spore Trap) in a *E. globulus* plantation (CH033B) used to trap ascospores of Mycosphaerella Leaf Disease, at Christmas Hills, in north-west Tasmania (Inset).
2.2.2 Data

Atmospheric ascospore density and meteorological data were collected for this study. Data were collected from the 5th September 2002 (Spring) through to 11th November 2004 (Spring) at the field site. Trees were 308 days old when data collection began.

**Atmospheric ascospore density**

Atmospheric ascospore densities were studied using a Quest Volumetric Spore Trap (Melpat International Pty Ltd, Canningvale, WA, Australia). The trap was adjusted to sample 20 litres of air per min through a 25 mm × 2 mm orifice that was approximately 50 cm above the plantation floor. The trap’s vacuum was powered by a 12-volt car battery that was attached to two solar panels allowing for continuous and constant power over the duration of the experiment. Perspex (toughened plastic) disks were placed into the trap and mechanically rotated exposing the disk to an opening through which air was impacted. Disks were divided into 8 segments corresponding to 8 individual days, and further divided into 24 hour bands. Disks were lightly sprayed with Vaseline lubricant to ensure spores would remain on the disk. The trap was placed in a central location of the plantation between tree rows (Figure 2.1) which were 2.5 metres apart. As the trees reached heights of 2 metres the trap was also raised to 2 metres, to be level with the top of the canopy. This was done to ensure branches from neighbouring trees did not obstruct the trap. The trap was raised on the 3rd of October 2003.

Ascospores were examined using a microscope using ×200 magnification and were identified as *M. nubilosa* according to descriptions given by Park and Keane (1982) (Figure 2.2). Though a complex of *Mycosphaerella* species were identified as infecting foliage at the site (Smith 2006), PCR molecular identification techniques verified *M. nubilosa* as the
most dominant (Glen, Smith et al. 2007). Samples were counted from each hour by counting one random field of view for each hour and multiplying up to give one hour (note: there were 54 fields of view in each hour). To examine the accuracy of the sampling method, ascospores were counted for the complete area designated to an hour and compared to the scaled up sample. The relationship between observed and estimated ascospore densities per hour was examined using linear regression.

Figure 2.2. Ascospore of *Mycosphaerella nubilosa* as seen under the microscope (a) at 200x magnification and a sketch (b).

**Meteorological data**

Hourly records of meteorological variables (see Table 2.1) were recorded at an onsite automated meteorological station (ENSIS, Hobart, Australia) (see Figure 2.1 for location of meteorological station at study site). Temperature and humidity were measured using a temperature/humidity probe (Vaisala HMP35A. Helsinki, Finland), solar radiation using a Licor L11200X pyranometer (Nebraska, USA), wind speed using a Met One 014A anemometer (Oregon, USA) and rainfall using a tipping-bucket rain gauge that was 200 mm in diameter and recorded rainfall in units of 0.2 mm per tip (Monitor Sensors TBRG Qld., Australia). Sensors were connected to a Campbell Scientific 21X multiplexer data
logger (Campbell Scientific, Logan, Utah, USA), which recorded the hourly data and calculated vapour pressure deficit (VPD). The logger also calculated daily averages for each of the climatic variables in addition to recording the maximum and minimum daily temperature and maximum rainfall every 5 minutes. The sensors and logger were located approximately 1.5 m above the ground inside a protective Stanley screen housing. The solar radiation sensor was located at the top of the screen (~2 m) and both the anemometer and rain gauge were located 3 meters away from the station in opposite directions. The anemometer was secured 2 meters above the ground and the tipping bucket rain gauge was fastened on a 30×30 cm concrete slab at ground level.

Table 2.1. Meteorological variables recorded at Christmas Hills (see Figure 2.1 for the location of the meteorological station at the study site).

<table>
<thead>
<tr>
<th>Meteorological variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = Rainfall (mm)</td>
</tr>
<tr>
<td>T = Average temperature (°C)</td>
</tr>
<tr>
<td>T_{MAX} = Maximum temperature (°C)</td>
</tr>
<tr>
<td>T_{MIN} = Minimum temperature (°C)</td>
</tr>
<tr>
<td>RH = Average relative humidity (%)</td>
</tr>
<tr>
<td>VPD = Average vapour pressure deficit (Kpa)</td>
</tr>
<tr>
<td>SR = Average solar radiation (KW/m²)</td>
</tr>
<tr>
<td>WV = Average wind velocity (ms⁻¹)</td>
</tr>
</tbody>
</table>

**Missing and unreliable data**

There were two periods where the weather station failed and data were not available. These were during the periods 23rd July 2003 to 11 September 2003 and 13 January 2004 to 26th February 2004. See section 2.2.3 Data analysis, for details on how the missing data were handled.

Solar radiation measured at the weather station was inconsistent between years and hence unreliable. These data were replaced with solar radiation data purchased from the Bureau
of Meteorology (this variable was denoted as BOMSolRad) for its Cape Grim meteorological station (40° 40’S, 144° 41’E) on the north west coast of Tasmania, approximately 30 km from the study site. The effect of the geographical difference between the two sites is assumed to be negligible.

Additional data variables

Additional variables were used in data analysis. These included age of the plantation and a number of variables further derived from the meteorological variables. These were chosen based on conceptual models from previous studies (Gottwald and Bertrand 1982; Papastamati, van den Bosch et al. 2002; De Wolf, Madden et al. 2003; Mondal, Gottwald et al. 2003; Prados-Ligero, Melero-Vara et al. 2003) and are included in Table 2.2.

Descriptions of the variables explain the conceptual model which is hypothesized to effect ascospore density. The temperature range variable (T_{Range}) was chosen considering the empirical distribution (10th and 90th percentiles) of the temperature data for days when ascospores were recorded.

Table 2.2. Additional variables computed from meteorological variables logged by the weather station and regarded as important for the conceptual model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH90</td>
<td>A binary variable indicating if relative humidity is above 90%</td>
</tr>
<tr>
<td>RH90Time</td>
<td>The length of time (measured in days) that the RH has been above 90%</td>
</tr>
<tr>
<td>Rain</td>
<td>A binary variable indicating if it is raining</td>
</tr>
<tr>
<td>RTimeSince</td>
<td>The length of time (measured in days) since the last rain event</td>
</tr>
<tr>
<td>R_DSR</td>
<td>Number of days since rain</td>
</tr>
<tr>
<td>TInRange</td>
<td>A binary value indicating if the temperature is in the range 5°C-15°C</td>
</tr>
<tr>
<td>T_HrsOutRange</td>
<td>The length of time (measured in days) that the temperature is outside the temperature range 5°C-15°C</td>
</tr>
<tr>
<td>Light</td>
<td>A binary variable indicating if it is light</td>
</tr>
</tbody>
</table>
2.2.3 Data analysis

Data used for analyses were ascospores per cubic litre per hour (spores L\(^{-3}\)hr\(^{-1}\)). Analyses were split into three parts 1) investigation of seasonal and diurnal patterns in spore density, 2) effects of meteorological variables on hourly ascospore density and 3) mathematical description of ascospore presence/absence and density patterns.

1) Investigation of seasonal and diurnal patterns in ascospore density

Dates when maximum ascospore densities were recorded were noted. Average seasonal and monthly values were calculated to identify patterns in the data. Hourly ascospore data were averaged for each hour of the day to investigate diurnal density patterns on days with and without rain.

2) Effects of meteorological variables on hourly ascospore density

Relationships between ascospore densities and meteorological variables were examined using scatter plots of the daily data and fitted with a trend line to extract outer boundaries. Additionally, a 100-day sequence of data was randomly chosen and used to examine daily lag time effects of rainfall and relative humidity on ascospore density.

3) Mathematical description of ascospore presence/absence and density patterns

This component was split into three sections and included fitting a mathematical function to (a) the patterns of ascospore presence with age of plantation, (b) the effect of meteorological variables on these patterns and (c) the density of the ascospore releases within the patterns identified. See Appendix 2 for more details on the functions used.
Patterns were investigated for daily and diurnal (hourly) data as follows:

Ascospore data were converted into a binary variable to investigating the presence (1) or absence (0) of ascospores. This eliminated the complication of including meteorological data and ascospore density for initial investigations into the effects of plantation age and/or season on ascospore density.

The mathematical functions (Appendix 2) used to describe the patterns, in the data were additionally used to calculate the probability of an ascospore release occurring and the density of ascospore density s once a release had been observed. Both calculations were based on the meteorological variable values during the time of ascospore release.

Appendix 2 gives an example of how to calculate the probability of ascospore release occurring excluding (Section 1) and including (Section 2) meteorological data, in addition to estimating the density of the ascospore release.

Function application that did not include meteorological effects used the full ascospore data set. Size of the set was reduced for analyses that did include weather variables, by removing the ascospore release observations that were taken within the two time periods when the ascospore trap failed (see section ‘Missing and unreliable data’ page 31).
2.3 Results

2.3.1 Ascospore counting method

Figure 2.3 depicts the fit of observed versus estimated *Mycosphaerella spp.* ascospore density data. Ascospores per unit volume from one field of view (scaled up to 1 hour) could be used to estimate the actual density of ascospores per hour. There was more unexplained error (variation) in the model at low ascospore densities.

![Figure 2.3. Density (observed) of trapped *Mycosphaerella spp.* ascospores per hour period versus estimated ascospore density at Christmas Hills, Tasmania in September 2003.](image)

2.3.2 Investigation of seasonal and diurnal patterns in ascospore density

Seasonal periodicity of *Mycosphaerella spp.* ascospores were recorded throughout the collection period of the experiment, and substantial peaks in ascospore densities were observed in the second year of the experiment compared to the first (Figure 2.4). The highest total ascospore density for a day occurred during the second year of the experiment on December 15\(^{th}\) with 23,976 ascospores being recorded. Peaks in ascospore density following December 15\(^{th}\) were frequently observed. In comparison, during the first year of the experiment, the highest daily total was 2,700 ascospores on November 24\(^{th}\), with the remaining ascospore density observations being low and infrequent during the first year.
Figure 2.4. Average monthly atmospheric *Mycosphaerella* spp. ascospore density (average ascospores/L$^3$ hr$^{-1}$ including standard error bars) throughout the data collection period (5$^{th}$ September 2002 through to 11$^{th}$ November 2004) at an *E. globulus* plantation at Christmas Hills, Tasmania. This graph shows greater ascospore densities during the second year (after November) compared to the first. It also shows that in the second year the highest density s were recorded in alternate months, beginning in December (summer).
The seasonal results indicate variability in ascospore density between years (Figure 2.5). During the second year of the trial, ascospore densities increased markedly during the summer then decreased over the subsequent seasons.

Figure 2.5. Seasonal average (including standard error bars; P<0.05) atmospheric ascospore density (spores L⁻³ hr⁻¹) throughout the data collection period. The red arrow in this graph shows the reduction in ascospore density from summer to spring during the second year of the trial. No pattern was evident from season to season during the first year.

Many of the peaks in ascospore density occurred up to 2 days after rainfall occurred was recorded and when average relative humidity was high (>90%). For example, Figures 2.6 and 2.7 include a 100-day sequence (randomly chosen) from the trial period depicting the increase in ascospore numbers within 2-3 days of a rainfall event and when relative humidity was high.
Figure 2.6 suggests the amount of rain does not affect ascospore release as low and high rainfall (mm) resulted in ascospores being trapped. The response to relative humidity (Figure 2.7) was similar (i.e. ascospores were recorded a few days after relative humidity increased), but not as obvious.

Figure 2.6. Observed daily ascospore density and rainfall data over a 00-day series (randomly chosen) from the 25th December 2003 – 3 April 2004. The red rectangle encompasses the days that include rainfall events and the associated ascospore record. The results suggest the density of the rainfall event (mm) does not influence the density of the ascospore release as most rainfall events (large and small) have an ascospore record associated with it. All of the ascospore densities were low.
Figure 2.7. Observed daily ascospore density and relative humidity data over a 00-day series (randomly chosen) from the 25th December 2003 – 3 April 2004. The base of the red arrows indicates when relative humidity begins to increase. The arrowhead points to the corresponding ascospore record which the increase in relative humidity under the arrow body could have initiated.
When all the data was average for a day (separating days with and without rainfall), two ascospore release patterns were observed. On days with no rain, a slight decrease in ascospores was observed during the middle of the day, and an increase was observed during the night (Figure 2.8). In comparison, on days where rainfall was recorded, ascospore observations peaked at a number of times during the day and night.

Figure 2.8. Hourly average (±SE) ascospore densities per unit volume for a day with and without rain. This graph shows that a diurnal ascospore density pattern exists in the field on days when there was rain, with a peak (green ellipse) in ascospore density occurring midmorning, and minor ascospore peaks occurring throughout the 24 hr period (blue ellipse). On an average day with no rain events ascospore density displays a subtle pattern with an increase occurring from approximately 1700 then decreasing in the early morning.
An example of the hourly effect of rainfall and relative humidity over a day is given in Figures 2.9 and 2.10 respectively. Ascospores were recorded a short time (~ 3-5 hours) after a rainfall event. Relative humidity had a similar effect but it was not as clear (Figure 2.10). Figure 2.10 includes a 10-hour period (0-1000 hrs) when relative humidity was above 80%, and the associated atmospheric ascospore release (beginning at 1900 h or 7 pm). Similarly later in the day ascospores were observed around 2200 h after an increase in relative humidity had begun at 1600 h).

![Graph showing hourly rainfall and ascospore release](image)

Figure 2.9. An example of a 2-day period (5th & 6th December 2003) during the trial depicting hourly rainfall and associated ascospore release (red parallelogram).
Figure 2.10. Hourly ascospore density and relative humidity for a 2-day period. Base of arrows indicate when relative humidity begins to increase and the associated ascospore release (blue ellipse).

2.3.3 Effects of meteorological variables on ascospore density

Scatter plots for each meteorological variable with atmospheric ascospore density are given in Figure 2.11. Relatively high hourly ascospore release (4 spores L^-3 hr^-1) were observed when (a) average daily temperature was between ~5 °C and 15 °C, (b) when average daily relative humidity was between ~80 and 95%, (c) when average daily VPD was between 0 Kpa and ~0.3 Kpa, (d) when average daily rainfall was up to ~23 mm, (e) when there was up to 3 days since a rainfall event, and (f) when average daily wind speeds were approximately 1-5 m/sec.

The outer boundaries from trend lines fitted for each meteorological variable within which a ascospore release was observed were (a) temperatures between 3.6 °C and 24.2 °C, (b)
relative humidity of 60% and 100%, (c) VPD of 0.1 and 0.6 Kpa, (d) rainfall up to ~ 40 mm (e) up to 10 days since rain and (f) winds between 0.5 m sec\(^{-1}\) and 11 m sec\(^{-1}\).

Figure 2.11. The effects of (a) average temperature; (b) average relative humidity; (c) average vapour pressure deficit; (d) average rainfall; (e) days since rain; (f) wind speed on atmospheric ascospore densities.
2.3.4 Mathematical description of ascospore density periodicities

The mathematical functions which were fitted to the raw data could not allow for serial autocorrelation (multicolinearity) due to the constraints of the type of functions fitted (Neter, Kutner et al. 1996). This means that all data at time \( x \) are assumed to be independent of values before and after this time. Figure 2.12a & 2.12b include the raw presence/absence data and daily ascospore density data over the time of the trial respectively. This data will be referred too in various parts of the proceeding sections.

*Presence/absence of ascospores with tree age not including meteorological variables*

There was a clear trend in the probability for observing an ascospore release with plantation age (Figure 2.13). The fitted cubic age trend was greater at midnight than midday. There was an increased probability of recording an ascospore release at the beginning of the measurement period (as seen in the raw data, Figure 2.14), during the first spring and summer, and during the second autumn (around ~900 days after planting) than during the first autumn and second spring.

Plantation age also influenced the probability of observing an ascospore release for diurnal data. This pattern is shown in Figure 2.15 for a number of days throughout the measurement period. The diurnal effect was more profound during summer days in the first year (e.g. Figure 2.15 day 312) compared to the second year when the diurnal pattern became less obvious (e.g. Figure 2.15 day 1092).
Figure 2.13. The pattern describing the probability of recording an ascospore release over time, for midday and midnight excluding meteorological variables. This graph shows the probability of observing ascospores is greater at midnight than midday throughout the trial period. Probabilities (indicated by red rectangle) of observing ascospores were also greater during the first spring and second autumn than other seasons. Note: Probability values are between 0 and 1. The y-axis on these graphs has been amended due to the small values.
Figure 2.14. Raw ascospore presence/absence data over time, indicating seasons and the year. The presence of an ascospore release is indicated by a black bar, and absence by no data point.
Figure 2.15. The diurnal patterns of probability for recording an ascospore release on selected days throughout the data collection period excluding meteorological data. The series of graphs show a clear diurnal pattern existed at the beginning of the trial (~day 312). This pattern decreased over time and was minimal by the end of the trial (~day 1092). Note: Probability values are between 0 and 1. The y-axis on these graphs has been amended due to the small values.
Predicting the presence/absence and the density of an ascospore release when meteorological variables are incorporated

The mathematical functions were used to analyse two aspects of an ascospore release, and are therefore presented as: firstly the probability of observing ascospores and secondly the size of the event once one occurred (Ridout, Demétrio et al. 1998). Figure 2.16 includes the raw data for ascospore density over time with the effects of meteorological variables.

Percentage relative humidity was excluded from the analysis as it was highly correlated with VPD (r = -0.931; p≤0.0001) and exhibited co-linearity when incorporated (Neter, Kutner et al. 1996). An alternative, binary relative humidity variable did not exhibit co-linearity and was included.

Figure 2.16. Raw ascospore density data over time, indicating seasons and the year.
The following conclusions relate to the effect of meteorological variables (from Tables 2.1 & 2.2) on atmospheric ascospore density and are based on estimates calculated as part of the analysis. Note: They should be considered as a complete model as many interactions exist between variables. The estimates are given in Table 3, Appendix 2. All results are significant to p<0.05.

**Relative Humidity above 90%**: If the relative humidity was above 90%, then the probability of observing ascospores increased.

**Rain time**: With increasing time since last rain event there was a decrease in the probability of observing ascospores.

**Wind velocity**: If an ascospore release was recorded, wind speed was positively correlated with ascospore density.

**Temperature**: Increases in temperature increased the density of an ascospore release, given that one occurred.

**Temperature within Range**: If the temperature was within the 5 °C – 15 °C range then the probability of observing ascospores was greater and the density of the ascospore release was likely to be increased.

**Hours that temperature is within range**: Increasing the number of hours that the temperature was within the specified range increased the probability of recording ascospores.

**VPD**: An increase in VPD generally decreased the probability of recording spores but this effect depended on the number of hours RH was above 90% and also on the level of solar radiation.

**Hours Relative Humidity has been above 90%**: Increasing the number of hours of high relative humidity generally increased the probability of recording ascospores but this effect was dependent on the level of VPD, light and amount of solar radiation. Hours that relative
humidity was above 90% also increased the density of the ascospore release when they were recorded.

**Solar Radiation:** Increasing the level of solar radiation generally decreased the probability of recording an ascospore release. However, this effect was dependent on the level of VPD and the number of hours of high relative humidity.

**Light:** Daylight generally decreased the probability of recording ascospore releases. However this effect was dependent on relative humidity being above 90% and the length of time high relative humidity was above 90%. Daylight also increased the density of an ascospore release when one was recorded.

The conclusions below explain the broad ‘global’ and ‘diurnal’ patterns that were identified in the data. In general, the inclusion of meteorological data resulted in the patterns observed when meteorological data was excluded becoming variable and no longer smooth.

**Global trend of ascospore releases with plantation age:** A age trend existed i) for predicting the probability of recording an ascospore release (Figure 2.17) and ii) the estimate of the density of the ascospore release once one had occurred (Figure 2.18). Results indicated that the probability of recording an ascospore release was greater when the plantation was younger, at midnight, compared to when it was older (~ age 1000) (Figure 2.17). The probability of recording an ascospore release was generally greater during the night, except around 900 days when the probability at midday increased slightly, then fell. In comparison, the estimated density of an ascospore release in a younger plantation was smaller than in an older plantation (Figure 2.18). The global trend also indicated the estimate was greater at midnight in a younger plantation and midday in an older plantation.
**Diurnal effects:** The diurnal effects changed over the period of the trial for presence/absence and density predictions. Figures 2.19 and 2.20 depict the diurnal effects for selected days over the time of the trial. Figure 2.19 illustrates the probability of an ascospore release being recorded, and Figure 2.20 shows the predicted density of an ascospore release if it occurs. These graphs show that in the initial stages of the trial the probability of recording ascospores (presence) was increased during the night and reduced during the day; e.g. for day 312, the probability at 1200 hrs is ~ 0.02, while at 2400 hrs it is ~0.15. This effect diminished as the trial progressed. Additionally, when an ascospore release was recorded, the density was relatively similar at all times throughout the day at the initial stages of the trial but then increased during the daytime during the later stages.
Figure 2.17. Probability of observing an ascospore release versus age of the plantation for the observed explanatory variables incorporating meteorological data. There was a greater probability of observing ascospore releases at midnight compared to midday, apart from during winter 2004 and a certain days in the period between spring 2003 and autumn 2004. Note: Probability values are between 0 and 1. The y-axis on these graphs has been amened due to the small values.
Figure 2.18. Estimated density of an ascospore release versus age of the plantation for the observed explanatory variables incorporating meteorological data. This graph shows the density of ascospore releases was generally greater at midnight compared to midday during the first year, compared to the second year of the trial, when the opposite trend prevailed. The density of ascospore releases began to increase during the second spring and summer.
Figure 2.19. Diurnal pattern of the estimated probability ($\mu$) of observing ascospores for selected days throughout the trial for observed meteorological conditions. Distinct diurnal patterns existed for the probability of observing ascospores throughout the trial period. The most profound diurnal pattern occurred early in the trial period (~Day 312). Note: Probability values are between 0 and 1. The y-axis on these graphs has been amened due to the small values.
Figure 2.20. Diurnal pattern of estimated ascospore density ($\lambda$) versus hour of the day for selected days throughout the trial including the observed meteorological conditions.

Diurnal patterns of ascospore density were present, with the most distinct patterns occurring between Day 505 and Day 886. The hourly ascospore densities over a day varied during the trial period e.g. Day 312 and Day 1065 had opposite diurnal patterns of ascospore release with increases occurring throughout the middle of the day on Day 1065.
2.3.5 Observed versus predicted ascospore periodicities

Although the patterns in the data (‘global’ and ‘diurnal’) were well described by the mathematical functions, there was spoor correlation between observed and predicted values, both for the presence of an ascospore release and the density of that event. This is examined in the following.

Presence/absence and density of an ascospore release with tree age including meteorological data

Global trend of ascospore release with plantation age: Figure 2.21 combines the two aspects of the analysis (probability of recording as ascospore release and the predicted density of the event) to examine the effects of the age of the plantation and meteorological variables on atmospheric ascospore density. There are two distinct features in Figure 2.21 which can also be observed in Figure 2.16. The first is the predicted increased density of ascospore releases at the beginning and around ¾ of the way through the experimental period. The predicted increase at the beginning reflects the increased probability of an ascospore release occurring (Figure 2.17) at this time. The higher peaks ¾ of the way through the experimental period were influenced by the predicted increase in the density of ascospore releases (shown in Figure 2.18). The second distinct feature is the peak at around 1000 days after planting, in the final Spring of the experiment. However the reasons for this were unclear. Figure 2.16, presented at the beginning of this section, is the raw data which was firstly split for modelling to produce the results in this section.

Figure 2.22 shows both the observed and predicted ascospore releases and density of ascospore releases over time. The predicted values show little relation to the observed values. This is further demonstrated in Figure 2.23.
Figure 2.21. Predicted ascospore density versus age of the plantation for the observed meteorological conditions. This graph shows predicted ascospore releases were greater than the observed during the first spring and second summer and autumn. Frequency of ascospore releases was generally greater during midnight compared to midday except during the second winter. There is no obvious explanation for the large peaks predicted at the end of the trial.
Figure 2.22. Observed and predicted ascospore releases and their density over time. Predicted values were calculated incorporating meteorological data.

Figure 2.23. Observed versus predicted ascospores over the time of the trial incorporating density of an ascospore release and meteorological variables.
**Diurnal effects:** The predicted diurnal patterns in ascospore release are shown in Figure 2.24. They reflect the combined patterns shown in Figures 2.19 and 2.20. In particular, the expected numbers of ascospore release during the middle of the day were less in the first year (e.g. day 312) compared to the second year (e.g. day 970). For a period just before the second summer, the predictions suggest an increase in the density of ascospore release at dawn and dusk (e.g. day 942). The fit of the observed versus predicted data in Figure 2.25 reflects a poor relationship between the observed and predicted data.

**Figure 2.24.** Predicted ascospore density versus hour of the day, for selected days throughout the trial for the observed meteorological conditions.
Figure 2.25. Observed versus predicted ascospores for diurnal data incorporating density of an ascospore release and meteorological variables.
2.4 Discussion

2.4.1 Epidemiology

This work has presented the first attempt to predict MLD atmospheric ascospore density in a *Eucalyptus* plantation. Investigations into the effect of meteorological variables on ascospore discharge are a fundamental first step towards developing a disease forecast model. The epidemiology of *Mycosphaerella* spp. on eucalypts has been researched in the past (Cheah 1977; Beresford 1978; Ganapathi 1979; Park and Keane 1982; Park 1984; Cheah and Hartill 1987; Park and Keane 1987) (Appendix 1). Thus far no attempts have been made to describe mathematically the atmospheric density patterns of MLD ascospores in the field and the effect of meteorological variables. The results presented here both confirm and extend the results of previous studies although there is some disagreement.

In many instances the results for the meteorological effects on ascospore density in this Thesis concur with those of other authors (Cheah 1977; Beresford 1978; Ganapathi 1979; Park and Keane 1982; Park 1984; Cheah and Hartill 1987; Park and Keane 1987). For example results in this Thesis indicate that moisture from rainfall is an important variable in regulating the presence of atmospheric ascospores. Moisture is essential for the mechanism which controls ascospore release in ascomycetes (Lucas 1998). Moisture is also critical for ascospore release in other *Mycosphaerella* species, such as *M. citri* (Mondal and Timmer 2002), *M. graminicola* (Shaw 1993), *M. pinodes* and *M. fijiensis* (Burt, Rosenberg et al. 1999), as well as other pathogens of eucalypts such as *Phaeophleospora eucalypti* (Hood, Chapman et al. 2002) (see Appendix 1 for more detail) and *Aulographina eucalypti* (Wall and Keane 1984).
The suggestion that rainfall intensity does not affect the density of ascospore density confirms the conclusion of Cheah and Hartill (1987), as well as those found by Hidalgo et al. (1997) with *M. citri* (see Appendix 1 for more detail). In this study, ascospores were recorded on days without rain in contrast to Cheah (1977) who only recorded ascospores during rain days. Ascospore presence in the absence of rain could be due to the influence of near saturation relative humidity that was common at site. Results from Park (1984) also support this conclusion, as ascospore release in the laboratory was observed under near saturation treatments (Park 1984). The importance of high relative humidity is supported by all laboratory studies (Cheah 1977; Park 1984; Cheah and Hartill 1987).

Ascospores were most commonly trapped when temperatures were between 5 °C and 15°C, with most release periods occurring around 10°C. This temperature range is low in comparison to past results from Cheah (1977), who observed the optimal temperature for ascospore release was 22°C with a large decline occurring at or below 15°C (Cheah 1977). Cheah (1977), the only other quantitative study that investigated the effects of temperature on *Mycosphaerella* ascospore release, was undertaken in the laboratory. The difference between the results in this Thesis and those of Cheah (1977) could be due to the exclusion of other environmental factors in his controlled environment trials, and that he was investigating *M. cryptica* and not *M. nubilosa*. Other investigations on the influence of temperature on spore release of *Mycosphaerella* have predominantly focussed on the optimal temperature required for ascospore germination and infection and it appears that temperatures are similar for
both germination and infection (Beresford 1978; Ganapathi 1979; Park and Keane 1982; Park 1984).

Wind velocity was a significant variable affecting ascospore density in this study. Results in this study were different to those found by Cheah (1977) who observed that an increase in wind speed resulted in a decrease in ascospore density in the tree canopy. Results from Beresford (1978) agreed with the current results, in that an increase in wind velocity results in an increase in ascospore density. The results presented in this chapter support theories in earlier work by Gregory and Lacey (1963). They suggested an increase in ascospore density was from greater wind speeds causing a reduction in thickness of the boundary layer at the leaf surface, thus exposing the leaf’s surface to greater effects of the wind and removing ascospores from fungal structures on the leaf surface. Beresford (1978) also concluded that light winds could produce enhanced vertical movement of ascospores between trees, also resulting in increased ascospore densities. This agrees with current results that suggest light winds (0.5 m/sec) result in an increase in atmospheric ascospore density, although if winds become too great, then there is a decrease. This decrease could be due to spores being blown out of the canopy beyond the spore trap or the inability of the spore trap to efficiently trap air in strong winds.

Cheah (1977) and Beresford (1978) found that ascospore discharge occurred immediately after rainfall and ceased soon after. The current results partially agree with this. Results indicate discharge was maximal one day after rain and then declined, until 6 days since rain when ascospore release ceased. These results, together with the diurnal pattern of ascospore records after a rain event (Figure 2.9),
suggest cycles of wetting then drying influence ascospore release and atmospheric
density, a conclusion also made by Park (1984).

In the absence of rain it was estimated that ascospore releases were more common
during the night than during daylight. This study is the first to report a diurnal
atmospheric ascospore density periodicity in MLD in the field, and hence, confirms
laboratory results from Park (1984). The formation of dew and increase in relative
humidity at night, in the absence of rain, may cause ascospore release to occur later in
the evening and continue during the night until mid morning and then reduce in
density during the afternoon. This suggests a wetting and drying cycle not reliant on
rain events, i.e. based on leaf wetness from the formation of dew, with drying
throughout the day that occurs from an increase in temperatures during daylight hours.
Rain events during the day, however can trigger a daytime ascospore release.

It was determined that the duration relative humidity is above 90% will affect the
density of a ascospore release; meaning that with wet and warm conditions
atmospheric ascospore density will be larger, especially in areas where there are
suitable microclimates, such as within densely foliated young eucalypt trees or young
coppices. This would lead to possible severe epidemics if suitable conditions
followed that favoured germination and penetration of spores that were deposited on
susceptible leaf tissue.

This was the first study where host age was incorporated into investigations of
Mycosphaerella spp. atmospheric ascospore density in a eucalypt plantation. Results
from the current study demonstrated that increasing plantation age resulted in
increases in density of ascospore releases. This could be due to the increased leaf volume of the older trees, as this means increased leaf area was available for infection, lesion formation and ascospore production. Trees in the plantation were nearing change to adult foliage and hence were also exhibiting the largest volume of juvenile leaf area, which is more susceptible to infection than adult foliage (Park 1984; Smith 2006). Leaf litter may also be an increasingly important source of inoculum as the plantation ages and the increase in ascospores associated with older age may be due to larger crowns and more leaf litter. Unfortunately the trees in the trial in this Thesis did not experience a severe epidemic but it is very important to include a factor such as plantation age as it will markedly influence inoculum production.

_Eucalyptus_ growth models such as CABALA (Battaglia, Sands et al. 1999) can be used to predict canopy size, which would aid in simulating ascospore density. Trials completed by Park (1984) concluded increased ascospore density resulted in increased disease incidence, with high density s $(10^5$ ascospores.ml$^{-1}$) resulting in blighting lesions. Simulated information on ascospore density could be further used to predict risk of infection occurring in epidemic proportions.

It is possible that ascospore densities at the research site investigated in this Thesis dropped in the months following the end of the experimental period. Reinfection of the trees would have been restricted by the tree’s change to adult foliage that is markedly less susceptible to _M. nubilosa_. Some remnant ascospore release could occur from leaf litter in consecutive months following adult phase change as found in leaf litter studies by Park (1984) and Beresford (1978). The epidemic should
therefore cease at the site resulting in trees with short term growth effects (Smith 2006). Ascospores ejected from leaf litter may continue to infect susceptible hosts, as observed in this study when seedlings were planted in a nearby plantation. Figure 1 in Appendix 3, illustrates the progression of infection into a neighbouring *E. nitens* plantation. The observed infection of the *E. nitens* is suggested to be by *M. cryptica* (Smith 2006). *E. nitens* has been found to show resistance to *M. nubilosa*, hence the newly planted *E. nitens* became infected by the less dominant *M. cryptica* that was at the site (Smith, 2006).

Mathematical functions were fitted to the data to quantify the effect of meteorological variables on atmospheric ascospore periodicity and density. Previous work has predominantly focussed on qualitative descriptions of the relationships, with no attempt being made to describe them quantitatively using population growth models. The global patterns in the current data were partially consistent with qualitative descriptions made by others, such as Park (1984), Beresford (1978), Cheah (1977) and Cheah and Hartill (1987), and the functions applied were successful in describing them. One of these patterns was the peak in ascospore releases occurring during autumn of the second year. This result partially confirmed that *M. nubilosa* displays a monocyclic infection pattern in *E. globulus* (Park 1984) as estimates of atmospheric ascospores peaked only once throughout the growing season. This information has implications on management strategies for planning timing of intervention to reduce the affects of the disease.

This study was completed from 2002-2004 and included 3 eucalypt growing seasons. During this period generally low levels of atmospheric ascospore densities were
recorded, and data collected on crown infection indicated crown damage was increasing with time but remained low (see Appendix 3 for results). In contrast, during the previous year, a blighting epidemic had occurred in an older plantation that was directly adjacent to the one investigated in this Thesis. To explain why a blighting epidemic occurred in the growing seasons previous to this Thesis study and not repeated in the years following, monthly rainfall distribution patterns for this site were investigated (see Appendix 4). The site received 123 mm (March - April) of rainfall at the end of the growing season of 2000-2001 and 198 mm (October - November) of rainfall at the start of the 2001-2002 growing season. This particular pattern of high rainfall, together with adequate temperature and relative humidity for ascospore release and infection probably ensured late growing season spore release and infection in 2000-2001 sufficient to prime an epidemic in the summer of 2001-2002, especially as the rainfall of the spring of 2001-2002 was also high.

In contrast during the Thesis study trees at the site did not experience a blighting epidemic. This could be due to the site not experiencing a similar monthly pattern of rainfall as observed from 200-2001. In this Thesis the trees at the site experienced a wet spring (2002-2003) however the previous autumn was less wet, which could have resulted in less favourable infection conditions, therefore reduced inoculum build up. The 2002-2003 autumn, however, was wet but it was followed by a less wet spring in the following growing season (2003-2004). Results suggest highly infected over-wintering trees that encounter a wet spring are more susceptible to experiencing a blighting epidemic.
2.4.2 Predictive model development

Model development for an agriculture or horticulture system may be easier to undertake and validate as the pathosystem can be experimentally controlled and manipulated. Given the level of control in this type of field study, the amount of variation from external sources can be minimised resulting in more accurate and precise data for modelling. Predictive models developed in agricultural systems may be effective when based on only 1-2 variables e.g. rainfall and temperature, and consequently they are less complex than the model presented in this Thesis. For example, leaf wetness models that are applied in forecasting *Plasmopara viticola* infection in grape vineyards (Dalla Marta, De Vincenzi et al. 2005; Hoppman and Wittich, 1997) use leaf wetness as an input variable to alert managers when leaf wetness exceeds a certain duration.

An *E. globulus* plantation is structurally complex and variable at the tree, canopy and plantation scale, compared to a horticultural crop. Growth of trees in eucalypt plantations is variable even with clonal plant material due to site, soil and aspect variation, competition from weed species and the presence of other vegetation around the plantation perimeter. All these factors which are also linked with plantation age will influence canopy microclimate to varying degrees and contribute to variability that makes for producing a predictive model very complicated (Scott Foster, Tasmanian Institute of Agricultural Research, pers. comm.). Even if trials were established in a highly controlled environment such as a glasshouse and trees were inoculated, the model would not be applicable to the field. A field model would need to take into account not only the structural site characteristics but also the
developmental stage and age of the plantation, a point previously discussed, considered and included in the current analysis.

In our study the poor ability of the predictive model to accurately predict the incidence and density of an ascospore release is explained by the marked variability in the ascospore data collected and large proportion of zero data, which is statistically challenging to analyse and requires complex models (Scott Foster, Tasmanian Institute of Agricultural Research, pers. comm.). Many of the large ascospore releases did not fit into the mathematical function and as a result data were smoothed. Many of the meteorological variables were correlated and could not be analysed in their continuous numerical form using the chosen model, but as derived binary variables, hence reducing the level of detail for that variable into the model.

The model in this study attempted to deal with the effect of meteorological variables on atmospheric ascospore density data by splitting the task into two separate models. The two models attempted to explain firstly the presence/absence of ascospores and secondly the density. While this led to useful interpretations that clarified the patterns in atmospheric ascospore density, the model fit remains subject to improvement. The results will be of use in further model development. One possible alternative approach might be Bayesian Markov Chain Monte Carlo (MCMC) techniques. The Bayesian approach (Brooks 2003) allows uncertainty about the estimates to be incorporated in a more natural way, which may improve the modeling of the variability of the ascospore densities. Also, the Bayesian approach can be combined with MCMC methods (Metropolis, Rosenbluth et al. 1953; Gelfand and Smith 1990) to greatly simplify computation over the corresponding classical tools, such as those
Bayesian MCMC methods are quite recent and allow very complex and more realistic models to be built (Gelman, Carlin et al. 1995). They may be of use in modeling the serial correlation within the ascospore densities and they permit hierarchical models that may help to construct a single model of the data, rather than two models as completed in this study.

The current form of the model is limited in its use and application in a predictive sense because; i) it was created using only one data set from one site with one spore trap; ii) it has not been validated on an independent data set obtained at sites that have a range of meteorological and disease conditions; iii) the plantation was not a closed system and spore patterns could have been misleading due to infiltration of spores from neighbouring infected plantations; iv) it did not take into account serial correlation, a constraint of the hurdle model (Neter, Kutner et al. 1996). In the future as many spore traps as possible should be used and placed strategically around the plantation. As a result of the above reasons, the methodology should therefore only be considered as a descriptive tool for the data presented in this Thesis until validated on a number of other sites in which case it could be developed into a risk model.

Given that free water has been shown to be an important variable controlling ascospore release, a number of leaf wetness sensor to measure time and duration of wetness (including dew), irrelevant of rain, would provide invaluable data for the model. Furthermore these sensors could detect variable drying times within the crown. A study involving young eucalypt trees has shown that leaf drying times varied depending on location within crown (Worledge & O'Grady et al. 2005). Leaf tagging and monitoring of subsequent lesion development would provide more
comprehensive data on infection progress but was not logistically possible in the context of this study.

**Conclusion**

The daily atmospheric ascospore pattern was most profound when the host was at its maximal juvenile vegetative growth phase, which is when conditions favourable for ascospore release are also common. Rainfall was the meteorological variable that triggered ascospore release among the variables measured in our study. In the absence of rain, it is suggested that moisture from the formation of dew resulted in the diurnal atmospheric ascospore density patterns. Atmospheric ascospores can be observed throughout the year as favourable warm and wet conditions, including wetting and drying cycles can occur for short periods of time during a day e.g. presence of small diurnal atmospheric ascospore periodicities during winter days. Based on exploratory investigations into historical data presented in Appendix 4, severe epidemic development requires a warm and wet autumn and spring from one growing season to another to allow 1) late growing season infection and therefore inoculum for the following season to build up and 2) conditions for further infection of already infected trees in the subsequent growing season.

This study provides the fundamental steps for developing a long-range forecast model to predict future times at high risk to MLD outbreaks in *E. globulus* plantations. A forecast system would allow forest managers to: monitor established sites in their most susceptible juvenile foliage years, adopt precautionary methods of disease management such careful timing of planting, or use the system for plantation site
selection through predicting if a specific site could be at risk to experiencing an epidemic based on the meteorological conditions at the site.

Practical problems in predicting the spread of plant diseases within and between fields require knowledge of the rate of release Q of pathogenic spores into the air. Many plant pathogenic fungus spores are released into the air from plant surfaces inside plant canopies, where they are produced, or from diseased plant debris on the ground below plant canopies, where they have survived from one growing season to the next. There is no direct way to specify Q for naturally released microscopic fungus spores. It is relatively easy to measure average concentrations of spores above a source, however. A two-dimensional Lagrangian stochastic (LS) simulation model for the motion of spores driven by atmospheric turbulence in and above a plant canopy is presented. The model was compared 1) with measured concentration profiles of Lycopodium spores released from line sources at two heights inside a wheat canopy and 2) with concentration profiles of V. inaequalis ascospores measured above ground-level area sources in a grass canopy. In both cases, there was generally good agreement between the shapes of the modeled and measured concentration profiles. Modeled and measured concentrations were compared to yield estimates of spore release rates. These, in turn, were compared to release rates estimated independently from direct measurements. The two estimates of spore release rate were in good agreement both for 1) the 30-min artificial releases of Lycopodium spores [significance level P = 0.02 (upper source) and P = 0.02 (lower source)] and for 2) the daily total release of V. inaequalis ascospores (P < 0.002). These results indicate that the LS model can yield accurate values of Q (or, conversely, of concentration). Thus, LS models allow a means of attacking a nearly intractable problem and can play an important role in predicting disease spread and in helping to reduce pesticide use in disease-management decisions.


Banana leaves showing different levels of black Sigatoka disease were collected from an unsprayed plantation in Costa Rica during two separate periods representing the wet to dry season transition (October 1993 - February 1994) and the dry to wet season transition (April - September 1995). Laboratory studies were used to investigate the relationship between the release of Mycosphaerella fijiensis ascospores and the amount of inoculum on
banana plants showing different levels of infection, as assessed by leaf necrotic area. The number of perithecia present in the necrotic area was used as an indication of potential ascospore loads and was investigated as a series of regression equations. A series of rewetting and incubation regimes was used to investigate spore release under field conditions (21 degrees C and 100% relative humidity in the early morning and 28 degrees C, 60% relative humidity on days when it rained in mid-afternoon). Results suggest that rainfall, combined with a high temperature, may lead to peaks of ascospore release but without necessarily increasing overall numbers released over periods of up to 4 days and that a high level of spore release was less sensitive to changes in temperature once it had been initiated. The exact role of temperature in spore release is still unclear, however, as leaf samples kept at atypically low temperatures also released non-germinating ascospores. An average of 4.5 ascospores was released per perithecium. This does not resolve ambiguities in the literature regarding the number of ascospores present in each perithecium. A linear model relating the average ascospore numbers and necrotic area, using quick estimates of the amounts of necrotic area on the leaves of a random sample of plants across a plantation, is proposed, to give an indication of the relative amount of airborne inoculum potentially available between different plantations.

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In groups of infected Eucalyptus regnans and E. delegatensis trees in the Univ. grounds, ascospores of *M. cryptica* were trapped at canopy height only during rainy periods; discharge commenced immediately after rain started and continued for up to 2 h after it ceased. The discharge rate was not directly related to rainfall intensity. Ascospores were trapped throughout the year, most from mid-summer to early winter (Jan.-June). The numbers were generally related to disease incidence within the canopy. There was no diurnal fluctuation in ascospore discharge. Experiments in controlled climate chambers corroborated these observations; leaf wetting was found to be the most important factor governing ascospore release and temp. played a secondary role, no spores being released at less than 10 or more than 30 degrees C. Light had no effect.


The progress of light leaf spot (Pyrenopeziza brassicae) epidemics on winter oilseed rape was monitored in 1998/99 and 1999/2000 at Rothamsted, and weather factors and P. brassicae ascospore concentrations were recorded daily. The data sets, which consisted of numbers of `apparently healthy' leaves, leaves with P. brassicae sporulation and dead leaves, were analysed using a structured population model with four compartments to investigate the effects of presence of P. brassicae inoculum (ascospores and conidiospores) and weather factors on the progress of light leaf spot on winter oilseed rape leaves. The model consists of ordinary differential or delay-differential equations to describe the rates of change per unit time in numbers of healthy susceptible leaves, infected leaves with no sporulation, leaves with sporulation, dead leaves (the four compartments) and the length of the latent period (which is temperature-dependent). The model allows for production of new susceptible leaves and leaf birth rate is assumed to be linearly dependent on temperature. The model incorporates an infection criterion depending on temperature and leaf wetness duration (expressed as rain duration). Rates of transition between the four compartments are related to rates of infection of 'susceptible leaves', sporulation of 'infected leaves' to produce 'sporulating leaves' and death of leaves from these three compartments. Parameter values were estimated by fitting the model to the data sets. The model fitted the disease progress data equally well in both seasons. The model fitting suggested that disease progress could be described only if both ascospore and conidiospore numbers were included. When either of the parameters representing the rates of infection by ascospores or conidiospores was eliminated, the model did not fit the data well. The sum of the model outputs for the first two leaf compartments was compared to the recorded numbers of 'apparently healthy' leaves; the fit to the data was better in 1999/2000 than in 1998/99. An assumption that the leaf birth rate changed around the time stem extension began (GS 2,0) improved the fit of the model. Seasonal variations in temperature had a large effect on the length of the latent period, which increased when mean daily temperatures were less than 5°C.


Two distinct types of leaf spot on juvenile leaves of Eucalyptus globulus were found to be caused by two species of Mycosphaerella. M. cryptica caused a small circular spot while M. nubilosa caused a larger, spreading lesion. M. parva Park & Keane was only found on older lesions caused by M. nubilosa, and appears to be saprophytic. M. cryptica was also shown to be the cause of large, blighting lesions on mature foliage of a wide range of species from both major subgenera of Eucalyptus. Observations on symptom development, sporulation, pathogenicity and leaf penetration for M. cryptica and M. nubilosa are presented.


The production of ascospores of M. nubilosa and M. cryptica was monitored at 2 field sites at monthly intervals. Ascocarp initiation and early development occurred throughout the year. Ascospore production was greatest from summer to mid-autumn; lower temp. then appeared to limit ascospore production from late autumn to early spring. Adequate leaf wetness is also necessary for ascocarp development and spore production. Under lab. conditions, ascospores of both fungi matured at 15degreesC with 12 h but not 1 h leaf wetness daily. Ascocarps of M. nubilosa continued to discharge spores for up to 17 months in the feld, while ascocarps of M. cryptica remained active for at least 8 months. Ascospore production by M. parva was often recorded on older lesions caused by M. nubilosa or M. cryptica, supporting the hypoThesis that this species is saprobic.


A 6-year study was carried out in an apple-growing region of North Italy by trapping airborne ascospores of Venturia inaequalis with a volumetric spore
trap operated continuously during the ascospore season, with the aim of better
defining the weather conditions that allow ascospores both to discharge and to
disperse into the orchard air. A total of more than 60 ascospore trapping events
occurred. Rain events were the only occurrences allowing ascospores to
become airborne (a rain event is a period with measurable rainfall greater than
or equal to 0.2 mm/h - lasting one to several hours, uninterrupted or interrupted
by a maximum of two dry hours); on the contrary, dew was always insufficient
to allow ascospores to disperse into the air at a measurable rate, in the absence
of rain. In some cases, rain events did not cause ascospore dispersal; this
occurred when: (i) rain fell within 4-5 h after the beginning of a previous
ascospore trapping; (ii) rain fell at night but the leaf litter dried rapidly; (iii)
nightly rainfalls were followed by heavy dew deposition that persisted some
hours after sunrise. Daytime rain events caused the instantaneous discharge
and dispersal of mature ascospores so that they became airborne immediately;
for night-time rainfall there was a delay, so that ascospores became airborne
during the first 2 h after sunrise. This delay did not always occur, and
consequently the ascospore trapping began in the dark, when: (i) the
cumulative proportion of ascospores already trapped was greater than 80% of
the total season's ascospores; iii) more than one-third of the total season's
ascospores was mature inside pseudothecia and ready to be discharged.

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The Smith Period, Negative Prognosis, Blitecast, Sparks and NegFry
forecasting schemes for potato late blight were evaluated over a 6-year period
at five locations representing a range of blight risk situations. Frequent
measurements were made by in-field meteorological stations and untreated,
blight susceptible, potatoes in small plots were regularly assessed for
symptoms of the disease. Although the Smith Period was the most reliable
scheme (warnings rarely in error) it often gave too long an advanced warning
of an eventual disease outbreak; NegFry was the most accurate scheme
assuming an ideal warning of 10 days was required by growers.


young E. globulus stand: observed, measured and calculated. Hobart, Cooperative
Research Centre for Sustainable Production Forestry.: 1-27.
Chapter 3.

Effects of Mycosphaerella leaf disease on the spectral reflectance properties of juvenile *Eucalyptus globulus* foliage.

Work from this chapter has been published* as:


3.1 Introduction

Species of the foliar pathogen *Mycosphaerella* can cause severe leaf necrosis and defoliation in young eucalyptus plantations in Australia, New Zealand, South Africa, Portugal, Spain and Chile (Park and Keane, 1987; Crous, 1996; Maxwell et al., 2000; Park et al., 2000; Mohammed et al., 2003; Potts et al., 2004; Tejedor, 2004) resulting in serious loss of productivity (Lundquist and Purnell, 1987; Carnegie et al., 1997; Carnegie and Ades, 2002; Maxwell et al., 2003; Mohammed et al., 2004). The two major species of *Mycosphaerella* infecting young plantations of *Eucalyptus globulus* (Labill.) in southern Australia are *Mycosphaerella nubilosa* (Cooke) Hansf. and *Mycosphaerella cryptica* (Cooke) Hansf. (Carnegie et al., 1994; Carnegie et al., 1997; Milgate et al., 2001; Mohammed et al., 2003).

Leaf reflectance and absorption of light are driven by two processes: the scattering of light as a result of a leaf’s surface and internal cellular structure, and radiant energy absorption dictated by leaf biochemistry (Datt, 1998). Many studies have successfully used spectral reflectance measurements as a non-destructive estimation of
photosynthetic pigment content (e.g. Gitelson and Merzlyak, 1994; Blackburn, 1998; Datt, 1998; Blackburn, 1999; Datt, 1999c, 1999a; Carter and Spiering, 2002; Sims and Gamon, 2002). This is achieved by measuring percentage reflectance at specific wavelengths that are sensitive to the composition and content of chlorophyll.

Several studies have investigated spectral changes specifically in response to fungal disease (e.g. Malthus and Madeira, 1993; Carter et al., 1996; Nilsson and Johnsson, 1996; Kobayashi et al., 2001; Kobayashi et al., 2003; Stone et al., 2003; Steddom et al., 2005). The specific nature of any fungal host–pathogen environment interaction will influence spectral changes, e.g. a fungal mat present on the surface of plant tissues such as in powdery mildews (Lorenzen and Jensen, 1989) will result in a different spectral response compared to a fungus that disrupts internal leaf tissue such as the causal agent of Dothistroma needle blight (Stone et al., 2003). In both these examples, there is a decrease in chlorophyll content with increasing disease severity. If chlorosis is not the dominant foliar symptom, the overall spectral response may be an amalgamation of radiation interactions arising from a complex of symptoms.

Mycosphaerella infection in E. globulus becomes visible to the naked eye or under a microscope as a small purple lesion approximately 7–21 days after infection. Approximately 21–35 days after infection, the purple area becomes necrotic although a zone of purple discoloration may delimit the necrosis (Park, 1988a, 1988b). Individual necrotic lesions present on a single leaf may increase significantly in size and coalesce. It is probable that the spectral response indicative of healthy E. globulus foliage and distinguishing it from symptomatic foliage of Mycosphaerella leaf disease (MLD) will be driven by the proportion of necrotic tissue to green tissue.
Reflectance in visible wavelengths (400–700 nm) in healthy leaf tissue is influenced by the light absorption of leaf pigments, including the chlorophylls, carotenoids and anthocyanins. In the past reflectance at wavelengths near 680 and 550 nm have been used as indicators of chlorophyll content of leaves; however, wavelengths near 680 nm become saturated at medium to high chlorophyll contents and the red foliar pigment anthocyanin also absorbs around 550 nm (e.g. Gitelson and Merzlyak, 1996; Datt, 1998; Sims and Gamon, 2002). Subsequently studies have shown that wavelengths between 690 and 705 nm are particularly sensitive to stress-mediated reduction in chlorophyll content (e.g. Carter, 1993; Carter and Miller, 1994; Gitelson and Merzlyak, 1994) (Datt, 1998, 1999a; Carter and Knapp, 2001; Sims and Gamon, 2002). The red edge (approx. \( R_{690} - R_{740} \) nm) (Curran et al., 1990) identifies where chlorophyll ceases absorbing (Datt, 1998), resulting in a steep increase in reflectance. The high sensitivity of reflectance in the spectral range of 690–705 nm to chlorophyll content results in a close relationship between red-edge behaviour and chlorophyll content (Gitelson and Merzlyak, 1996). The red-edge inflection point is the wavelength of maximum slope on the red-edge curve and is usually defined on the maximum of the first derivative of a reflectance spectrum (Curran et al., 1990). A decrease in chlorophyll content as a result of stress-induced chlorosis is accompanied by the well-documented shift of the red edge towards shorter wavelengths (Rock et al., 1988; Hoque and Hutzler, 1992; Gitelson & Merzylak, 1996). The top of the red edge is where the near-infra red (NIR) shoulder occurs and the NIR plateau continues from approximately \( R_{700} \) to \( R_{1300} \) nm (Lillesand and Kiefer, 2000). The reflectance spectra in the NIR region are influenced by internal cellular structure and leaf surface properties, and to a lesser degree by moisture content.
It is hypothesized that many of the changes to foliar properties induced by *Mycosphaerella* infection will influence the spectral response in the visible and NIR regions. The aims of this study were therefore, to: (i) investigate the spectral response of *E. globulus* foliage infected with MLD; (ii) identify wavelengths sensitive to various levels of severity at the leaf scale; and (iii) use these wavelengths in spectral indices and model development to predict MLD severity. Key wavelengths identified from this study will form the basis of developing spectral indices for identifying and monitoring this disease using remotely sensed imagery.

3.2 Methods

3.2.1 Study Site

This study was conducted in an 82-ha plantation site near the north-west coast of Tasmania (41°07′22″S, 144°45′28″E) with an annual rainfall of 1300 mm and a grey-brown acidic clay dermosol (Figure 3.1).

![Figure 3.1 Field site at Temma on the northwest coast of Tasmania (41°07′22″S, 144°45′28″E).](image)
Before clearing the area was under native wet sclerophyll forest. *Eucalyptus globulus* seedlings of mixed family seedlots were planted during July 2000 at 1100 stems/ha following standard local industry establishment procedures (T. Wardlaw, Forestry Tasmania, personal communication).

3.2.2 Leaf sampling

*Eucalyptus globulus* juvenile foliage was sampled in November 2002 from 18 trees infected with *Mycosphaerella*. At least three medium-sized branches (1–1.5 m long) were cut at each of three locations (lower, middle, top) in the crown of each tree. The position of a branch in the crown was noted before cutting and the branch tagged with this information. Branches were placed in buckets of water, transported to a field base 20-min drive from the site and placed in the shade for no more than 1 h before processing.

Fully expanded juvenile leaves were removed from each of the sampled branches and sorted into disease severity classes that were based on the percentage of leaf area with necrosis caused by MLD (Table 3.1 & Figure 3.2). *Mycosphaerella nubilosa* and *Mycosphaerella cryptica* were identified as being the predominant causal agents of MLD. It was neither necessary nor feasible to attribute necrotic lesions to individual species. The symptoms of each species are macroscopically very similar and their lesions can only be clearly differentiated by examination of reproductive structures (if present on a lesion) with the aid of a hand lens. Infected leaves with no obvious visual symptoms may have therefore been classed as healthy leaves.
Before amalgamation of leaves from all the branches into the disease severity classes we compared key spectral wavelengths within leaf severity classes originating from the different locations in the crowns and from different trees.

Table 3.1 Disease severity classes 1-5 (visual estimate of % leaf area covered with necrotic lesions), corresponding severity score and number of samples collected within each severity class. 0 = healthy leaves.

<table>
<thead>
<tr>
<th>% Leaf area with necrosis</th>
<th>0</th>
<th>1-5</th>
<th>6-12</th>
<th>13-25</th>
<th>26-50</th>
<th>51+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease severity class</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Number of samples</td>
<td>7</td>
<td>19</td>
<td>20</td>
<td>17</td>
<td>17</td>
<td>4</td>
</tr>
</tbody>
</table>
Figure 3.2 Example of juvenile leaf sample in each *Mycosphaerella* leaf disease damage severity class.

Photographs courtesy of Anna Smith.
3.2.3 Spectral measurements

Leaf reflectance measurements were conducted in a dark room using a UniSpec FR spectroradiometer (PP Systems Analytical Spectral Devices, Inc., Boulder, CO, USA). The UniSpec measures radiation in the visible and near-infrared regions (250–1100 nm) at a spectral bandwidth of 3.3 nm. Measurements were taken from a target sample located on a reference panel (Spectralon) that was made of barium (BaSO4). Two light sources, each with a 150-W halogen visible light bulb were used to illuminate the target and reference panels. The reference panel and a black surface were measured before analysis for calibration purposes and between samples to ensure all reflectance values remained standardized.

Leaf samples were arranged in a stack that covered an area of 10 cm × 10 cm, with adaxial surfaces facing the UniSpec lens. Adaxial leaf surface measurements taken at this orientation best simulate their position in a crown as viewed from above. The target sample was placed on a flat surface under the light source and approximately 70 cm from the UniSpec lens. Leaf stacks rather than single leaf layers were used so as to simulate the reflectance obtained from leaves in a canopy; in this study three leaf layers were used consistently for all samples. For each sample, three reflectance measurements were taken before it was reshuffled. This process was repeated five times (15 measurements per sample) and later averaged to obtain a mean spectrum for that sample. All spectra were converted to reflectance (Reflectance ‘R’ = light emitted from sample/light emitted from reference panel) (e.g. Stone et al., 2003).
3.2.4 Chlorophyll measurements

A SPAD chlorophyll meter (Model 502; Minolta Co., Ltd, Osaka, Japan) provides a relative estimate of chlorophyll content (Peryea and Kammereck, 1997; Richardson et al., 2002). In healthy *E. globulus* leaves this relative estimate is good with a coefficient of determination greater than 0.88 (Pinkard et al., 2006a).

Immediately after taking the reflectance measurements SPAD readings were recorded systematically from the same three areas of each leaf assessed irrespective of the condition of the leaf tissue (at the tip and either side of the mid-vein in the lower half of the leaf). For each measurement, an index (from 1 to 100) in SPAD units (based on absorbance at 650 and 940 nm) was obtained for a small portion of leaf tissue (approx. 0.06 cm²) held within the SPAD chamber at each of the three sample areas per leaf. SPAD readings were collected from 10 leaves per severity class sample and then averaged across all samples for a disease severity class.

3.2.5 Data analysis

All statistical analyses were completed by procedures in SAS Version 8 (SAS Institute Inc, 1989). A total of 84 samples (n = 84) were collected and used in the data analysis (Table 3.1).

An analysis of variance (ANOVA) was completed on different samples within severity classes to determine whether tree origin or location within the crown had an effect on leaf reflectance at key wavelengths. Normal probability plots were examined to ensure assumptions of normality and homoscedasticity held, and residuals were analysed. Data transformation was not necessary as distributions
proved normal, unimodal and symmetric. There were no significant among-trees or within-crown effect on the leaf reflectance values of severity classes (data not shown) and hence in the final analysis data were averaged for each severity class across all samples in that class (Table 3.1).

The data were used to investigate which wavelengths: (i) caused the largest differences between severity classes; and (ii) were most sensitive and responded to increasing levels of *Mycosphaerella* infection (Cibula and Carter, 1992; Carter, 1994; Kobayashi et al., 2001). Differences were calculated for each class by subtracting the mean reflectance of uninfected leaves at each wavelength from the mean reflectance for each severity class of infected leaves. Reflectance sensitivity was calculated as the reflectance difference between severity classes divided by mean reflectance of uninfected leaves.

Sensitivity curves showed which wavelengths were most sensitive (curve maxima) and least sensitive (curve minima) to increasing levels of symptomatic tissue for each severity class. These maxima and minima wavelengths from the sensitivity curves were used as numerator and denominator reflectance values for developing indices to be used in the general linear regression analysis and model development (Carter, 1994; Datt, 1999a; Coops and Stone, 2005). Ratios of leaf reflectance measured at sensitive and insensitive wavebands may correct for unwanted variation in irradiance, leaf orientation, irradiance angles and shadows features that can reduce the precision of radiometric measurements (Carter, 1994; Nilsson, 1995).
To investigate the influence of leaf disease severity on the red-edge feature, first
derivative spectra were examined by calculating the slope between two neighbouring
channels along the entire wavelength for each mean curve.

An ANOVA was undertaken to determine the wavelengths where severity classes
were significantly different. The indices derived from the sensitivity analysis, and
relevant published spectral indices, were also included in the ANOVA to determine
which indices significantly differentiate between severity classes. The published
indices selected (Table 3.2) are those reported as being sensitive to chlorophyll
content (Carter, 1994; Lichtenthaler et al., 1996; Datt, 1999a). Multiple comparison
t-tests (p = 0.05) were completed to identify which severity classes were significantly
separated and by which spectral variables.

Correlations were examined by computing Pearson’s correlation coefficients between
disease severity classes, relative chlorophyll concentration content as measured by the
SPAD meter, key wavelengths, published spectral indices and those derived from our
study.
Table 3.2 Comparison of reflectance mean values calculated using both reported and derived leaf reflectance indices between healthy leaves and the five *Mycosphaerella* disease severity classes.

<table>
<thead>
<tr>
<th>Leaf reflectance indices</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R678/R550) (Datt, 1998)</td>
<td>0.660a ± 0.040</td>
<td>0.735a ± 0.017</td>
<td>0.837b ± 0.019</td>
<td>0.924b ± 0.026</td>
<td>1.093c ± 0.029</td>
<td>1.197d ± 0.061</td>
</tr>
<tr>
<td>(R672/R550) (Datt, 1998)</td>
<td>0.657a ± 0.042</td>
<td>0.727a ± 0.018</td>
<td>0.827b ± 0.019</td>
<td>0.913b ± 0.026</td>
<td>1.075c ± 0.028</td>
<td>1.177d ± 0.058</td>
</tr>
<tr>
<td>(R800- R680)/R800 (Lichtenthaler et al., 1996) (R800)</td>
<td>0.768a ± 0.026</td>
<td>0.733ab ± 0.012</td>
<td>0.690bc ± 0.013</td>
<td>0.633c ± 0.018</td>
<td>0.548d ± 0.018</td>
<td>0.471e ± 0.035</td>
</tr>
<tr>
<td>(R795/R656) (Leckie et al., 2004)</td>
<td>0.216a ± 0.037</td>
<td>0.207a ± 0.009</td>
<td>0.244ab ± 0.011</td>
<td>0.291b ± 0.015</td>
<td>0.376c ± 0.017</td>
<td>0.453d ± 0.036</td>
</tr>
<tr>
<td>(R694/R760) (Carter, 1994)</td>
<td>0.259a ± 0.043</td>
<td>0.240a ± 0.009</td>
<td>0.278ab ± 0.011</td>
<td>0.326b ± 0.016</td>
<td>0.413c ± 0.017</td>
<td>0.485d ± 0.035</td>
</tr>
<tr>
<td>(R570-R531)/(R570+R531) PRI; (Gamon et al., 1990) (R570)</td>
<td>0.025ab ± 0.008</td>
<td>-0.017a ± 0.002</td>
<td>-0.026ab ± 0.002</td>
<td>-0.030b ± 0.002</td>
<td>-0.040c ± 0.002</td>
<td>-0.047c ± 0.003</td>
</tr>
<tr>
<td>(R850/R710) (Datt, 1999a)</td>
<td>2.350ab ± 0.207</td>
<td>2.413a ± 0.058</td>
<td>2.267ab ± 0.045</td>
<td>2.120bc ± 0.057</td>
<td>1.912cd ± 0.050</td>
<td>1.825d ± 0.086</td>
</tr>
<tr>
<td>(R750-R710)/(N750-710) = Upper slope of red edge; (Merton, 1999) (R750)</td>
<td>0.005a ± 0.001</td>
<td>0.005a ± 0.000</td>
<td>0.005a ± 0.000</td>
<td>0.004b ± 0.000</td>
<td>0.003bc ± 0.000</td>
<td>0.002c ± 0.000</td>
</tr>
<tr>
<td>(R750-R690)/(N750-690) = Slope of red edge; (Merton, 1999) (R750)</td>
<td>0.005a ± 0.001</td>
<td>0.005a ± 0.000</td>
<td>0.005a ± 0.000</td>
<td>0.004b ± 0.000</td>
<td>0.003bc ± 0.000</td>
<td>0.003c ± 0.000</td>
</tr>
</tbody>
</table>

Note: Reflectance means are given in each column (mean ± standard error). Different letters indicate significant difference \((p < 0.05, \text{Duncan's Multiple Range Test}; n = 84)\). PRI, Photosynthetic Reflectance Index; N, number of spectral channels between R710 and R750.
3.2.5.1 Model development and validation

Stepwise forward linear regressions were applied to identify the best model to explain the relationship between disease severity classes and reflectance spectra. This was done firstly by automatic computation and second by forcing specific wavelengths and indices into the analysis based on biological significance. Data were tested for normality and residuals were analysed. Data transformation was not necessary. The model with the least number of significant variables giving the best coefficient of determination and root mean square error (RMSE) was used to predict severity class. Predicted values were plotted against the observed data.

Infected leaves from an *E. globulus* plantation in southern Tasmania were collected to provide a validation data set (n = 54). Leaf sample collection, spectral measurements and data collation were as described above. The goodness-of-fit of the model to the data was determined by calculating the correlation coefficient between observed and predicted disease severity classes. The slope of the regression line in Figure 3.8 does not deviate significantly from 1. The residual errors of the predicted data were calculated. The percentages of severity classes underestimated, overestimated, correctly classified and misclassified were also calculated.

3.3 Results

3.3.1 Influence of leaf disease severity on leaf reflectance spectra

Figure 3.3 illustrates mean reflectance curves for each severity class and key features along the wavelength. Although there is a trend for sequentially increasing reflectance with increasing severity levels, differences between curves for each severity class are generally small in the blue and green visible wavelength region.
(R400–R600 nm). In the red region (R 600– R 700 nm) the curves begin to separate and then increase almost 10-fold in the red edge before reaching a plateau in the NIR region (R 700– R1300 nm) but the curves are not significantly different. In the red edge, not only is there a significant increase in reflectance but also curves cross-over (R708 nm) resulting in reversed pattern to the curve positions observed in the blue and green region.

This visual examination of the six mean spectra for each severity class was confirmed when individual wavelengths were examined. Significant correlations (p < 0.0001) with severity class occurred in the red wavelength region (e.g. between 631 and 690 nm; r ≥ P 0.65). The most highly correlated wavelength with disease severity class was R 678 nm (r = 0.760, p < 0.0001). Wavelengths longer than R 690 nm, located in the red-edge region, were not significantly correlated with disease severity class (p > 0.05). In the NIR region low, negative correlations existed (r = -0.3, p < 0.05). There were no significant correlations between relative chlorophyll content and individual wavelengths (r = -0.192, p = -0.08).

Figure 3.4 shows the mean reflectance difference curves. The maximum differences in reflectance values between all severity classes are in the NIR at R760 nm. Notable differences between reflectance values for different severity classes also occur at R678 nm and to a lesser extent around R480 nm. Sensitivity curve analyses (Figure 3.5) indicate that the wavelength most sensitive to changes in disease severity is R678 nm. Wavelengths near R500 nm also display increased sensitivity to disease severity whereas wavelengths near R708 and R550 nm appear relatively insensitive.
Figure 3.3 Mean reflectance spectra for healthy juvenile *Eucalyptus globulus* foliage (‘0’) and each of the five severity classes of *Mycosphaerella* leaf disease. Mean reflectance and ±SE bars are shown at $R_{490}$, $R_{550}$, $R_{678}$, $R_{708}$ and $R_{780}$ nm. Significant difference between mean values is denoted by ***$p \leq 0.0001$; **$p \leq 0.01$; NS, not significant; n = 84.
Figure 3.4 Mean reflectance difference curves for healthy juvenile Eucalyptus globulus foliage ('0') and each of the five severity classes of Mycosphaerella leaf disease (progressing in severity from ‘1’ to ‘5’ with '5' having more than 50% leaf area with necrosis). Difference curves were calculated by subtracting mean reflectance of healthy foliage from that of infected foliage at each wavelength.
Figure 3.5 Mean reflectance sensitivity curves for healthy juvenile *Eucalyptus globulus* foliage ('0') and each of the five severity classes of *Mycosphaerella* leaf disease (progressing in severity from ‘1’ to ‘5’ with ‘5’ having more than 50% leaf area with necrosis). Sensitivity is calculated as mean reflectance difference of infected foliage classes divided by mean reflectance of uninfected foliage classes at each wavelength. Arrows indicate sensitive (peaks) and insensitive (dips and crossing of x-axis) wavelengths.
Wavelengths of maxima and minima sensitivity were used to calculate a series of reflectance indices. The best performing index was $R_{678}/R_{550}$ (Tables 3.2 and 3.3).

This ratio is similar to Datt’s index ($R_{672}/R_{550}$) (Datt, 1998) and both indices differentiated between four infection classes: 0–1, 2–3, 4 and 5 (Table 3.2). The other indices selected from the literature did not discriminate between most severity classes, although there were always significant differences between the most extreme severity class (5) and healthy leaves (0) for each index (Table 3.2). All the indices examined were significantly ($p < 0.0001$) correlated with disease severity class except for the DATT index ($R_{850}-R_{710}$ nm)/($R_{850}-R_{680}$ nm) (Datt, 1999a; Table 3.3). $R_{678}/R_{550}$ gave the highest correlation with severity class ($r = 0.841$). A plot of mean $R_{678}/R_{550}$ values against MLD severity classes is illustrated in Figure 3.6. Several other indices derived from the sensitivity maxima and minima (Figure 3.5) performed well with correlation coefficients above $r = 0.7$, for example $R_{750}/R_{687}$ and $R_{708}/R_{681}$. These indices, however, were not significantly correlated with relative chlorophyll content except for the weak correlation of two indices developed by Datt (1999b; Datt, 1999a) (Table 3.3).

The first-order derivative curves show a sequential decrease of the derivative curve maxima in the red-edge region with increasing Mycosphaerella infection (Figure 3.7) arising from the sequential ‘flattening’ of the red-edge feature. However, for healthy leaves and most infected severity classes the red-edge inflection points were at 711 nm, and at 714 nm for the remaining classes. There was not a consistent shift to shorter wavelengths of the red-edge peak in the first-order derivative reflectance curves (Figure 3.7).
Table 3.3 Pearson’s correlation coefficients ($r$) with reported and derived reflectance indices and a) disease severity class for Mycosphaerella leaf disease and b) relative chlorophyll concentration (SPAD values).

<table>
<thead>
<tr>
<th>Leaf reflectance indices</th>
<th>a) Disease</th>
<th>b) Chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(R678/R550)$</td>
<td>0.841 ***</td>
<td>-0.148 NS</td>
</tr>
<tr>
<td>$(R672/R550)$ (Datt, 1998)</td>
<td>0.836 ***</td>
<td>-0.147 NS</td>
</tr>
<tr>
<td>$(R800-R680)/(R800+R680)$ (Lichtenthaler et al., 1996)</td>
<td>-0.784 ***</td>
<td>0.133 NS</td>
</tr>
<tr>
<td>$(R795/R656)$ (Leckie et al., 2004)</td>
<td>-0.755 ***</td>
<td>-0.087 NS</td>
</tr>
<tr>
<td>$(R694/R760)$ (Carter, 1994)</td>
<td>0.733 ***</td>
<td>-0.200 NS</td>
</tr>
<tr>
<td>$(R699/R756)$ (Stone et al., 2005)</td>
<td>0.713 ***</td>
<td>-0.224 NS</td>
</tr>
<tr>
<td>$(R570-R531)/(R570+R531) = PRI; (Gamon et al., 1990)</td>
<td>-0.602 ***</td>
<td>0.177 NS</td>
</tr>
<tr>
<td>$(R850/R710)$ (Datt, 1999a)</td>
<td>-0.582 ***</td>
<td>0.254 **</td>
</tr>
<tr>
<td>$(R750-R710)/(N750-710) = Upper slope of red edge; (Merton, 1999)</td>
<td>-0.503 ***</td>
<td>0.093 NS</td>
</tr>
<tr>
<td>$(R750-R690)/(N750-690) = Slope of red edge; (Merton, 1999)</td>
<td>-0.501 ***</td>
<td>0.043 NS</td>
</tr>
<tr>
<td>$(R850-R710$ nm)/(R850-R680 nm) (Datt, 1999a)</td>
<td>-0.022 NS</td>
<td>0.343 **</td>
</tr>
</tbody>
</table>

Note: ***$p \leq 0.0001$; ** $p \leq 0.01$; NS, not significant; $n = 84$. N, number of spectral channels between $R710$ and $R750$. PRI, Photosynthetic Reflectance Index.
Figure 3.6. Mean values of the reflectance index \((R_{678}/R_{550})\) plotted against class '0' (healthy foliage) and each of the five severity classes of Mycosphaerella leaf disease (progressing in severity from '1' to '5' with '5' having more than 50% leaf area with necrosis). Vertical bars represent standard errors; \(n = 84\).
Figure 3.7 Mean first-order derivative reflectance spectra for healthy juvenile *Eucalyptus globulus* foliage ('0') and each of the five severity classes of Mycosphaerella leaf disease (progressing in severity from ‘1’ to ‘5’ with '5' having more than 50% leaf area with necrosis)
3.3.2 Regression model and validation

The model that best explained the relationship between disease severity classes and spectral response was:

\[
\text{Severity} = -5.5 + (426.4 \times R570) - (415.4 \times R575) - (0.2 \times R795 \div R656) + (8.1 \times R678 \div R550)
\]

\( (R^2 = 0.83, \text{RMSE} = 0.51, p < 0.0001) \)

When the model was tested using the independent validation data set to predict disease severity class (Figure 3.8), the predicted data was highly correlated with the observed \( (r = 0.95, P < 0.0001) \). The model predicted plus or minus one severity class in 80% of cases, and 59% were within 0.5 of the observed severity classes. Overall, 13% of the predictions were exact, 43% overestimated and 44% underestimated severity classes.
Figure 3.8 The relationship between observed and predicted disease severity classes (mean ± SE) using the model and independent validation data. Disease Severity = -5.5 + (426.4 × R570) - (415.4 × R575) - (0.2 × R795/R656) + (8.1 × R678/R550), \( R^2 = 0.83 \), \( p < 0.001 \), \( p = < 0.0001 \), RMSE=0.512. The solid line represents a perfect fit; \( n = 54 \).
3.4 Discussion

Numerous studies have demonstrated that the composition and content of leaf pigments change when exposed to a range of environmental stresses such as air pollution, soil moisture deficit, certain nutrient deficiencies and those phytophagous agents that induce chlorotic-like symptoms (e.g. Carter and Knapp, 2001; Li et al., 2004; Wen et al., 2004; Ayala-Silva and Beyl, 2005; Coops and Stone, 2005). The non-destructive estimation of leaf pigments based on reflectance measurements has been well established and many studies have reported a large number of reflectance-based algorithms or vegetative indices developed for the estimation of leaf pigment content (e.g. Table 2 in Le Maire et al., 2004). This diversity relates, in part, to the fact that at wavelengths where absorption coefficients of pigments are high, reflectance is more sensitive to low concentrations, while spectral regions with low absorption are more sensitive to higher pigment concentrations (Gitelson and Merzlyak, 1996; Blackburn and Steele, 1999). There is general agreement, however, that wavelengths near \( R_{700} \) nm are the most sensitive to small changes in chlorophyll content (Gitelson et al., 1996; Carter and Spiering, 2002). Sims and Gamon (2002), for example, found that \( R_{705} \) was sensitive to chlorophyll content irrespective of leaf structure and developmental stage. Chlorosis is a key symptom presented by eastern hemlock foliage (\( Tsuga canadensis \) Carriere) infested with the hemlock woolly adelgid and Pontius et al. (2005) successfully developed a reflectance based algorithm that included wavelengths near \( R_{700} \) nm.

Our results, however, do not suggest such a well-defined relationship between spectral reflectance and chlorophyll content. Different types of host/pathogen interactions result in a wide spectrum of symptoms at the canopy scale in addition to the loss of
chlorophyll content (Agrios, 2005) which may influence such a relationship. Mycosphaerella Leaf Disease in eucalypts is characterized in most infection events by localized, non-uniform patches of necrotic tissue interspersed with green or discoloured tissue. Wavelengths near 678 and 500 nm were identified as being sensitive to an increasing per cent of leaf tissue infected by *Mycosphaerella* infection while relatively insensitive wavelengths occurred near 708 and 550 nm. Chlorophyll *a* absorbs strongly around $R_{680}$ nm while the absorptivity of all the chlorophyll pigments is low near $R_{550}$ nm, and at $R_{500}$ nm reflectance is controlled by combined absorption of both chlorophyll *b* and carotenoids (Merzlyak et al., 1999). Reflectance near 675 nm was nominated by Merzlyak et al. (1999) as being sensitive to chlorophyll breakdown but is also influenced by the retention or accumulation of the carotenoid pigments during senescence. Kobayashi et al. (2003) also identified the wavelength 675 nm as being sensitive to the severity of rice blast disease (*Magnaporthe grisea*). In our study, neither the wavelengths near $R_{700}$ nm or near $R_{550}$ nm were identified having high sensitivity to MLD severity classes. Both regions have low absorptivity of the chlorophyll pigments and have previously been reported as influenced by relative small changes in chlorophyll *a* content and early symptoms of chlorosis (e.g. Datt, 1998; Gitelson et al., 2003). The absence of sensitivity near the wavelength 700 nm helps explain the observed lack of movement of the red edge to shorter wavelengths (Fig. 5). Malthus and Madeira (1993) also reported the red-edge position to be poorly correlated with infection of field bean leaves by the fungus *Botrytis fabae* and demonstrated that this was because infection was not significantly correlated with chlorophyll *a* content.
The poor correlation between relative chlorophyll content and the selected reflectance indices (Table 3.3) may have also been influenced by the methodology used in this study. The sampling methodology, as measured by the SPAD meter, relied upon point measurements at predefined locations on the leaf whereas the reflectance measurements were of multiple leaves arranged in a small stack. Measurement of total leaf chlorophyll content and content of chlorophyll a and b may have been more appropriate. The greatest difference between severity classes, not surprisingly, was found in the NIR region, near $R_{760}$ nm. This is similar to the results reported by Kobayashi et al. (2003), Stone et al. (2003a) and Steddom et al. (2005) when examining the spectral reflectance response to *Dothistroma* infection on pine needles; rice blast disease and *Cercospora* leaf spot on sugar beet foliage respectively. Leaf reflectance in the NIR is affected primarily by the leaf surface and internal cell structure (Datt, 1999a). Both these leaf features are grossly affected within the *Mycosphaerella* lesions. Our results also concur with Kobayashi et al. (2003) and Carter et al. (1996), in that sensitivity of reflectance to leaf disease severity in the NIR region was generally low.

When the sensitive wavelength $R_{678}$ nm was combined with the insensitive $R_{550}$ nm wavelength, the resulting index ($R_{678}$ nm/$R_{550}$ nm) was found to be highly correlated with disease severity. This index was used to formulate a regression model for predicting *Mycosphaerella* severity. The predicted data were well correlated with the observed data, with most predictions falling within one class of the observed value. A similar reflectance ratio was reported as being correlated to disease severity for rice blast disease (i.e. $R_{550}$ nm/$R_{675}$ nm; Kobayashi et al. (2003)).
*Mycosphaerella* leaf disease on juvenile *Eucalyptus globulus* foliage presents a complex of symptoms, including a decrease in chlorophyll content, increase in non-green pigments such as anthocyanin (Stone et al., 2001; Close and Beadle, 2003), breakdown of cellular integrity and subsequent desiccation. All these cellular features contribute to the overall reflectance of visible and NIR radiation from infected leaves. Very few studies have actually attempted to quantify necrotic spot diseases by spectral changes in leaf reflectance (Malthus and Madeira, 1993; Stone et al., 2003a; Steddom et al., 2005). Therefore, it is likely that reliance on empirical studies of leaf spectra for specific foliar pathogens will continue to form the basis of identifying potential spectral algorithms suitable for testing remotely at the canopy scale.


Park RF. 1988a. Effect of certain host, inoculum, and environmental factors on infection of *Eucalyptus* species by two *Mycosphaerella*. Transactions of the British Mycological Society 90:221-228.

Park RF. 1988b. Epidemiology of *Mycosphaerella nubilosa* and *M. cryptica* on *Eucalyptus* spp. in South-Eastern Australia. Transactions of the British Mycological Society 91:261-266.


Chapter 4.

Predicting *Mycosphaerella* leaf disease severity in a *Eucalyptus globulus* plantations using Digital Multi-Spectral Imagery.

Work from this chapter has been submitted to “Southern Hemisphere Forestry Journal” and is in press:


Predicting symptom severity of *Mycosphaerella* leaf disease in a *Eucalyptus globulus* plantation using Digital Multi-Spectral Imagery.

4.1 Introduction

In recent years there has been a rapid advancement in the development of new tools and techniques for assessing the condition of forest plantations (e.g. using the Crown damage Index, Stone et al., 2003b). Current methods used to monitor forest health include aerial sketch mapping, ground-based field surveys and roadside assessments. However these can be time consuming, labour intensive and costly (Stone and Coops, 2004). At present, aerial sketch mapping remains the most cost effective surveillance method and has provided useful information for plantation managers at a broad scale (Stone and Haywood, 2006). The results of such surveys are often subjective and the accuracy is strongly influenced by the experience and skill of the airborne surveyor to
precisely interpret forest crown damage symptoms and further relay this information onto a map (Coops et al., 2003a). This information can be digitised instantaneously using an electronic sketchpad (or at a later time) and then incorporated into a Geographical Information Systems (GIS). This series of processes is open to error due to loss of information from one step to the other (Stone and Haywood, 2006).

Recent advances in digital remote sensing has lead to the technology being investigated as a tool for assessing forest health (e.g. Lefsky et al., 2001; Stone, 2002; Levesque and King, 2003; Mohammed et al., 2004; Stone and Coops, 2004; Stone and Haywood, 2006; Wulder et al., 2006a). The data is in a digital format and represents the magnitude of the energy emitted from a surface or target in specified regions of the electromagnetic spectrum. By targeting specific regions of the spectrum to collect electromagnetic data (e.g. chlorophyll well, red edge, NIR plateau) information can be gained on the physical structure and condition of the target vegetation. Remote sensing could potentially overcome some of the limitations of aerial surveys by providing data that is of known spatial accuracy and precision, and can be analysed with and incorporated into other spatial data sets with the potential for spectrally identifying early stages of stress (Rock et al., 1988).

A key component of successfully using digital remotely sensed data for assessing crown health is the development of models that spectrally characterise disease symptom severity. To date, spectral data has been used in a number of studies to identify plant damage levels from biotic and abiotic causes, including fungal pathogens (e.g. Stone et al., 2003a; Leckie et al., 2004; Coops et al., 2006), insect attack (e.g. Leckie et al., 1992; Skakuna et al., 2003; Stone et al., 2005; Blanchfield et
al., 2006; Wulder et al., 2006b), structural damage (e.g. Levesque and King, 1999; Cosmopoulos and King, 2004; King et al., 2005; Levizou et al., 2005), and changes in chlorophyll (e.g. Daughtry, 2000; Carter and Knapp, 2001; Maccioni et al., 2001; Coops et al., 2003b) and water content (Danson et al., 1992; Peñuelas et al., 1993; e.g. Datt, 1999b; Chaerle and Van der Straeten, 2000; Pu et al., 2004).

Recently a study was completed on the spectral effects of Mycosphaerella Leaf Disease (MLD), a major pest of juvenile Eucalyptus globulus trees in Tasmania (Pietrzykowski et al., 2006). This work indicated that when the sensitive chlorophyll absorbing wavelength 678nm was combined with an insensitive wavelength (550 nm), they formed an index that discriminated between healthy and infected foliage (Pietrzykowski et al., 2006).

Translating results from the leaf to crown scale can be problematic as crown scale reflectance is influenced by structural (e.g. branches, shadows cast within a crown and by neighbouring trees and multiple leaf angles) and physiological attributes (e.g. leaf age, crown flushing and a complex of disease symptoms that can be expressed within a crown at the one time), site factors (e.g. substrate and understorey characteristics), the atmospheric conditions (e.g. clouds, pollution, sun angle) and the sensor (Guan & Nutter, 2001). Leaf based spectral investigations provide the preliminary information needed to begin investigating the effects biotic stress agents have on whole crown reflectance (Gitelson and Merzlyak, 1994; Rock et al., 1994; Carter and Knapp, 2001; Stone et al., 2001; Sims and Gamon, 2002; Coops et al., 2003b; Stone et al., 2003a; Stone et al., 2005).

Once spectral symptom characterisation has been successful at the crown scale and imagery has been translated to depict predicted severities e.g. a plantation, the
information needs to be in a form that is useful. Depending on the intended use of image, data can either be classified into disease severity classes or depicted on a continuous scale. Continuous data, illustrates the distribution of damage severity, which is useful for investigating patterns in severity distribution. Classified data (e.g. low, moderate, severe) can be used to calculate the proportions of crowns in each damage class, which if related to impact on productivity can be further used in yield modelling.

Issues relating to model prediction accuracy for continuous and categorical data, at the crown and plantation scales need to be investigated. The objective of this study was to use high-resolution digital multispectral imagery to quantify disease severity (defoliation and necrosis) caused by *Mycosphaerella* leaf disease in a *Eucalyptus globulus* plantation. The potential benefits of using this technology as a forest health surveillance tool for plantation management were examined.

4.2 Methods

4.2.1 Study site

The study site was an 82 ha *E. globulus* plantation, 85 meters above sea level and was established in 2000 at Temma on the northwest coast of Tasmania, Australia (41°07’22”S, 144°45’28”S) (refer to Chapter 3. Section 3.2.1 Study Site for additional site preparation details). The plantation was chosen, as it appeared to exhibit a disease gradient of MLD severity from severe (greater than 50% severity) to low (10% severity) (Figure 4.1). The east-west MLD gradient of decreasing MLD severity appeared to be the result of a dispersal gradient, i.e. less inoculum with increasing distance from inoculum source (i.e. an adjacent plantation) (Figure 4.1)
(Wardlaw, 2002). The two dominant *Mycosphaerella* species identified at the site were *M. cryptica* and *M. nubilosa* (Wardlaw, 2002).

At the time of the experiment, mean tree height and diameter (± standard deviation) at breast height were 4.95 m (± 1.45 m) and 7 cm (± 3 cm) respectively. Understory vegetation included native grasses, small woody shrubs and ferns.

Figure 4.1. Aerial photograph showing the study site at Temma (TEO7C-41°07′22″S, 144°45′28″S). Low, moderate, and severe labels indicate MLD severity gradient from west to east. The photograph also includes a plantation planted in 1999 (TEO7D) exhibiting a severe MLD epidemic.
4.2.2 Assessment of Mycosphaerella Leaf Disease crown damage for model development

Three sites were chosen along the observed MLD severity gradient (low, moderate and severe) in the *E. globulus* plantation (Figure 4.1). At each site, a bay* tree was randomly chosen to mark the corner of a plot consisting of 24 trees (four rows of six trees, one row being in a windrow*). Windrow trees were included as they displayed healthier crown condition than trees in bays (Wardlaw, 2002) (Figure 4.2).

A total of 72 trees were visually assessed for severity of Mycosphaerella Leaf Disease. The variables to assess crown severity are in Table 4.1. The *Mycosphaerella* defoliation score was the estimated percentage of the crown defoliated and the *Mycosphaerella* Necrosis Index was the percentage of the crown’s leaves with necrosis incidence multiplied by the average severity of necrosis on leaves. Estimates were completed following protocols outlined by Stone et al., (2003b).

4.2.3 Spatial assessment of Mycosphaerella Leaf Disease

A survey of crown damage due to MLD was completed assessing every 5th tree in every 5th row. The location of each crown sampled was determined with a GPS (Garmin, e-trex) weigh point and assessed for incidence and severity of defoliation and necrosis (Table 1). The data was used to produce a map of the site that would depict the observed spatial pattern of MLD crown damage.

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*Bays and windrows are created as a result of site preparation procedures. Windrows are formed when debris is mounded and burned. The areas between windrows are called bays.*
Figure 4.2. Ground photograph showing severe MLD diseased trees at the study site.

Bay trees (foreground, right) exhibited severe crown defoliation and necrosis on remaining leaves. The windrow trees (background, left) exhibited considerably less crown defoliation, though the levels of necrosis were only slightly less. Photo courtesy of Dr. Timothy Wardlaw, Forestry Tasmania.

Table 4.1. Assessment variables used to quantify damage in *E. globulus* caused by Mycosphaerella Leaf Disease.

<table>
<thead>
<tr>
<th>Damage variable</th>
<th>Description</th>
<th>Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defoliation</td>
<td>The percentage area of the crown with absent leaves</td>
<td>0 – 100%</td>
</tr>
<tr>
<td><em>Mycosphaerella</em> Index</td>
<td>Percentage of the crowns leaves exhibiting necrosis</td>
<td>0 – 100%</td>
</tr>
<tr>
<td><em>(Necrosis Incidence × Necrosis Severity)</em></td>
<td>For leaves with necrosis, the average percentage of the leaf with necrosis</td>
<td></td>
</tr>
</tbody>
</table>

Note: Visual average estimates are based on the majority of leaves still attached to branches.
4.2.4 Image acquisition and pre-processing

The imagery used in this project was acquired using a Digital Multi-Spectral Camera (DMSC; SpecTerra Services, 2004) under cloud free conditions between 11am and 1 pm on 24 March 2003. The imagery was captured over an area of approximately 400 ha at a pixel size of 0.5 m by 0.5 m, which resulted in several pixels per tree crown and a 512-pixel swath width for the complete image. The camera was mounted on a single engine light aircraft. The DMSC had four individual 1024 ×1024 charge-coupled device (CCD) arrays capable of acquiring data at 12-bit digitisation. Flight paths were oriented parallel to the solar azimuth to reduce bi-directional reflectance artefacts and approximately 1,000 meters above the ground.

Narrow bandwidth (approximately 10 nm full width half maximum bandwidth) interference filters were fitted to the DMS camera, which allowed detection at 550 nm, 680 nm, 740 nm and 780 nm wavelengths (Table 4.2). These wavelengths were chosen as they proved successful in characterising MLD symptoms at the leaf scale (Pietrzykowski et al., 2006).

Pseudo-Invariant Features (PIFs – one white and one black sheet; size =3m²) with known spectral properties were placed in the field of view of the camera within the target area. Reflectance spectra were collected from the PIF’s at the time of image capture using a hand-held spectroradiometer (PP Systems Analytical Spectral Devices, Inc. Boulder, Colorado, USA 1999). An empirical line calibration was then used to convert image intensity values for pixels in the image to reflectance.
Trees sampled and assessed in the field were individually identified in the image and each whole crown was manually delineated into a region of interest (ROI) in ENVI (ENVI 2001) as per the method described by Leckie et al., (1992). Statistics calculated from each ROI included the mean reflectance and variance in each band for which imagery was captured.

Table 4.2. Reflectance variables investigated for the usefulness in spectrally characterising symptoms of *Mycosphaerella*. \( R = \) Reflectance (nm), \( \text{Var} = \) Variance, NDVI=Normalised Difference Vegetation Index.

<table>
<thead>
<tr>
<th>Data</th>
<th>Spectral variable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflectance mean</td>
<td>( R_{550} )</td>
</tr>
<tr>
<td></td>
<td>( R_{680} )</td>
</tr>
<tr>
<td></td>
<td>( R_{740} )</td>
</tr>
<tr>
<td></td>
<td>( R_{780} )</td>
</tr>
<tr>
<td>Reflectance variance</td>
<td>( R_{\text{Var550}} )</td>
</tr>
<tr>
<td></td>
<td>( R_{\text{Var680}} )</td>
</tr>
<tr>
<td></td>
<td>( R_{\text{Var740}} )</td>
</tr>
<tr>
<td></td>
<td>( R_{\text{Var780}} )</td>
</tr>
<tr>
<td>Reflectance Indices</td>
<td>((R_{740}-R_{680})/60) = Lower slope of red edge (Merton, 1999)</td>
</tr>
<tr>
<td></td>
<td>((R_{780}-R_{740})/40) = Upper slope of red edge (Merton, 1999)</td>
</tr>
<tr>
<td></td>
<td>((R_{780}-R_{680})/(R_{780}+R_{680})) = NDVI (Lichtenthaler et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>((R_{550}/R_{680})) (Leckie et al., 1992)</td>
</tr>
<tr>
<td></td>
<td>((R_{680}/R_{550})) (Pietrzykowski et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>((R_{780}-R_{680})/100) = Total slope of the red edge (Merton, 1999)</td>
</tr>
</tbody>
</table>

4.2.5 Data analysis

Model development

A range of variables were used in building the model, including the mean reflectance and reflectance variance of the ROI’s at each waveband acquired (550 nm, 680 nm, 740 nm and 780 nm), and a variety of spectral indices that compare reflectance in two or more bands (Table 4.2). Initially many indices were considered as they have been shown, in other work, to be highly correlated with the health of vegetation (e.g. Leckie et al., 1992; Franklin and Raske, 1994; Peñuelas et al., 1995; Peñuelas et al.,
These were modified to utilize the four spectral bands acquired for this study. The red edge indices (upper, lower and total slope) were modified from those developed by Merton (1999), and the NDVI from that of Lichtenhaler (1996). Only indices highly correlated with severity were kept for final model development and are shown in Table 2.2. One index ($R_{680}/R_{550}$) tested was derived from a previous leaf scale investigation using foliage sampled from this plantation (Pietrzykowski et al., 2006).

An ANOVA was completed on both symptom data sets to investigate the level of variance a given variable contributes to the total variation. Wavelengths and indices that explained the most variation and with no co-linearity were included in a stepwise multiple regression analysis. Normal probability plots were examined to ensure assumptions of normality and homoscedasticity held, and residuals were analysed. Data transformation was not necessary as distributions proved normal, unimodal and symmetric. Subsequently, the most appropriate models chosen were those with the least number of significant variables giving the best coefficient of determination and root mean square error (RMSE). All statistical analyses were completed using SAS Version 8 (SAS Institute Inc, 1999).

**Map development**

Linear spectral mixture analysis was used to identify the proportion of bare soil, shadows and sunlit crown in the imagery, and a mask was created to eliminate pixels containing less than 40% sunlit crown (ENVI, 2004). It was not possible to manually delineate every crown throughout the image, so a circular filter, with a diameter approximating the average crown diameter, was used in ArcGIS (ArcGIS ESRI, 2001).
to create images representing the mean pixel value and pixel variance at the crown scale. A mask of the model was then applied to the calibrated and registered image to depict predicted defoliation and *Mycosphaerella* index severity.

*Model validation*

A bootstrap validation procedure in SAS was completed to test the predictive accuracy of the model. This is a widely accepted method for model validation when data sets are limited in size (Davison and Hinkley, 1997). The bootstrap-validation method (macro sourced from: http://merlot.stst.uconn.edu) was applied to the full data set (n=72) and was set at 1000 iterations.

*Classification accuracy assessment*

Model accuracy was assessed using the predicted severities for the 72 trees. Once the models were applied to the raw DMSI data, the predicted defoliation and *Mycosphaerella* index values were extracted from each tree crown. A coefficient of determination was calculated to indicate the fit of the equation to the stimulus-response data. Additionally, an accuracy analysis (confusion matrix) was completed on data classified into classes. Details are given in the following paragraphs.

When considering breaking the continuous data into classes to look at *Mycosphaerella* symptom severity it is important to consider critical thresholds where the pathogen is having an effect on the host’s growth. To date, research has identified two thresholds relating to the effect of defoliation (Lundquist and Purnell, 1987; Rapley, 2005; Pinkard et al., 2006; Smith et al., 2006) and leaf necrosis severity in eucalypt growth (Smith, 2006). Results have shown that crown defoliation levels over ~20% begin to
have an effect on stem growth and volume (Lundquist and Purnell, 1987; Pinkard et al., 2006) but if levels remain below ~70%-80%, then damage is not long term and the trees recover (Rapley, 2005; Smith et al., 2006). Beyond ~70% defoliation, tree growth is indefinitely reduced and damage yield is permanent (Smith et al., 2006). By identifying trees with 20% crown severity, forest managers would be alerted to possible growth reductions and could more intensely monitor their health status. Hence, it is logical to establish an action threshold (e.g. 50%) to allow time for interventions before tree crowns reach a critical ~70%-80% level of damage.

For this study, data were split into three (0-20%, 20-70%, 70%+) and four (0-20%, 20-50%, 50-70%, 70-100%) classes. The fourth class was added to compare level of accuracy with an additional class. A confusion error matrix was produced (Congalton, 2001). An error matrix is a square arrangement of numbers comparing training data (observed) used to describe each class during classification with the output, classified image (predicted). Two statistics from error matrix analysis are used to describe the classification accuracy of the map in general: (1) the Overall Accuracy (OA) score and; (2) Kappa (or ‘Khat’) statistic from Kappa analysis (Congalton and Green, 1999). The OA score shows the proportion of pixels that have been correctly classified according to the training data. This is calculated from the sum of the correctly classified pixels (the main diagonal in the matrix) divided by the total number of pixels. Kappa analysis attempts to account for chance agreement by incorporating the row and column totals, (which indicate the probability of getting a correct classification by random chance) into the accuracy assessment (Congalton and Green, 1999). The Kappa statistic ranges from -1 to +1, and can be interpreted in terms of the percentage of agreement between the training data and the classified map.
(e.g. Strong agreement Kappa = >0.80 (80%); Moderate agreement Kappa = ≥ 0.40 (40%) and ≤ 0.80 (80%); Poor agreement Kappa = < 0.40 (40%) (Landis and Koch, 1977). The Kappa coefficient is calculated as: \( \text{kappa} = (\text{observed agreement} - \text{chance agreement}) / (1-\text{chance agreement}) \). OA values tend to overestimate the accuracy of classified images (Ma and Redmond, 1995) and Kappa analysis tends to underestimate map accuracy (Foody, 1992). Therefore, the true predictive accuracy of the image usually exists between the OA and Kappa statistics.

To describe the precision of individual classes the Producer’s and User’s accuracy were calculated. Producer’s accuracy was calculated by dividing the total number of correctly classified pixels by the total number of training pixels in that class (the column total). Producer’s accuracy indicates commission errors, where pixels have been incorrectly included in the target class. User’s accuracy indicates errors of omission, where pixels have been incorrectly omitted from the target class (Congalton, 2001). For example, a class (e.g. defoliation severity 1) with a producer’s accuracy of 80% and a user’s accuracy of 85%, would indicate that 80% of areas of defoliation severity ‘1’ on the ground have been correctly classified, but that only 85% percent of areas labelled ‘1’ on the map are actually areas of severity ‘1’ on the ground. This map would therefore underestimate the extent of areas with defoliation severity ‘1’.
4.3 Results

4.3.1 Observed spatial pattern of Mycosphaerella Leaf Disease damage

A table summarising the damage from surveyed trees is given in Table 4.3. At 995 days after planting, 13% of the crowns surveyed displayed no evidence of infection from MLD (Table 4.3(a)). Of the 87% with damage, 52% had less than 45% damage (Table 4.3(b)). Only a small proportion of crowns had damage greater than 75% (Table 4.3(b)).

A non-random spatial pattern of crown severity existed for crown defoliation and *Mycosphaerella* index at Temma (Figure 4.3). Figures 4.3a and 4.3b both depict the east-west gradient for defoliation and *Mycosphaerella* index severity respectively. The south-eastern area of the plantation had increased levels of defoliation and *Mycosphaerella* index severity. This area of the plantation was close to the inoculum source, a 1999 *E. globulus* plantation that was severely infected with MLD.
Table 4.3. Summary of results from GPS survey assessing CDI of *E. globulus* trees showing infection symptoms caused by MLD. (a) Percentage of total trees with and without damage; (b) Distribution of trees in each damage class.

| (a) | Assessment date:  
<table>
<thead>
<tr>
<th></th>
<th>25th March 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. globulus</em></td>
</tr>
<tr>
<td>Days since planting</td>
<td>995</td>
</tr>
<tr>
<td>Trees with NO damage (%)</td>
<td>13</td>
</tr>
<tr>
<td>Trees WITH damage (%)</td>
<td>87</td>
</tr>
</tbody>
</table>

| (b) | Assessment date:  
<table>
<thead>
<tr>
<th></th>
<th>25th March 2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days since planting: 995</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Damage score (mid-point of range)</th>
<th>Crown damage range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0-2</td>
</tr>
<tr>
<td>5</td>
<td>3-7</td>
</tr>
<tr>
<td>10</td>
<td>8-15</td>
</tr>
<tr>
<td>20</td>
<td>16-25</td>
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<td>26-35</td>
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<td>40</td>
<td>36-45</td>
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<tr>
<td>50</td>
<td>46-55</td>
</tr>
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<td>56-65</td>
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<td>70</td>
<td>66-75</td>
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<td>80</td>
<td>76-85</td>
</tr>
<tr>
<td>90</td>
<td>86-92</td>
</tr>
<tr>
<td>95</td>
<td>93-97</td>
</tr>
<tr>
<td>100</td>
<td>98-100</td>
</tr>
</tbody>
</table>
Figure 4.3. Observed spatial distribution of crown a) defoliation and b) *Mycosphaerella* index severity at the study site. Data points represent every fifth tree in every fifth row.
4.3.2 Model development to predict Mycosphaerella Leaf Disease severity

Pearson correlation coefficients for the relationships between crown defoliation and Mycosphaerella index, with reflectance wavebands and indices extracted from the DMS imagery, are given in Table 3. Reflectance variance at 780 nm was the most highly correlated waveband with defoliation \((r = 0.600, P>0.0001)\). The remaining correlations between wavebands and defoliation were low, with the index R680/R550 having the next highest correlated \((r = 0.501, P<0.0001)\). The Mycosphaerella index was most correlated with \(R_{680}\) nm, \((0.405, P<0.001)\) and least correlated with \(R_{550}\) nm (NS). The sensitive and insensitive wavelengths formed the index \(R_{680}/R_{550}\) which were highly correlated with the Mycosphaerella index \((r = 0.507, P>0.0001)\) in this study and in past work at the leaf scale (Pietrzykowski et al., 2006). The relationship between other wavebands and Mycosphaerella index correlations was poor. Figure 4(a) and 4(b) depict the response of \(R_{Var780}\) and \(R_{680}/R_{550}\), the two reflectance variables most correlated with defoliation severity and Mycosphaerella index respectively. Both of these variables were used in model development (Table 4.3).

The regression models that best explained the relationships between spectral response and a) crown defoliation severity and b) the Mycosphaerella index were:

\[
\text{Defoliation Severity} = 51.84 + (193.60 \times R_{Var740}) - (294.39 \times R_{Var780}) \\
R^2=0.5, P<0.0001 \ (a)
\]

\[
\text{Mycosphaerella Index} = -33.35 + (206.62 \times R_{680}/R_{550}) \\
R^2=0.3, P<0.0001 \ (b)
\]
Table 4.3. Pearson’s Correlation coefficients ($r$) single reflectance wavebands and reported indices for a) defoliation and b) *Mycosphaerella* index. $R$ = Reflectance (nm); Var = Variance.

<table>
<thead>
<tr>
<th>Waveband</th>
<th>a) Defoliation</th>
<th>b) <em>Mycosphaerella</em> Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R550$</td>
<td>-0.226 NS</td>
<td>0.010 NS</td>
</tr>
<tr>
<td>$R680$</td>
<td>0.206 **</td>
<td>0.405 **</td>
</tr>
<tr>
<td>$R740$</td>
<td>-0.307 **</td>
<td>-0.183 **</td>
</tr>
<tr>
<td>$R780$</td>
<td>-0.287 *</td>
<td>-0.256 NS</td>
</tr>
<tr>
<td>$R_{Var}550$</td>
<td>-0.371 **</td>
<td>-0.151 NS</td>
</tr>
<tr>
<td>$R_{Var}680$</td>
<td>-0.166 NS</td>
<td>0.094 NS</td>
</tr>
<tr>
<td>$R_{Var}740$</td>
<td>-0.370 **</td>
<td>-0.168 NS</td>
</tr>
<tr>
<td>$R_{Var}780$</td>
<td>-0.600 ***</td>
<td>-0.363 NS</td>
</tr>
</tbody>
</table>

**Indices**

- $(R740-R680)/60 = \text{Lower slope of red edge}$ (Merton, 1999)
- $(R780-R740)/40 = \text{Upper slope of red edge}$ (Merton, 1999)
- $(R780-R680)/(R780+R680) = \text{NDVI}$ (Lichtenthaler et al., 1996)
- $(R550/R680)$ (Leckie et al., 1992)
- $(R680/R550)$ (Pietrzykowski et al., 2006)
- $(R780-R680)/100 = \text{Total slope of the red edge}$ (Merton, 1999)

Note: **$P \leq 0.001$;  *$P \leq 0.01$;  *$P \leq 0.05$; NS, not significant; } n = 72.
Figure 4.4. Spectral responses for (a) reflectance variance at $R_{780}$ nm with defoliation and (b) $R_{680}/R_{550}$ (Pietrzykowski et al., 2006) with *Mycosphaerella* index. Also shown are simple regression lines and the $R^2$ of the relationship between each spectral variable and *Mycosphaerella* disease symptom.
4.3.3 Map Development

An image depicting the predicted defoliation severity and *Mycosphaerella* index using each of the algorithms is shown in Figures 4.5(a) & 4.5(b). The image clearly indicates trees in windrows have 1) less defoliation and 2) lower *Mycosphaerella* index than trees in bays. The image also illustrates the damage severity gradient from the eastern (more severe) to western (less severe) parts of the plantation as well as increased Mycosphaerella index severity in trees along the road in the eastern part of the plantation. These figures can be compared to the observed distribution pattern of damage severity in Figures 4.3(a) and 4.3(b), which depict a similar pattern. Each of the predictive models indicates a reasonable fit with an $R^2$ of 0.5 (P<0.0001) and 0.3 (P<0.0001), which are low but not uncommon with this form of data (e.g. Yuan et al., 1991).

4.3.4 Image classification

Classified images for both models are shown in Figure 4.6a & 4.6b. Despite the continuous data being classified into 3 classes, the distribution of damage severity still indicates bay trees have higher levels of defoliation severity (Figure 4.5a) and *Mycosphaerella* index (Figure 4.5b) than windrow trees.

The defoliation image additionally shows severity is greater in windrows (less green pixels) in the eastern of the plantation compared to the west. A similar, gradient is obvious in the *Mycosphaerella* index image.
Figure 4.5. Digital multi spectral imagery of a *E. globulus* plantation infected with *Mycosphaerella* leaf blight. Imagery predicting (a) Defoliation Severity = 51.84 + (193.6 × RVar740) – (294.39 × RVar780), $r^2=0.5$, $P=<0.0001$, RMSE=15.25) and (b) a *Mycosphaerella* index = -33.35 + (206.62 × R680/R550), $r^2=0.3$, $P=<0.0001$, RMSE=21.95). The strips of green are healthy windrow trees.
Figure 4.6. Classified digital multi spectral imagery of a *E. globulus* plantation infected with MLD. The imagery depicts the distribution of damage severity classified into three classes of a) crown defoliation and b) crown *Mycosphaerella* index.
Figure 4.7(a) and 4.7(b) shows the proportion of the plantation (ha) in each defoliation severity and *Mycosphaerella* index class. Analysis of the classified data shows that the majority of the plantation has defoliation severity levels greater than 70% and that the distribution of damage was highly skewed towards higher defoliation severity levels.

Figure 4.7. Crown distribution in each severity class for defoliation and *Mycosphaerella* index damage caused by MLD. Values represent the percentage proportions and areas in hectares for each bar.
Approximately 78% of the plantation, or ~33 ha of the crowns exhibited 70% or more crown defoliation. Only 0.1% of the plantation had less than 20% defoliation severity. In comparison, *Mycosphaerella* index severity results displayed a bell curve distribution, with approximately 90% of the crowns exhibiting 20-70%, or ~ 39ha, damage severity. Only 0.2% of the crowns had less than 20% damage. These results indicate that the plantation was exhibiting a severe epidemic of MLD.

4.3.5 Model validation

The model validation procedure indicated both models were highly significant. Significance of all parameter estimates for each variable in both models was less than P<0.05. A summary of the results is shown in Table 4.

4.3.6 Classification accuracy

Results for the accuracy analysis when data was classed is given in Table 5. Note there were no observed crowns in *Mycosphaerella* index class 1. The level of accuracy and reliability of classifications was based on the severity classes (1-3) being correctly classified. This was observed in both the defoliation and *Mycosphaerella* index data sets. The overall accuracy of the defoliation predictions was 71%, with a Kappa coefficient of 0.63, which indicates moderate chance agreement. The producer’s accuracy for the defoliation error matrix indicated a variation in probabilities of a sample being correctly classified. It showed that trees with low levels of defoliation (class 1) would be classified with 100% accuracy, compared to crowns with higher levels of defoliation (classes 2 & 3), which had a 71% and 44% probability of being correctly classified (Table 5). In comparison the users accuracy indicated all three classes had similar probabilities of being accurately predicted.
Table 4.4. Summary of results for Bootstrap validation procedure for each variable in each model. The Raw column includes the original P-value from the raw data and the Bootstrap column contains the bootstrap resample adjusted P-value.

<table>
<thead>
<tr>
<th>Model variable</th>
<th>Raw</th>
<th>Bootstrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>R680/R550</td>
<td>&lt;0.0001</td>
<td>0.0015</td>
</tr>
<tr>
<td>RVar740</td>
<td>0.0015</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RVar780</td>
<td>&lt;0.001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4.5. Predicted accuracy of (a) Defoliation and (b) Mycosphaerella Index models using 3 severity classes. n=72.

(a) Defoliation

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Row Σ</th>
<th>Users Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>8</td>
<td>0</td>
<td>24</td>
<td>67</td>
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<td>2</td>
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<td>27</td>
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<tr>
<td>3</td>
<td>0</td>
<td>3</td>
<td>8</td>
<td>11</td>
<td>73</td>
</tr>
<tr>
<td>Column Σ</td>
<td>16</td>
<td>38</td>
<td>18</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Producers Accuracy (%)</td>
<td>100</td>
<td>71</td>
<td>44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Mycosphaerella Index

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Row Σ</th>
<th>Users Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>6</td>
<td>0</td>
<td>6</td>
<td>100</td>
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<td>40</td>
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<td>Column Σ</td>
<td>0</td>
<td>54</td>
<td>18</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Producers Accuracy (%)</td>
<td>0</td>
<td>65</td>
<td>72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Producers accuracy is calculated by dividing the total number of matched score tree crowns by the total number of trees measured (Column Σ) in the field. Users accuracy is calculated by the total number of matched score trees by the total number of field-measured trees identified in the imagery (Row Σ).
Overall accuracy of predictions for the *Mycosphaerella* index was 67%, with a Kappa coefficient of 0.54, which indicated moderate agreement. Table 4.5 includes the Users and Producer’s accuracies for the *Mycosphaerella* index. Producer’s accuracy indicated crowns with high values for the *Mycosphaerella* index (class 3) were more accurately classified (72% accurate) than crowns with low levels (1 and 3). Users accuracy indicated there was a varied reliability (50-100%) in accurately classifying damage severity.

The data was split into four classes as a comparison to observe how the predictive accuracies and Kappa coefficients would be modified. Results indicated that classification into 4 rather than 3 classes would reduce the overall accuracy of both models from 71% and 67% to 57% and 49% for the defoliation and *Mycosphaerella* index models respectively. The Kappa coefficients also reduced to 0.5 (moderate agreement) and 0.4 (moderate agreement). Similarly the producers and users accuracies decreased in each of the classes as the reliability to correctly classify into 4 classes was reduced (Table 4.6). Therefore, by increasing the number of classes more information might be illustrated in the image (Figure 4.8), however the probability of a correct prediction was reduced.
Table 4.6  Predicted accuracy of (a) Defoliation and (b) Mycosphaerella Index models using 4 severity classes. n=72.

(a) Defoliation

<table>
<thead>
<tr>
<th>Severity</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Row ∑</th>
<th>Users Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>8</td>
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<td>0</td>
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<td>73</td>
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<td>Column ∑</td>
<td>16</td>
<td>21</td>
<td>17</td>
<td>18</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Producers Accuracy (%)</td>
<td>100</td>
<td>48</td>
<td>41</td>
<td>44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) Mycosphaerella Index

<table>
<thead>
<tr>
<th>Severity</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Row ∑</th>
<th>Users Accuracy (%)</th>
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<td>0</td>
<td>25</td>
<td>29</td>
<td>18</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Producers Accuracy (%)</td>
<td>0</td>
<td>52</td>
<td>31</td>
<td>72</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Producers accuracy is calculated by dividing the total number of matched score tree crowns by the total number of trees measured (Column ∑) in the field. Users accuracy is calculated by the total number of matched score trees by the total number of field-measured trees identified in the imagery (Row ∑).*
Figure 4.8. DMSI of a *E. globulus* plantation infected with MLD. The imagery depicts the distribution of damage severity classified into four classes of a) crown defoliation and b) crown *Mycosphaerella* index.
4.4 Discussion
Currently, forest health surveillance programs are completed using traditional methods that are labour intensive and often subjective. These methods remain the most cost effective and most widely implemented programs to monitor forest health (Stone and Coops, 2004). The acquisition of DMSI at an *E. globulus* plantation has allowed development of a model to assess the condition of trees affected by Mycosphaerella Leaf Disease. The model was further applied to assess the health status of a complete plantation to illustrate the power of remotely sensed data. This study provides an example of how high resolution remotely sensed imagery can be used as a crown health surveillance tool in a young eucalypt plantation, whilst eliminating many issues relating to current surveillance methods.

The capability of remotely-sensed methods to predict at the crown scale is controlled by the spatial resolution of the imagery. In this study, the 50 cm DMSI resulted in several pixels per tree crown. The complexity of symptoms being assessed within and between crowns was identifiable at this scale, particularly when using reflectance variance data.

The accuracy of each model to predict MLD infection symptoms at the individual tree scale was moderate. This was probably due to the complexity of symptoms being expressed, in addition to background noise from soil and undergrowth. The visual accuracy was however improved when the image was viewed at a broader scale such as a stand of trees, or an area of the plantation. This site clearly illustrates this point, as stands of defoliated bay trees were highly distinguishable from highly foliated windrow trees even though some more foliated trees did occur in the bays. Generally
it is hypothesized that accuracies improve when looking at a stand or region, as pixels delineating the target area are more representative of the on-ground situation. If individual tree crowns are targeted then the delineation may not always include enough pixels and hence misrepresent what is occurring in reality.

Partial success in characterizing the MLD stress symptoms at the crown scale is due to prior knowledge on the wavelengths that are sensitive to MLD at the leaf scale (Pietrzykowski et al., 2006). These investigations gave insight into wavelengths sensitive to MLD that aided the selection of wavebands to be used when acquiring the digital imagery. In many cases researchers rely on previously identified sensitive wavelengths and indices and ratios from the literature, e.g. the NDVI (e.g. Lichtenthaler et al., 1996; Skakuna et al., 2003; Pontius et al., 2005) to use in their investigations, which may or sometimes may not be successful. In this study prior knowledge that $R_{680}$ nm and $R_{550}$ nm (sensitive and insensitive wavelengths respectively) were highly significant in spectrally characterizing MLD leaf necrosis, led to success of the Mycosphaerella index model.

The additional component to crown spectral investigations in this study was the inclusion of reflectance variance variables. These investigations found $RV_{ar780}$ nm was highly sensitive to increased levels of crown defoliation. The increase in reflectance variance at 780nm, with increasing defoliation severity, occurs due to the increased mixture of substrates within a defoliated crown (e.g. branches, trunk, pigment changes of near senescing leaves). The use of variance and textural data to identify crown structural attributes has been successfully used in other studies such as
defoliation in aspen (Moskal and Franklin, 2004) and sugar maple decline (Yuan et al., 1991).

A map presenting continuous data demonstrates the quantitative resolution and the
detail of the information. For managers, this information is invaluable as it can be
used to identify infection outbreak hot spots, site nutrient factors and monitor the
progression of epidemics. Management strategies can then be implemented based on
accurate and thorough knowledge of the status of the plantation. At the site used in
this study, this may include fertilization of bay trees.

Concurrently, classified information is also valuable as plantation managers can
interpret the disease status of their estate in a summarised format relevant to their
operational needs. For example, managers can identify the proportion of their estate
in particular health ranges, which can be further used to aid in management decisions,
such as the need to adjust yield models to account for growth loss, restrict trees to a
pulpwood crop or apply fertilizer in attempt to enhance tree growth and recovery
(Dr. Timothy Wardlaw, pers. comm., 2006). Classification to indicate a particular
threshold range (i.e. 0-20%) can be set to alert managers to areas of the plantation at
risk to progressing to higher damage levels and suffering long-term growth effects.
An alert category gives managers’ sufficient time to implement management
strategies to reduce disease impact.

The application of DMSI and the methods used to develop the crown scale predication
models in this study were limited in several ways. Firstly, a larger number of samples
would have ensured an adequate number of samples were included for the full range
of attributes being targeted and hence develop a more robust model. Though the models were developed using an adequate sample size (n = 72) a minimum of 75 is recommended (Sims & Culvenor, 2004). An additional sample, preferably from another plantation, would also have enabled an independent validation to assess the predictive abilities of the models. Secondly the measurements were taken from young *E. globulus* trees (exhibiting juvenile foliage) in north-western Tasmania exhibiting a range of MLD symptoms and hence the model should only be used in the same situation. The model would need to be validated at a number of other sites before it could be applied operationally. Thirdly, measuring levels of crown discolouration could improve the accuracy of the ground-based assessments. At the remote scale, crown discolouration may be a more distinguishable attribute to characterise when image processing.

The increasing availability of affordable, high-resolution data from satellites will make digital remote sensing more operationally feasible plantation health assessment tool and highly competitive to current assessments methods. It is envisaged that in the future, remotely sensed images of plantations would be routinely acquired, analysed and processed by plantation managers. The medium could form the basis of site risk assessment and be integrated into management decision support systems for enhanced plantation management.

King DJ, Olthof I, Pellikka PKE, Seed ED, Butson C. 2005. Modelling and mapping damage to forests from an ice storm using remote sensing and environmental data. Natural Hazards 35:321-342.


Chapter 5.

Towards precision forestry: the application of risk prediction models and remote sensing to the management of forest health

5.0 Research outcomes

This chapter recapitulates the outcomes from the research undertaken in this thesis. A conceptual framework of developing new tools for managing the health of eucalypt plantations is outlined. Thesis outcomes are discussed in terms of their potential for the health management of eucalypt plantations, specifically in developing strategies for reducing the impact of Mycosphaerella leaf disease (MLD).

Investigations were completed into a) identifying the level of site risk to MLD based on meteorological variables, and b) using remote sensing to assess and quantify levels of damage once infection has occurred.

The outcomes are:

1. A prototype model to predict atmospheric ascospore density (in the top of the tree canopy). This was completed following the application of mathematical functions to describe the effects meteorological variables have on diurnal and global trends of atmospheric ascospore concentrations. The outcomes of initial investigations are:

   a. Atmospheric ascospore concentrations are most prevalent when average temperatures are between 5 °C-15 °C and relative humidity is ~93% and rainfall is present.
b. In the absence of rain, ascospore events are more prevalent and abundant during the night than during the day. In the presence of rain, ascospore events can occur at any time.

c. Atmospheric ascospore events are more prevalent during spring, summer and autumn.

It must be emphasised that this prototype model was developed with data from a single site over 28 months and must be validated at other sites.

2. MLD severity classes were spectrally characterised at the leaf scale with the spectral ratio $R_{678}/R_{550}$.

3. Remotely-sensed DMSI data was used to develop models to spectrally characterise the severity of MLD symptoms (defoliation and necrosis) at the crown scale. Such models could be applied to all crowns with MLD in a young eucalypt plantation thus giving 100% sampling coverage.

5.1 Forest health management: current and future trends

Australia has adopted internationally recognized sustainable forest management certification. This has pushed the forest industry to move towards sustainable forest management in order to secure a sustainable supply of raw material and to ensure marketplace acceptance of Australian products.

Managers of eucalypt plantations need to protect trees from economically significant levels of damage caused by a large number of biotic agents native to the environment that may cause a wide range of damage types; stem damage, shoot and tip damage,
leaf damage and different levels of defoliation. Damage can also be dispersed in various patterns within the crown, such as the upper or lower crown defoliation which can result from different levels of MLD severity in eucalypts (e.g. Wardlaw, 2001).

The symptoms caused by different biotic agents (or hazards) are often similar, for example insects, mammals and fungi all cause crown defoliation and necrosis. Commonalities in terms of symptoms have lead to the concept of a quasi generic approach to health management in eucalypt plantations (Figure 5.1). This approach involves modelling tools to guide decision-making in respect to both resource allocation planning and the requirement for intervention (the nature of interventions will however differ). Resource allocation will be dictated by simulation and predictive models that quantify site productivity and site hazard (i.e. hazard = a risk rating for a site descriptive of the long term (or rotation length) risk assessment in respect to damage from biotic agents).

Historically severe damage caused by the majority of Australia’s native eucalypt pests and pathogens (e.g. *Mycosphaerella* spp.) has been sporadic both in native forest and plantations. There has been little proactive management of damage, in fact little response to damage - other than to bear the cost. As more studies are carried out into the epidemiology of pest and pathogens, predictive models could be widely used to indicate the short term risk of biotic damage during the life of the plantation. Hot spots indicated by such predictive risk modelling (and any change in health status detected by remote sensing technologies) could be used to trigger ground-based
investigations, either on a regular basis or when change is detected. The application of the forest health module in CABALA (input from either ground based or remote sensing data) can indicate rotation length impact (currently only in terms of volume) (Battaglia and Sands, 2006). This impact information could be used to guide decisions on intervention (if this is possible in the actual circumstances). Due to the low margins of profit involved in many eucalypt plantations, the high cost of intervention procedures such as aerial spraying and the paucity of other types of environmentally-friendly control strategies available, it is likely that the decision will be to take no action. The latter is most likely in Tasmania where public opinion is hypersensitive to chemical spraying in forestry. However, impact information is important in respect to planning e.g. the rotation length for trees can be extended to compensate in terms of growth for a reduction in volume associated with a previous biotic epidemic.

Tools such as growth models, risk prediction models and remote sensing have been used in many agricultural systems (e.g. Nagarajan et al., 1984; Gamon et al., 1990; Guan and Nutter, 2002; Apan et al., 2004; Koller and Upadhyaya, 2005; Magarey et al., 2005; Beeri and Peled, 2006; Xavier et al., 2006). Apart from the recent adoption of CABALA for site productivity modelling (Battaglia et al., 1999; Battaglia et al., 2004), their use in operational eucalypt plantation forestry has been limited to researchers improving current management strategies and investigating the viability and accuracy of this new methodology in a forest plantation landscape (Battaglia et al., 1999; Candy, 1999; Coops et al., 1999; Coops, 1999; Stone, 2002; Stone et al., 2004; Stone and Coops, 2004; Van Staden et al., 2004; Whittock et al., 2004; Goodwin et al., 2005; Tuominen, 2005; Coops et al., 2006a; Coops et al., 2006b;
One of the primary reasons for not using remote sensing for health surveillance is the cost involved in acquiring imagery with sufficient resolution to indicate fairly subtle changes in crown health (symptoms such as discoloration and defoliation) on a routine basis. With the current and rapid development of the technology for forestry applications, internationally and nationally, a compromise will soon be reached between cost effectiveness and usefulness of information in respect to forest health (Stone, 2002; Stone et al., 2003b; Stone and Coops, 2004; Coops et al., 2006a). In comparison, research in respect to the prediction of damage in eucalypts (at the local site scale) has been limited to the development of ground based monitoring methods of pest presence which trigger action when a certain threshold of presence is observed (e.g. Elek, 1997; Stone et al., 2003a; Loch, 2005, 2006).

5.2 Mycosphaerella Leaf Disease management in the 21st century

This thesis explored Mycosphaerella Leaf Disease as a case study to pioneer the approach to forest health management, specifically in eucalypt plantations, as outlined in section 5.1. Commercially favoured eucalypt species in Tasmania and New Zealand are no longer grown due to the impact of MLD. By reference to a disease with a high potential for damage such as MLD, the outcomes of this thesis have been used to promote the importance of an integrated pest management system to eucalypt plantation growers.
5.2.1 MLD risk prediction

*Predicting MLD in eucalypt plantations.*

Forestry Tasmania has ceased planting *E. globulus* in MLD prone regions of Tasmania and substituted this species with *E. nitens*. Until the risk of damage from MLD and associated impact is better understood they will not plant *E. globulus* at such sites. A site-specific model to predict MLD - such as the prototype model presented in this thesis - could be applied operationally in eucalypt plantation forestry in two ways; i) to avoid establishing plantations at locations categorised as being high MLD sites using as many spore traps as possible or ii) match a species or genotype to site risk e.g. plant *E. nitens* not *E. globulus* on high risk sites or a family of *E. globulus* exhibiting resistance to MLD (e.g. Potts et al., 2004; Milgate et al., 2005).

Once tree crowns of *E. globulus* change into adult foliage (at the approximate age of 3 years) they are markedly more resistant to *M. nubilosa*, and to a lesser degree, resistant to *M. cryptica* (Park, 1988b). A prediction of site risk to avoid planting in areas at high risk to MLD would therefore require inputting meteorological forecast data during the 3 years after planting. If the risk status were low for this time frame this would mean that susceptible juvenile foliage is ‘safe’ from severe infection.

A pre-planting risk prediction would allow managers to implement a pro-active management strategy at establishment such as applying additional fertilizer to MLD prone sites to reduce the risk of damage resulting from a severe epidemic. Improved soil nutrition appears to significantly promote growth in *E. globulus* subject to biotic stress such as shown by the faster recovery rates of artificially defoliated *E. globulus* trees (Pinkard et al., 2006). In the trial at Temma (Chapter 4), trees were evidently
less impacted by MLD when planted in the nutrient rich windrows (where the debris from clearing a plantation is piled) compared to bays in between windrows (Chapter 4).

**An additional** application of a MLD risk model would be at the post planting stage. A risk model could be incorporated as part of existing forest health surveillance programs. Similar surveillance systems are used for sudden oak death in California (Meentemeyer et al., 2004), wheat blight in Argentina (Moschini, 1996) and for predicting the start of the pollen season in Melbourne, Australia for hay fever sufferers (Eng et al., 1997).

Once a high-risk MLD warning has occurred, managers can target their surveillance programs to specific eucalypt plantations e.g. using ground based and/or remote assessment of symptoms (targeted surveillance is currently carried out by helicopters). Using the forest health module in CABALA (Battaglia and Sands, 2006), it is possible, at any particular site, to predict the volume loss associated with a certain percentage of necrosis and defoliation (Pinkard, pers. comm.).

An economic evaluation of this volume loss will dictate whether managers can afford to apply additional fertilizer to reduce the possible effects caused by MLD (Pinkard et al., 2006). It is unlikely that a single MLD epidemic in juvenile eucalypts, whatever its severity, will require intervention - unless it occurs late in a growing season and/or the tree crop is scheduled to be pruned (MLD leads to premature senescence of branches which have to be pruned live for high quality veneer production). However the capacity to predict and offset damage from a subsequent and second epidemic is
very important. This second epidemic could have a significant impact on volume at rotation length in a pulp crop and result in the downgrade to pulp of a crop planned for veneer production.

Progress this thesis has made towards developing a risk prediction model for MLD
This study focussed on the meteorological variables, dictating the presence and magnitude of M. nubilosa atmospheric ascosporic loads. The outer boundaries of meteorological variables for the presence of atmospheric ascospore loads were determined, in addition to diurnal and seasonal atmospheric ascospore patterns. The mathematical descriptions of diurnal and global trends and the effects of meteorological variables on atmospheric ascospore concentrations are the first steps in developing the prototype MLD risk model (Chapter 2). It is acknowledged that this prototype model requires further development to improve the predictive accuracy and validation at the sites of other eucalypt plantations.

As expected from previous authors, the meteorological variables that favour atmospheric ascospore concentrations included daily rainfall events, high relative humidity (>90%) and warm temperatures (5-15°C). These conditions are present in many locations around Australia as indicated by preliminary work that mapped the climatic regions conducive to MLD infection (see Figure 1.1, Chapter 1). Risk prediction at such a broad scale (Figure 1.1) may be useful, however finer scale predictions such as determined by the research in this thesis are necessary for developing a site specific risk model for management at the plantation scale.
Climate conditions conducive to ascospore release were consistently present at the site investigated in this thesis, and ascospores were always observed in the atmosphere. However the daily atmospheric ascospore loads recorded at the trial site were small compared to those reported in the literature, rainfall was low during the two growing seasons and trial trees at the site did not experience a serious defoliating epidemic - although spotting was obvious. *Mycosphaerella* spp. rarely cause high levels of damage in native forest unless this ecosystem suffers disturbance. In an artificial system, such as a eucalypt plantation in a disease prone region, MLD sporadically causes high levels of damage. There appears to be unidentified factor(s) that sporadically weaken the equilibrium between this native pest (MLD) and its host resulting in a severe and defoliating epidemic. The data associated with a low level of MLD can be informatively compared to data sets from eucalypt plantations that have experienced or will experience an epidemic of MLD - especially an epidemic of *M. nubilosa* in *E. globulus* and give an indication of the unidentified factor(s) that sporadically weaken the equilibrium between the two resulting in a severe and defoliating epidemic. Thus despite the low severity of disease, the study described in Chapter 2 provides an extremely valuable data set of meteorological data and associated atmospheric ascospore concentrations.

A sporadic severe epidemic was witnessed in the 2001-2002 growing season with 2 year old trees at the Christmas Hills site (Wardlaw et al., 2006). A comparison of climatic conditions during the 4 growing seasons between 2000 and 2004 at Christmas Hills (Chapter 1; Appendix 4) may explain i) the occurrence of a severe MLD epidemic in the growing season of 2001-2002 and ii) the low severity in the subsequent period of time throughout which the spore trapping was carried out.
Temperature and relative humidity between 2000 and 2004 did not vary in any significant way. These climatic factors always fell within the boundaries of conditions known to be conducive to MLD during the 4 growing seasons under observation.

Patterns of rainfall did however, differ significantly between growing seasons; a high autumn 2001 (March) rainfall at the end of a growing season for a 2 year old plantation (with infected senescent leaves), followed by a high 2001 spring rainfall (September) at the start of the subsequent growing season may explain the blighting epidemic during the 2001-2002 growing season. The trees (those investigated in this thesis) planted in winter 2001 did not experience the same pattern of rainfall coming into their second growing season i.e. both a low pre-season autumn rainfall (during 2001-2002) and a high spring rainfall, only a spring rainfall. These trees did not experience a blighting epidemic.

The unusually high rainfall in the autumn preceding the 2001-2002 epidemic may have increased the number of lesions or latent infections before winter and the exponential effect of high rainfall in the following spring would have further enhanced the effect of the inoculum originating from the autumn infections. This meant that atmospheric ascospore concentrations reached a sufficiently high threshold to cause the blighting epidemic in 2001-2002. Autumn – spring patterns of rainfall will be further explored in relation to other known epidemics at various sites in Tasmania using the set of data associated with low disease levels and presented in this thesis to determine those differences that can indicate the triggers for a *Mycosphaerella* epidemic.
Predictive accuracy for the magnitude of ascospore events could be improved by: collecting atmospheric ascospore concentration and meteorological data from a growing season during a severe MLD epidemic; detailed monitoring of infection including the tagging of leaves as they become infected (the youngest 2-3 pairs of eucalypt leaves are most susceptible to MLD); relating eucalypt leaf phenology at a site to the appearance of symptoms, individual leaf lesions, meteorological data and atmospheric ascospore loads. Given the significance of wetting and drying cycles in ascospore release mechanisms (Cheah, 1977; Beresford, 1978; Cheah and Hartill, 1987; Park and Keane, 1987; Park, 1988), leaf wetness sensors would provide additional information on the effect wetness duration has on atmospheric ascospore concentrations. Such measurements could be used to improve the prototype model through incorporating the rate of evaporation from the canopy and modelling volume of water held in the canopy (Battaglia et al., 2004; Worledge et al., 2005).

5.2.2 Remote sensing technology for assessing MLD symptoms

Spectral characterisation of MLD: from leaf to plantation.

Results from the leaf-based studies in Chapter 3 were fundamental to the successful interpretation of data from Chapter 4. These investigations identified wavelengths highly sensitive to necrosis caused by MLD in *E. globulus* foliage. This work provides signature wavelengths that could be applied to other biotic stresses which exhibit similar symptoms as MLD in eucalypts e.g. crown defoliation caused by insects (Loch and Floyd, 2001).

The models developed at the crown scale successfully differentiated crown defoliation and necrosis caused by MLD. The models were further applied to assess all trees in a
E. globulus plantation. As a result, the different proportions of the plantation in various damage severity categories could be identified.

The results gained from the DMSI on crown health at the E. globulus plantation indicated 78.2% of the plantations trees exhibited 70-100% crown defoliation, and 89.9% had a Mycosphaerella index severity of 20-70%. At the time of the study plantation managers took the decision to downgrade the trees from a solid wood end product to pulp as well as extended the rotation time to allow trees extra volume yield (Wardlaw, Forestry Tasmania, pers. comm.). The information extracted from analysis of the DMSI in this study demonstrates how remotely sensed data can be used to make accurate decisions based on a 100% coverage such as that made at the time of the study. Additionally, the digital nature of DMSI means it can be easily incorporated into existing GIS layers, such as geology, topography and soil. Integrating GIS and remotely sensed data would be advantageous when investigating the possible topographical and edaphic effects on spatial distribution of MLD.

Currently, the application of DMSI as a routine forest health surveillance tool would be prohibited by the cost of image acquisition (AU$8,000 for the study site in Chapter 4) and also the fact that the platform for the camera is a light aircraft. This form of image acquisition on a routine basis is not viable for plantation companies. Although DMSI might be considered economically feasible for targeted one-off assessment of plantations post damage, for quantifying losses, aerial sketch mapping remains the most cost effective tool for routine canopy health assessment (e.g. Stone and Haywood, 2006).
The 0.5 m resolution of the DMSI used in this study successfully detected within and between variation in crown MLD symptoms. Table 5.1 includes specifications and costs of other sensors used for environmental monitoring. If pre-epidemic symptoms of MLD or any other biotic agent (low levels of localised infection symptoms) are present in isolated tree crowns then high spatial resolution (0.5 m) imagery is essential. In comparison, symptoms identifiable at the compartment or tree stand scale would require less resolution, which in turn reduces the cost of the imagery.

Table 1. Spatial and spectral characteristics of some sensors used for environmental monitoring.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Ground resolution</th>
<th>Spectral bands</th>
<th>Wavelength range (nm)</th>
<th>Cost/ha ($)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landsat</td>
<td>30m</td>
<td>9</td>
<td>450-1250</td>
<td>&lt;0.01</td>
<td>Landscape mapping and monitoring</td>
</tr>
<tr>
<td>Hyperion</td>
<td>30m</td>
<td>220</td>
<td>400-2500</td>
<td>0.09</td>
<td>Landscape scale canopy condition modelling</td>
</tr>
<tr>
<td>Compact Airborne Spectrographic Imager (CASII)</td>
<td>80cm-5m</td>
<td>12-78</td>
<td>400-1050</td>
<td>&gt;1.50</td>
<td>Crown scale condition modelling</td>
</tr>
<tr>
<td>Digital Multi-Spectral Imager (DMSI)</td>
<td>&gt;20cm</td>
<td>4</td>
<td>400-1100</td>
<td>0.90</td>
<td>Crown scale condition modelling (e.g. Chapter 4)</td>
</tr>
<tr>
<td>LIDAR</td>
<td>2m pulse spacing</td>
<td>1</td>
<td>NA</td>
<td>&gt;5.00</td>
<td>Elevation modelling</td>
</tr>
<tr>
<td>QuickBird</td>
<td>0.61m</td>
<td>4</td>
<td>450-900</td>
<td>0.13</td>
<td>Landscape land use changes e.g. agriculture, engineering, construction</td>
</tr>
<tr>
<td>SPOT</td>
<td>2.5m-20m</td>
<td>4</td>
<td>500-1750</td>
<td>0.25</td>
<td>Environmental monitoring</td>
</tr>
<tr>
<td>IKONOS</td>
<td>0.8m</td>
<td>4</td>
<td>400-900</td>
<td>0.13</td>
<td>Environmental monitoring e.g. agriculture, forestry, mining</td>
</tr>
<tr>
<td>ASTER</td>
<td>15-90</td>
<td>14</td>
<td>400-900</td>
<td>0.01</td>
<td>Environmental monitoring e.g. geology, soil, hydrology</td>
</tr>
<tr>
<td>Aerial Photography</td>
<td>1:15,000 Scale Compartment, plantation</td>
<td>1</td>
<td>400-750</td>
<td>0.50</td>
<td>High resolution spatial mapping</td>
</tr>
<tr>
<td>Aerial sketch-mapping</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>0.20</td>
<td>Forest health inventory</td>
</tr>
</tbody>
</table>

Note: Prices (per hectare) are based on cost of acquiring and archiving a standard scene in 2007.
For example, in this study soil nutrition effects on tree health were identified in the DMSI with highly foliated trees growing in nutrient rich ash beds of windrows being clearly distinguishable from less foliated trees growing in poor nutrient bays. In such a situation lower resolution imagery (e.g. 30 m pixels using Landsat or Hyperion imagery) could identify the differences between bay and windrow trees and would be cheaper. This information would provide plantation managers with precise locations for fertilizer application to reduce the growth effects of MLD infection. Sensor specifications need to match the type and distribution of damage symptoms to satisfy the requirements of plantation managers as well as be cost effective (e.g. Moskal and Franklin, 2004; Coops et al., 2006a; Wulder et al., 2006a).

Further work required to improve model accuracy

The accuracy of the various reflectance models to predict crown defoliation and Mycosphaerella index severity at the crown scale was moderate and could be improved in a number of ways. Firstly, more accurate indices could be derived using more detailed ground-based crown assessment data e.g. information on crown architecture and discolouration. Secondly alternative crown delineation techniques could be investigated, such as CSIRO’s ‘halo’ technique (Coops et al., 2003a). This method may improve accuracy of signals emitted from eucalypts. Apical flush colour will inevitably confuse the crowns health status signal and the halo method would remove the flush region from the delineation.
Progress this thesis has made to identify MLD using remote sensing in plantation forestry

This study is the first to use remotely sensed data for assessing crown health in a eucalypt plantation. Such a study using high resolution imagery is a prerequisite and lays the foundation for subsequent investigations of alternative sensors (e.g. Hyperion; Pu et al., 2005) that are cheaper and possibly suitable for use in routine forest health monitoring. The next step towards applying this technology is to investigate the balance in cost and resolution to obtain imagery that can identify required target features.

Image acquisition from space can increase the frequency of data capture and data availability and potentially reduce the cost of image collection. For example the satellite born sensor QuickBird has recently proven to produce results similar to those in Chapter 4 (e.g. QuickBird; Coops et al., 2006c) and is cheaper per hectare than DMSI. Many more sensors with similar or better specifications are planned for launch in the near future e.g. WorldView I and II to be launched in late 2007 and early 2008 respectively. The development of this technology and launch of more sensors is likely to increase the adoption of this kind of technology for forest management in future.

In summary the outcomes of this thesis and the lessons learnt will assist forest scientists in achieving standardised, operationally cost-effective, remotely sensed reflectance data, which is spatially explicit and pertains to the physiological status of plantations. Hyperspectral and LIDAR platforms will be combined so that imagery informative of both canopy structure and condition can be simultaneously acquired.
Integration of this digitised information with decision support systems will permit site-specific health ratings, quantification of the impact of any damaging agent, future productivity and improved economic forecasts for resource management. Detection of an early stage of stress may permit intervention to either prevent or offset damage. In Australia the prognosis for operational systems as outlined above is 2-3 years for plantation pines and 5-10 years for plantation eucalypts.
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