Abstract

Tuart (*Eucalyptus gomphocephala*) is a woodland tree endemic to the coastal plain of south-western Western Australia (WA). However, it has been undergoing a severe health decline since the early 1990's, confined to Yalgorup National Park, but like many eucalypt species that occur in native forests, little is known about the foliar fungi infecting these trees. The genus *Mycosphaerella* contains some of the most widespread and destructive foliar pathogens of eucalypts, including *M. cryptica* and *M. nubilosa*, both of which have been largely responsible for disease epidemics in Australia. This study examines the potential contribution that *Mycosphaerella* species have in the decline of *E. gomphocephala* (tuart) in south-western WA, and examines the accuracy of standard methods used for the identification of species based on germination patterns. A method for the successful production of ascospores of *M. cryptica* in culture was also examined.

Three survey sites along the natural distribution of tuart, ranging from Yanchep National Park in the north to Ludlow Forest in the south and including Yalgorup National Park were surveyed for the incidence and severity of Mycosphaerella Leaf Blotch (MLB). *Mycosphaerella cryptica* was the only species isolated at all of the sites surveyed. Yalgorup National Park recorded the highest incidence and severity ratings of the three sites surveyed, with Ludlow Forest recording the lowest incidence and severity ratings. There have been previously no published accounts of *Mycosphaerella* species occurring on tuart in native forests of WA.

Pathogenicity trials were then carried out on the *M. nubilosa* and *M. cryptica* to investigate infection processes and subsequent disease development. Three methods of inoculation were used; Experiment 1 spore inoculation on intact plants, Experiment 2 lesion inoculation on intact plants, and Experiment 3 spore inoculation on excised leaves. *Mycosphaerella cryptica* was shown to infect adult and juvenile tuart leaves through direct penetration of the epidermal layer or via

stomatal openings. This species will begin to germinate on leaves up to 8 days following inoculation and will produce appressoria and infect leaves 13 days after inoculation. This is the first study to examine how *M. cryptica* infects tuart. *Mycosphaerella nubilosa* was not found to infect tuart. In order to examine infection processes with accuracy and reliability an attempt was made to examine the ability of *Mycosphaerella* species to produce ascospores *in vitro*. Nine different agar media were used as substrates for *M. cryptica* and *M. nubilosa*. *Mycosphaerella cryptica* produced ascospores *in vitro* on Eucalyptus Agar (EA) after 6 weeks of incubation at 20°C. Initially pseudothecial initials were produced on EA after 4 weeks, when these were then stressed by cutting up the culture with a sterile scalpel; ascospores were produced 4 weeks later. This is believed to be the first account of *Mycosphaerella* species producing sexual spores in culture. This finding has huge potential for future studies to increase our understanding of how *M. cryptica* and other *Mycosphaerella* species infect eucalypts and cause disease.

The identification of *Mycosphaerella* species has previously relied heavily upon characters such as ascospore morphology and the mode of ascospore germination. However, there is wide overlap and variation among these characters, which can lead to morphologically similar species being identified as the same. The accuracy of spore germination patterns as a reliable identification tool was examined. Germinating ascospores of 2 known and 2 unknown but provisionally named species of *Mycosphaerella*; *M. cryptica, M. nubilosa, M. pseudonubilosa* prov. nom and *M. tumulus* prov. nom. were examined every 6 hours over a 48 hour period to provide a detailed description of their germination patterns. *Mycosphaerella cryptica* and *M. pseudonubilosa* prov. nom continuously released ascospores over 48 hours which would allow for greater dispersal under ideal environmental conditions. Germ tube growth rate was shown to influence the identification of the isolates based on germination patterns. *Mycosphaerella tumulus* prov. nom. grew very rapidly, which caused

the ascospores to become distorted and so cloud germination patterns possibly leading to incorrect identification.

Mycosphaerella nubilosa germinates from both ends, with germ tubes growing perpendicular to the long axis of the spore. Ascospores are discharged within the first 6 hours, with germination beginning during the following 6 hours. *Mycosphaerella pseudonubilosa* prov. nom. also germinates from both ends with germ tubes growing perpendicular to the long axis of the spore. In contrast to *M. nubilosa, M. pseudonubilosa* prov. nom. discharges ascospores after 24 hours, with germination beginning in the following 6 hours. Initially there is a similar germination pattern between the two species, however after 18 hours the differences become apparent with *M. pseudonubilosa* prov. nom. developing lateral germ tubes while *M. nubilosa* does not.

This study has shown that *M. cryptica* is widespread in the tuart forest and is a potential contributor to the decline of regenerating tuart and highlights the need for further studies on *Mycosphaerella* species and their impact in native forests of Western Australia. This study also showed the importance of monitoring the timing and duration of spore release, germination, and germination patterns over time when identifying *Mycosphaerella* species based on spore germination patterns alone. In future studies, it is recommended that a 'new' standard be put in place to ensure that germination patterns are examined at specific time periods. This study has also shown that ascospores can be produced *in vitro* by *Mycosphaerella* species, which has significant implications for the production of pure ascospore inoculum for use during infection studies and pathogenicity trials.