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SPORULATING MYCELIUM OF *DAVIDSONIELLA AUSTRALIS* ON THE BARK OF *NOTHOFAGUS CUNNINGHAMII*, AND ROLE AS INOCULUM FOR NEW INFECTIONS

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(with three tables and one plate)

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Sporulating mycelial mats on the bark of lower stems are a common and notable sign of *Davidsoniella australis* infection of *Nothofagus cunninghamii* in cool temperate rainforest in Tasmania and Victoria. Inoculation studies indicate viable conidia from mats may be present in the rainforest during most of the year. Air- or water-borne conidia from sporulating mats and in the frass of the ambrosia beetle *Platypus subgranosus* that attacks infected trees, is the likely source of new infections in *N. cunninghamii*.

Key Words: *Davidsoniella australis, Nothofagus cunninghamii*, mycelial mats, inoculum.

INTRODUCTION

Davidsoniella australis (J. Walker & Kile) Z.W. de Beer, T. A. Duong & M.J. Wingf. (Basionym *Chalara australis* J. Walker & Kile) causes mortality of the cool temperate rainforest tree *Nothofagus cunninghamii* (Hook.) Oerst. (Myrtle Beech) in Tasmania and Victoria (Kile & Walker 1987). Cumulative tree mortality ranged from 9.4% to 53.4% in the stands surveyed by Elliott *et al*. (1987). The disease symptoms in individual infected trees include wilting of the crowns and dark brown discolouration of the outer wood of the mid- to lower stems and roots. The disease is regarded as an important element in rainforest regeneration and replacement (Packham *et al*. 2008).

D. australis may develop on the outer bark of the lower stems of infected trees initially as patches of dark grey to black sporulating mycelium (hereafter called mycelial mats) external to the underlying infected xylem tissue (Kile & Walker 1987). After sporulation, the residual black mycelium remains evident on the bark. Mycelial mats may also develop on the cut surfaces of recently infected trees or on other freshly exposed wood surfaces in the forest (Kile & Walker 1987, Kile 1989).

The ambrosia beetle *Platypus subgranosus* Schedl attacks the stems and exposed roots of infected trees although it is not a vector of the pathogen (Kile & Hall 1988). Viable conidia of *D. australis* occur in the frass of *P. subgranosus* from infected trees as the fungus sporulates in the beetle tunnels in infected wood (Kile & Hall 1988), and frass is also commonly dispersed over the mycelial mats. Wounds to *N. cunninghamii* stems from falling trees and branches are the probable infection courts (Kile & Walker 1987, Kile & Hall 1988, Kile *et al.*1989).

Mycelial mats are the most conspicuous reproductive stage of the fungus in nature and hence a potential source of inoculum for new infections. The incidence of mycelial mats and their potential as an inoculum source was assessed on naturally infected and artificially inoculated *N. cunninghamii* in Tasmania.

MATERIALS AND METHODS

The presence or absence of mycelial mats on *N. cunninghamii* naturally infected by *D. australis* was assessed as part of the survey of disease occurrence in relation to cool temperate rainforest subtypes and site and stand parameters undertaken by Elliott *et al*. (1987). The seasonal pattern of mycelial mat development was studied by inoculating 40-year-old trees of *N. cunninghamii* (tree age based on fire records) with *D. australis* in a stand in the Arve Valley, near Geeveston, Tasmania. Ten trees were inoculated once in autumn, winter and spring and summer by injecting 0.1 ml of conidial suspension (approximately ($5x10^6$ conidia per ml) into 3-mm-diameter by 25-mm-deep radial holes drilled around the stem circumference (one hole per cm of stem circumference) at 50 cm above ground level. The *D. australis* isolate (DAR 50148) was from an infected tree in a nearby location and the conidial suspension was prepared as described in Kile & Walker (1987).

Mycelial mat development was assessed on inoculated trees at 2–4-week intervals for six months following inoculation and at more irregular intervals for 6–18 months after inoculation. The maximum height above the inoculation zone at which mats developed and the proportion of the stem circumference around which mats developed was measured and the area of stem on which mats developed estimated in 25% of classes.

The viability of conidia from mycelial mats on inoculated trees was tested by taking bark samples (approx. 2 x 1 cm) from the margin of the mat. A small section was examined microscopically to assess for the presence of phialides and

conidia. The residual material was placed in 5 ml of a 3% malt extract solution in a vial and rubbed with a glass rod to disperse conidia. After incubation at 20°C for 15–18 hours, the first 50–100 conidia encountered were scored for germination at 200x magnification. The rod-shaped conidia of *D. australis* were morphologically relatively distinctive from the spores of other organisms that contaminated ageing mats. Where possible the same mats, or mats of approximately the same age and condition, were used for repeat sampling.

RESULTS

Mycelial mats occurred on 38% of *D. australis*-killed trees estimated to have been dead less than three years in the survey of Elliott *et al.* (1987). The extent of mats varied but the majority developed on the lower stems (up to 1.0–1.5 m above ground level) and exposed roots but sometimes occurred up to 3–4 m above ground level (pl. 1). The proportion of trees with mats was independent of tree diameter (table 1) but there was a significant orientation effect with a greater number of trees having the major extent of mats on the south side of the stems ($X^2 = 45.5$, $p < .001$) irrespective of tree diameter class. There was no significant difference in mat occurrence between the rainforest subtypes surveyed by Elliott *et al.* (1987).

Mycelial mats were frequent and extensive on artificially inoculated trees and were typically initiated around the full circumferential inoculation zone but later developed above and below that zone. Mat development showed a seasonal pattern with the most extensive and protracted development occurring on autumn-inoculated trees (table 2). The time to mat initiation was similar for autumn and spring inoculations and marginally longer in winter and summer.

The pattern of mycelial mat development was similar for all seasons of inoculation: an incubation period (7–12 weeks); mat initiation (usually on still living trees); expansion and cessation of mat development; some further development of mats during the next autumn period particularly after summer inoculation (table 2). Height of mat development on stems was less than the height of *D. australis* discolouration within the stems shown when dead trees were felled and crosscut. There was no orientation effect on mat development in the inoculated trees.

Newly developed mycelial mats emitted the characteristic iso-butyl acetate aroma of *D. australis* cultures (Kile *et al.* 1992). The aroma was relatively transient being detectable by nose in the first 1–3 weeks of mat initiation and expansion and most notable following autumn inoculation given the extensive mat development (table 2). No insect species were consistently associated with mats. On some trees that died following autumn, winter and spring inoculation, *P. subgranosus* attack occurred on the stems in the following summer but this was not specifically associated with mats.

PLATE 1 – Black mycelial mats of *Davidsoniella australis* and frass of *Platypus subgranosus* on the exposed roots and stem of naurally infected *Nothofagus cunninghamii*. (Photo: CSIRO Inc.)

TABLE 1 – Occurrence and major orientation of mycelial mats of *Davidsoniella australis* on the stems of 190 *Nothofagus cunninghamii* estimated to have been dead less than three years in 16 rainforest stands in Tasmania

Diameter range (cm)	No. trees	Percentage with mats	North*	East	South	West
15–35	50	32	1	2	13	0
36–55	49	49	4	3	15	2
56–75	37	43	4	1	9	2
76–95	25	36	1	5	3	0
96–115	18	22	1	1	1	1
> 115	11	57	2	0	2	0
Totals	190	$\bar{x}$=38	13	12	43	5

* Orientation of the major occurrence of mats.

Microscopic examination of mycelial mat samples showed abundant phialophores and conidia of *D. australis* on newly formed mats and especially around the margins of expanding mats. Numbers of phialophores and conidia diminished with ageing of the mats and exposure to rainfall and contamination by other microorganisms in the forest.

TABLE 2 — Mycelial mat development on the stems of *Nothofagus cunninghamii* inoculated with *Davidsoniella australis*

Season of inoculation	No trees with mats	Max. height of mats(cm) above inoculation zone	Estimated mat cover (%)*	Weeks to mat initiation	Main mat development period	No. trees killed	Ave. weeks to tree death
Autumn (March 1984)	9/10	68	50–75	7–10	April – Aug.	10	19 (12–41)
Winter (July 1984)	6/10	15	< 25	9–12	Sept. – Oct.	10	18 (17–26)
Spring (Nov. 1984)	6/10	41	< 25	6–9	Dec. – mid-Jan.	10	11 (10–14)
Summer (Jan. 1985)	6/10	33	< 25	9–11	March- April and May to July	8	11 (6–11)

Note: Mean tree height 11.2 m, mean diameter at 1.3 m over bark 8.9 cm.
* Visual estimate of stem area with mats between the inoculation zone 50 cm above ground level and the maximum height of mats.

TABLE 3 – Average percentage germination of *Davidsoniella australis* conidia from mycelial mats initiated in different seasons on inoculated trees of *Nothofagus cunninghamii*

Season	May 1984	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan. 1985	Feb.	Mar.	Apr.	May	June
Autumn (n=5*) (March)	52# (14–74)	56 (16–94)	8 (1–15)	3 (0–12)	14 (0–46)	2.4 (0–8)	0							
Winter (n=2) (July)						27 (8–46)	5 (3–6)	0						
Spring (n=3) (Nov.)									28 (26–30)	8 (4–11)	2 (1–2)	0		
Summer (n=3) (Jan.)												13 (13)	22 (14–30)	6 (3–12)

* Number of trees from which mycelial mats were sampled.
#Average and range in the samples tested.

Viable conidia were present on mycelial mats formed after inoculation in all seasons (table 3) but with the natural exposure of mats, variability in the number of conidia and germination rate was very high and not amenable to statistical analysis. Viable conidia were present on mats formed following autumn inoculation for up to six months but the number and viability of conidia on mats formed after inoculation in other seasons declined rapidly (table 3).

DISCUSSION

The occurrence of mycelial mats of *D. australis* on naturally infected *N. cunninghamii* is concentrated on the lower stems and exposed roots and on the more shaded southern sides of the trees. Mats are a notable feature of the disease. The generally more profuse and more even circumferential development of mats on the inoculated trees probably results from the large well-distributed inoculum dose killing large volumes of host tissue relatively quickly.

In seasonal terms the most extensive and most protracted mycelial mat formation on inoculated trees occurred after autumn inoculation. Summer-inoculated trees also showed some further mat development during autumn. This suggests that temperature and moisture conditions during the autumn–early winter period were most conducive to growth of the pathogen through to the external surface of the bark from the infected tissue beneath. This contrasts with winter inoculation when time to tree death was similar to autumn and bark wetness would frequently be high but temperatures lower. Spring and summer inoculation resulted in the most rapid tree death, but it is likely higher temperatures and more rapid drying of the bark restricted mat formation.

Other pathogenic species that cause similar vascular staining and tree wilt symptoms as *D. australis* may form

localised mycelial mats on infected trees or cut surfaces of infected tissue (see illustrated examples for *Ceratocystis fimbriata* in CABI (2020) and *C. albifundis* M. J. Morris, de Beer, M.J. Wingf. in Roux *et al*. 2007 and de Beer *et al*. 2014). *Bretziella fagacearum* (Bretz) Z.W. de Beer, Marinc. T.A. Duong & M.J. Wingf., the cause of oak wilt, forms distinctive mycelial mats beneath the bark of infected trees that leads to bark cracking and spore dispersal (Curl *et al.*1953). *D. australis* appears unique as an endemic pathogen in natural forests where mat formation is common and sometimes extensive on the bark of infected trees.

Mycelial mats produced viable conidia although the number and viability declined quickly for spring, winter and summer inoculation. In autumn, the ongoing development of mats over several months resulted in higher conidial viability for longer, although variability was high due to the exposure to the natural environment. The seasonal pattern of mat development in natural forest is unknown but in recently infected trees could be expected to respond to the seemingly favourable environmental conditions in the autumn–early winter evident in the artificially inoculated trees.

Mycelial mats are the most conspicuous reproductive phase of *D. australis* known in nature, although viable conidia are found particularly in the adult frass from *P. subgranosus* attack in infected trees (Kile & Hall 1988). These conidia come from sporulation of the fungus in the beetle tunnels in the host with conidia expelled with the frass but also potentially frass contact with sporulating mats. The results from artificial inoculation and frass studies (Kile & Hall 1988) suggest the potential for viable conidia of *D. australis* to be present in rainforest for much of the year from mats and frass, the quantum depending on the number and status of infected and dying trees. Conidia and ascospores of species such as *B. fagacearum* and *Hymenoscyphus fraxineus* (T. Kowalski) Baral, Queloz, Hosoya are dispersed by wind (Curl 1955, Timmermann *et al*. 2011). Given the often wet and windy environment of the Tasmanian rainforest, both wind and water are likely to be important for *D. australis* dispersal.

Infection of fresh billets of stem wood of *N. cunninghamii* exposed in the rainforest occurred at any time of the year although with a winter minimum and summer–autumn peak (Kile *et al.*1989). This suggests a maximum inoculum load during spring to autumn, potentially from a combination of conidia from mats and from frass. It is impossible to readily determine the contribution of conidia disseminated from mats and conidia transferred with frass to the initiation of new infections. However, not all trees produce mycelial mats but all infected trees are attacked by *P subgranosus.* Given fresh frass is likely readily carried in air or water, transfer of conidia in frass may be the more important source of inoculum for new infections. In this respect the disease cycle appears similar to that of the wilt disease of Cacao *Theobroma cacao* L. caused by *Cerotocystis cacaofunesta* Engelbrecht & Harrington where pathogen-contaminated frass was considered the main means of spread but where, unlike *D. australis*, there was little external sporulation of the pathogen (Iton 1960).

Davidsoniella australis spreads below ground via root grafts leading to the development of clumps of dead and dying trees (Elliott *et al.* 1987, Packham 1994). The ready infection through the seasons of fresh exposed sections of *N. cunninghamii* in rainforest (Kile *et al.*1989), the natural infection in different seasons of artificial wounds in *N. cunninghamii* (Kile & Walker 1987, Packham 1994) and the rapid development of disease in disturbed areas (logging, thinning, roading) of *N. cunninghamii*-rich rainforest where the likelihood of wounding of the residual or bordering *N. cunninghamii* is increased (Packham 1994, Elliott *et al*. 2005), indicates suitable tree wounds and air- or water-borne inoculum from mycelial mats and frass is the source of new above-ground infections of *N. cunninghamii* in the rainforest.

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