MYCOSPHAERELLA LEAF DISEASES OF TEMPERATE EUCALYPTS AROUND THE SOUTHERN PACIFIC RIM*

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ABSTRACT

Research with *Mycosphaerella* spp. on eucalypts has been historically and strongly focused towards taxonomical species descriptions, extension of host, and geographical range. To date there is insufficient information to develop management prescriptions that can be applied operationally.

The research concept we have adopted is an integration of empirical studies (detection, impact, epidemiology, and physiology) and designed experiments that provide a knowledge base from which models can be developed and validated. Our empirical studies and designed experiments form a core response to current industry priorities in Australia.

Keywords: Mycosphaerella leaf disease; disease management tools; *in planta* molecular detection; process-based growth model; host resistance.

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INTRODUCTION

This paper reviews the status of Mycosphaerella leaf diseases on temperate eucalypt species in the south Pacific Rim countries. We describe research based in Tasmania that is being conducted using Mycosphaerella leaf disease as a case study. We aim to provide a knowledge base, a new approach, and tools for developing viable prescriptions for disease management in plantation eucalypts.

TAXONOMY, EPIDEMIOLOGY, AND BIOLOGY

Over 30 species of *Mycosphaerella* have been recorded on eucalypts (Carnegie & Keane 1998; Crous 1998; Dick & Dobbie 2001; Milgate *et al*. 2001; Maxwell *et al*. 2003). Commonly a suite of leaf pathogens is found together in an area although only a small number, particularly *M. nubilosa* (Cooke) Hansf. and *M. cryptica* (Cooke) Hansf., appear responsible for significant damage (Park *et al*. 2000).

Mycosphaerella leaf disease causes loss in photosynthetic area (leaf spotting, shoot blight, and defoliation). It can lead to poor tree form and occasionally death. Damage may be "one-off" or sustained over fairly long periods. It is often difficult to confidently differentiate species of *Mycosphaerella* — especially as more than one can be present on a leaf — based on field symptoms such as the morphology of lesions or the distribution of fruit bodies. A more reliable characteristic is the development of germ tubes during ascospore germination. For example, in *M. cryptica*, germ tubes develop from only one cell of the ascospore, while in *M. nubilosa* germ tubes develop from both cells (Crous 1998). This type of character, however, is of no value for diagnosis in the field.

The only in-depth studies of the epidemiology and biology of Mycosphaerella leaf disease have been for *M. cryptica* and *M. nubilosa* (Cheah 1977; Beresford 1978; Ganapathi 1979; Cheah & Hartill 1987; Park & Keane 1987; Park 1988a, b; Carnegie 2000). Ascospore germination and subsequent leaf infection for *M. cryptica* and *M. nubilosa* have been reported by Park to be optimal under conditions of 5–7 days' leaf wetness at 15–20°C. Ascospore release requires the presence of free water (Beresford 1978; Cheah 1977; Park 1988a). Flushes of new growth appear more susceptible to infection and so disease development also depends partially upon the growth cycle. Severe epidemics are often seen in plantations with trees in juvenile foliage. Significant leaf disease, therefore, develops only in areas that have a climate that provides sufficient periods of leaf wetness and temperatures within the optimum range for leaf infection, lesion development, and spore discharge which coincide with a disease-prone stage of the host.

The areas in the southern Pacific Rim where damage from *Mycosphaerella* spp. has been reported to be severe are:

- **The coastal regions of Chile** where severe (complete) defoliation of young *E. globulus* Labill. has been observed. Surveys have identified a suite of species, including known damaging species such as *M. cryptica* and *M. marksii* Carnegie & Keane, and seven other species of *Mycosphaerella* (six new to Chile) associated with leaf disease symptoms (Ahumada *et al*. 2003). *Mycosphaerella nubilosa* was not reported by Ahumada.
- **The central North Island of New Zealand**. *Mycosphaerella* problems have been reported as serious on those eucalypt species that are no longer widely planted (e.g.,

E. delegatensis R.T.Baker). *Mycosphaerella cryptica* is the major cause of Mycosphaerella leaf disease in New Zealand, although *M. nubilosa* is present (Dick 1982; Dick & Gadgil 1983). *Phaeophleospora eucalypti* (Cooke & Massee) Crous, Ferreira & Sutton is the most significant leaf pathogen at the moment on the main species currently planted (*E. nitens* (Deane & Maiden) Maiden) (Margaret Dick pers. comm.).

- **South-eastern Australia**, where Mycosphaerella leaf disease has been of sporadic concern over the last three decades in the eucalypt plantation estate. In Tasmania severe damage from *M. cryptica* and *M. nubilosa* is concentrated in *E. globulus* plantations in north-west Tasmania where recent expansion of these plantations has exacerbated the problem. *Mycosphaerella tasmaniensis* Crous & M.J.Wingf. (Crous *et al*. 1998) is associated with severe leaf disease and shoot blight of *E. nitens* on cold high-altitude sites. Historically in Victoria severe *Mycosphaerella* epidemics occur in *E. globulus* plantations in Gippsland (Reinoso 1992; Carnegie 2000; Carnegie & Ades 2002). The incidence of *Mycosphaerella* is now increasing in the Green Triangle of western Victoria (and eastern South Australia) following the rapid expansion of *E. globulus* plantations during the late 1990s (Paul Barber pers. comm.).
- **South-western Australia**, where the number of *Mycosphaerella* species associated with Mycosphaerella leaf disease appears to have increased considerably over the past 10 years. Ten years ago studies by Abbott *et al*. (1993) and Carnegie *et al*. (1997) reported the disease to be more severe in regrowth areas of native forests and relatively low in plantation areas. Since Abbott's study in 1993, the *Mycosphaerella* inoculum load has increased and disease, especially that caused by *M. cryptica*, is now prevalent in plantations. Not only has the number of reported species increased, but first records and new descriptions of potentially damaging species (i.e., *M. nubilosa*) are frequent (Maxwell *et al*. 2003).

Mycosphaerella leaf disease is not considered a major health issue in eucalypt plantations elsewhere in Australia. In south-east New South Wales, Mycosphaerella leaf disease causes sporadic problems in *E. globulus* plantations. *Mycosphaerella cryptica* can be a problem on *E. nitens* in the higher altitude areas of New South Wales but is not widespread. *Eucalyptus pilularis* Smith is the host most susceptible to *Mycosphaerella* spp. in northern New South Wales (Angus Carnegie pers. comm.). In Queensland, the sub-tropical hardwood plantation estate is relatively small and in a state of expansion. The immediate and most evident damaging disease agent is the foliar pathogen *Quambalaria pitereka* (J.Walker & Bertus) J.A.Simpson. Mycosphaerella leaf disease does not appear to cause much damage but its presence could constitute a potential threat.

IMPROVED IDENTIFICATION AND DETECTION

Difficulties in taxonomic identifications have led to inaccurate reports of the incidence and impact of different species. The interpretation and, most importantly, comparison of much past research are problematic. Accurate, rapid, and cost-effective molecular tools provide a valuable link in the differentiation of fungal associations and highlight the potential for detecting multiple pathogenic species simultaneously occupying the same niche. Nested PCR technology has recently been adapted to detect five of the most

pathogenic and commonly identified species in Tasmanian *E. globulus* and *E. nitens* plantations (Glen, Langrell, Tommerup, Smith & Mohammed unpubl. data). *Mycosphaerella* spp. can be detected and discriminated in 25- to 200-mm^2 samples of leaf, and in stem slivers with or without visible lesions. Up to five *Mycosphaerella* spp. occurred together, even in small leaf samples, and up to three were found in small samples without macroscopically visible lesions. The detection of *Mycosphaerella* species from lesions which are too immature to produce ascospores also opens up the possibility of using the nested PCR technology to identify species at pre-visual and pre-necrotic stages, and to develop a predictive tool for plantation management months in advance of the current ability to identify Mycosphaerella leaf disease in plantations. The detection technology applied to epidemiological research would greatly enhance the development of accurate early-warning disease-forecasting systems. Whilst there is limited knowledge of the pathogenicity of some *Mycosphaerella* species, the effects of a complex of species within a leaf and within a lesion are unknown and require investigation.

RISK AND IMPACT MODELLING

Near-complete defoliation certainly captures the attention of forest managers but it is the impact of a range of damage levels on growth rates and wood quality that is the key issue in intervention decisions.

There was an attempt to establish *E. globulus* plantations in north-western Tasmania in the late 1970s but planting was discontinued after 2 years because of unacceptably high damage from Mycosphaerella leaf disease (David de Little pers. comm.). The high spring-summer rainfall event that led to severe Mycosphaerella leaf disease in this initial period of plantation development has recently been calculated by Wardlaw as a 1 in 25-year event. This level of risk today would be considered acceptable for short-rotation high-value crops.

The preference for planting *E. globulus* on non-frost-prone sites because of its higher pulp yields led Forestry Tasmania to plant *E. globulus* in 1997 in the Circular Head area of Tasmania instead of *E. nitens* which is less susceptible to Mycosphaerella leaf disease. Assessments of crown damage in 2002 by Wardlaw found severe Mycosphaerella leaf disease (>50% leaf area loss) in nearly 50% of 2-year-old plantations in the Circular Head area. The spring-summer rainfall event of 2002 corresponding with epidemic Mycosphaerella leaf disease was calculated to be a 1:3.3 year event. Forestry Tasmania has ceased planting *E. globulus* in north-western Tasmania until MLD can be managed.

A very small number of empirical studies have been done to measure the impact of Mycosphaerella leaf disease on eucalypt growth. Defoliation levels of 25% were shown to reduce wood volume in *E. nitens* infected with *Mycosphaerella* spp. in South Africa (Lundquist & Purnell 1987). Carnegie *et al*. (1998) maintained that levels of diseased leaf area as low as 10% result in up to a 17% reduction in the height of *E. globulus* plantation trees. Since the first study was not sufficiently similar to the disease syndrome on *E. globulus*, and the second did not measure growth for a sufficiently long period to allow confident predictions of impact, we have recently established two long-term impact trials in Tasmania:

- An exclusion trial is measuring the impact of one or two light epidemic events on the growth of *E. globulus* (between ages 1 and 2);

- Growth plots have been established in two adjacent compartments that suffered contrasting damage. One plot escaped epidemic disease, and the other suffered severe disease (>70% crown loss) during its second growth season.

In the absence of immediate empirical data we have to resort to best-guess predictions using growth models and extrapolated impacts based on empirical data for other pests. More recently we have used process-based growth modelling (Sands *et al*. 2000; Pinkard & Battaglia 2001; Battaglia *et al.* 2002).

The economic performance of different disease and management scenarios was modelled by Wardlaw using stand management software linked to an *E. globulus* growth model based on sites in northern Tasmania (peak mean annual increment (MAI) varying from 22.5 to 32.5) pruned for solid wood, thinned at age 9 years, and harvested at age 22. Two 50% bottom-up defoliation events (at ages 2 and 3) were predicted to reduce net present value (NPV) from $1099 to $60/ha on high-quality sites targeted for sawlog regimes.

While a reduction in tree growth is the usual consequence of loss of leaf area, the initial responses affect basic physiological processes in the host. Photosynthetic responses are likely to vary between defoliation due to insects, to pruning, or to leaf fungal infection. Defoliation events in *E. globulus* (pruning / simulated insect defoliation) result in increased photosynthetic response in residual foliage (Elek 1997; Pinkard *et al.* 1998). However, in preliminary investigations, no compensatory response has been measured in leaves that have lost functional leaf area due to necrotic lesions caused by *Mycosphaerella* spp. (Pinkard unpubl. data). These differences in response are likely to affect source/sink relationships and influence the partitioning of biomass. The most recent dynamic growth model, CABALA, is particularly well suited to investigating the effects of diseases on growth (Battaglia pers. comm.). Biomass allocation is linked strongly to the extent to which resources limit growth, and the biomass of roots and foliage determines the rate at which these resources can be acquired. The model predicts a number of stand components other than wood volume, such as leaf area index, crown length, and the distribution of tree sizes. These can be used to measure the impact of disease incidence. Furthermore the linkages between capture of resources and the processes of allocation mean that the model has the capacity to deal with or be adapted to the impact of disease on tree function. We have used CABALA to model the effect of a 50% bottom-up defoliation of a 50-ha plantation on a high-quality site in western Australia (MAI 31.5 m^3/ha) grown for pulpwood on a 10-year rotation. A single defoliation event decreased the value at harvest by $424/ha. We are also applying CABALA to calculating leaf wetness ("water-holding capacity" of the canopy) to better predict risk on any one site.

DISEASE ASSESSMENT

In a multi-disciplinary research programme such as the one we are conducting it is essential that there is a common understanding and language to describe and measure crown damage — that caused by *Mycosphaerella* spp. and by other biotic damaging agents.

The description of damage must be accurate and must include quantitative and qualitative assessment of the type of damage (defoliation, necrotic lesions), location in the crown (e.g., top-down, bottom-up), and temporal processes (blighting *versus* senescence responses). Visual standards have been developed to provide precise and repeatable

measures of such damage. Assessment has to be carried out at both tree and stand levels. A group of forest pathologists and entomologists in Australia are, as a matter of priority, promoting national standards for the assessment of a crown damage index (CDI) on young eucalypts (Stone et al. 2000, 2001; Stone, Matsuki, & Carnegie, 2003; Stone, Wardlaw, Floyd, Carnegie, Wylie & Little 2003). The CDI is a single value, which represents the total amount of damage present on a tree. The three general categories to calculate this value are considered to be:

(1) necrosis,
(2) discoloration, and
(3) defoliation.

However, in more specialised assessments these can be split into specific categories. For example, if a company was concerned about spread of Mycosphaerella leaf disease, and the field staff could easily differentiate the symptoms, we could separate the necrosis measurement into two scores (Mycosphaerella leaf disease necrosis, and other necrosis) without compromising the final CDI score. For each category an incidence (percentage of leaves affected in the tree crown) and a severity (percentage of leaf area damaged on affected leaves) are estimated. A detailed Mycosphaerella leaf disease visual standard (developed by Smith *et al.* in prep.) is used to obtain more accurate results (Stone, Matsuki, & Carnegie 2003).

We have tested the accuracy, repeatability, and subjectivity of assessing Mycosphaerella leaf disease and other damage at tree level to ensure the reliability, objectivity, and repeatability of the crown damage index method (Smith *et al.* in prep.). Nine assessors, with varying levels of experience, estimated damage on three plots of 50 trees each to obtain an understanding of the subjectivity of assessing damage caused by insects and fungal pathogens (e.g., *Mycosphaerella* spp.). The repeatability of estimates by the same assessor was determined by estimating damage in the same plot of 50 trees in the morning and in the afternoon. Information on the accuracy of estimates was achieved by destructively sampling nine of the assessed *E. globulus* and measuring damage levels. The most experienced assessors provided the most repeatable estimates and were generally the most accurate. The incidence of foliar necrosis was the least subjective measure, while defoliation was the most subjective and the least accurate of the indices measured. All assessors, regardless of experience, were able to predict the crown damage index to within 12%. Software that provides diagnostic assistance, training, quality assurance, and standardisation for forest health research projects and surveys in sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), black cherry (*Prunus serotinia* Ehrh.), white oak (*Quercus alba* L.), and northern red oak (*Quercus rubra* L.) has been developed in Canada (Nash *et al.* 1992). We recommend that similar assessment-training software be developed for application to the health assessment of eucalypts.

While field inspections enable the accurate identification of the damaging agents, the ability to objectively quantify the spatial extent of damage and the impact on stand productivity is more limited. In order to develop our decision-support models that provide management with options to respond to changes in plantation health, there is a need for quantitative data on tree health and condition. The application of high-resolution, multi-spectral, reflectance imagery offers a means to obtain spatially-explicit data on the physiological status of plantations (Martin & Aber 1996).

The integration of digitised canopy condition coverage with other physical and environmental GIS layers presents the real possibility of spatial modelling of site-specific classification and health risk ratings (Stone, Wardlaw, Floyd, Carnegie, Wylie & Little 2003). We are currently investigating the ability and operational feasibility of remote sensing to determine Mycosphaerella leaf disease severity levels and the physiological status of diseased trees.

CONTROL OPTIONS

Having predicted a high risk of recurrent epidemic disease and substantial economic losses, the next challenge is to identify possible approaches to manage the disease. The range of options in forestry is no different from agriculture but there are significant constraints in their practical implementation.

Exclusion / Eradication

Exclusion / eradication is impractical — significant *Mycosphaerella* pathogens are already widely dispersed. New introductions in Australia would be exceedingly difficult to detect and map.

Chemical

Fungicides are useful to control Mycosphaerella leaf disease in the nursery (Dick & Gadgil 1983; Carnegie 2000) but are not an environmentally or economically viable option in large-scale plantations. For experimental purposes, exclusion in the field can be achieved, weather and location permitting, with 2- to 3-weekly spraying of a common fungicide such as benomyl. Preliminary trials by the Forest Research Institute, New Zealand, found that two relatively new fungicides (Flusilazole and Azoxystrobin) showed promise for the control of *M. cryptica* on 2- to 3-year-old *E. nitens* (Dick pers. comm.).

Included in the trials at the Forest Research Institute was the environmentally benign, chemical, plant-defence activator phosphonate which has been sprayed aerially in National Parks and World Heritage areas to control *Phytophthora* spp. If effective, but non-phytotoxic, levels can be applied then this chemical, or similar plant defence activators such as Benzothiadiazol (Bion®), could offer protection for a considerable period of time as they operate by inducing host resistance.

Resistance

There are some clear prospects with inter-specific differences already widely used on high-risk sites (e.g., the use of *E. nitens* in north-western Tasmania). Intra-specific differences exist but have yet to be exploited operationally.

We examined the quantitative genetic variation in susceptibility to infection by *M. nubilosa* in a genetically diverse population of *E. globulus* families growing in a field trial in north-western Tasmania. The trees were 2 years old and still in the juvenile foliage stage when a heavy epidemic of *M. nubilosa* occurred. Disease incidence and severity were assessed on juvenile foliage. Disease incidence was uniform across the trial, and the mean leaf area damage was very high at 34%. Significant genetic variation in susceptibility was

detected, with a narrow-sense heritability of disease severity (0.6) being the highest yet reported for Mycosphaerella leaf disease of eucalypts. We followed the effects of this disease outbreak on growth up to age 7 years and found that *M. nubilosa* damage had a significantly deleterious impact on tree growth at both the phenotypic and genetic levels. At age 7, the top 10% of families had a mean diameter at breast height 20.8% greater than the trial mean. Approximately half of this gain would have been achieved by early selection for disease resistance (9.1%) or height (11.0%) at age 2, with a time advantage of 5 years. This is similar to the selection of above-average families. It is likely such gains would be reduced in homogeneous plantings of resistant genotypes, or if genotype × environment interactions are significant. Nevertheless, a large component of this gain is likely to be due to disease resistance per se, and collection of seed from resistant seed orchard parents offers the potential for rapid gains in productivity in plantations at risk of disease.

Silviculture

Silvicultural treatments are considered to offer good prospects for control of Mycosphaerella leaf disease — they are operationally feasible and potentially economically viable. However, their effectiveness needs to be demonstrated experimentally.

There is some evidence that better nutrition may help to prevent or offset the effects of biotic damage (e.g., Stone & Birk 2001; Stone 1993; Carnegie & Ades 2002) but this is far from proven and the mechanisms behind such a response are not known. There is still much debate associated with the outcomes of nutritional stress and insect herbivory. Silvicultural treatments intended to improve tree vigour may also directly influence herbivorous populations, either positively or negatively. Sap-sucking insects, aphids in particular, show a positive response to nitrogen fertiliser. However, fertiliser application is perhaps the silvicultural option with most promise in countering damage associated with foliar pathogens or insect defoliation. It is well known that fertiliser treatment, in the absence of other site limitations, promotes crown development, an option largely untested for reducing the impact of fungal infections and chewing insects. Any additional resistance or accelerated onset of more-resistant adult foliage is a bonus where Mycosphaerella leaf disease is concerned.

On sites with sub-optimal soil nutrient reserves we find different responses to Mycosphaerella leaf disease that suggest early secondary fertiliser application may be a useful management tool. Height and diameter growth of trees planted in windrows is insensitive to the severity of disease, whereas trees planted in the bays between windrows record significant reductions in height increment as disease severity increases. Trees planted in windrows do not appear to display premature senescence of spotted leaves.

There is little known about the physiological effects of plant nutrition on host response to leaf infection and defoliation. A better understanding of the role of nutrition in such responses may provide operationally feasible cultural controls for use in young plantations.

CONCLUSIONS

The traditional approach using empirical studies can predict (realistically) only within the same range of conditions. Our studies on Mycosphaerella leaf disease on *Eucalyptus*

globulus are being linked with a process-based model called CABALA being developed by the Co-operative Research Centre for Sustainable Production Forestry in Hobart. This allows the effect of damage on tree physiology to be linked with growth responses. Using Mycosphaerella leaf disease as a case study will, we hope, allow realistic scenario-building with the potential to more reliably predict the impact of similar damage caused by other pests and pathogens and the effect of modifying silvicultural prescriptions, without the need to resort to individual empirical studies.

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