Spore germination and early gametophyte development of the soft tree fern *Dicksonia antarctica*

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Abstract

The influence of different organic substrates on germination and early stages of gametophyte development of Dicksonia antarctica *was investigated. All substrates tested (agar, burnt soil, unburnt soil,* D. antarctica *trunk and decaying log) supported 100% germination of spores after 14 days. Variation in germination rates between substrates was reduced when substrates were overlaid with filter paper, which removed differences in water availability. Gametophyte development after 10 weeks was further advanced on soil (both burnt and unburnt) than on tree fern trunk or decaying log. Substrate conditions such as moisture availability, rather than the nature of the substrate itself, may limit gametophyte development in* D. antarctica.

Introduction

Dicksonia antarctica (man fern, soft tree fern; Photo 1) is the most abundant and widespread tree fern found in Tasmania (Garrett 1996). It is an ecologically important understorey species of rainforest and wet eucalypt forests (including wet sclerophyll forest and mixed forest). It is a robust fern that can tolerate fire and reshoot after burning, often attaining ages greater than 300 years (Mueck *et al.* 1996). Its fibrous trunk provides a suitable microhabitat for many epiphytes (e.g. Peacock 1994; Ough and Murphy 1996; Ford and Gibson 2000; Ough 2001; Ough and Murphy 2004; Roberts *et al.* 2005) and acts as a nursery site for the establishment of some tree species (Ough and Murphy 2004).

Dicksonia antarctica is a valuable horticultural product. Each year tens of thousands of *D. antarctica* stems are harvested from Tasmania's native forest and exported nationally and internationally. As the future of the tree-fern harvesting industry will increasingly rely on areas where native forest silviculture is applied (including second and subsequent timber-harvesting rotations in regrowth forest), it is important to know what factors influence the recruitment of *D. antarctica* from spore, as this recruitment will be important for sustainable harvesting of the species in the future.

Many factors, such as water, temperature and light, influence the germination of fern spores in natural habitats (Page 1979; Neyland 1986). The relative roles of each of these factors cannot be separated practically in the field. In this paper, we examine the early stages of development of *D. antarctica* on five organic substrates under controlled light and temperature regimes, and assess how the physical differences between substrates influence germination and gametophyte development.

Photo 1. Mature plants of Dicksonia antarctica *(manfern, soft tree fern).* Dicksonia *occurs naturally in the understorey of wet eucalypt forest and rainforest, and in damp gullies in drier forest types.*

Methods

Spore collection

Whole fronds from individuals of *D. antarctica* were collected from native forest sites in the Florentine Forest Block in central Tasmania. The fronds were stored on clean paper in a dry environment with a constant temperature of 18°C until the sporangia opened. Spores were collected from the paper and stored in cool, dry conditions for no more than a week before use.

Substrate collection and preparation

Unburnt soil, a partially decayed log (*Eucalyptus regnans*) and a *D. antarctica* trunk were collected from mixed eucalypt forest from the Florentine Forest Block. Burnt soil was collected from a logging coupe immediately following a regeneration burn in the same region and forest type. Each organic substrate was air-dried then mixed with distilled water using substrate to water weight ratios of 50:50 for burnt soil and unburnt soil, and 25:75 for decayed log and *D. antarctica* trunk, then placed in a Petri

dish. These ratios followed those used by Duncan and Dalton (1982) for germinating moss spores, with higher water to substrate ratios for the decayed log and *D. antarctica* trunk as these rough-textured substrates had a lower water-holding capacity than burnt and unburnt soil. The burnt and unburnt soil had a fine texture and covered the Petri dish, leaving a flat, even surface that held water. The *D. antarctica* trunk and the decayed log had a rough texture, resulting in a patchy distribution of water across the surface.

From previous work (Duncan and Dalton 1982), it was known that high rates of spore germination could be achieved using a plain agar substrate. To establish a standard against which to compare rates of spore germination on the other substrates, a set of Petri dishes was prepared using a plain agar substrate. Desmid agar was made from stock solutions of:

0.1 g $MgSO_4.7H_2O$ in 100 ml H_2O,
0.1 g K_2HPO_4 in 100 ml H_2O, and
1.0 g KNO_3 in 100 ml H_2O.

10 ml of each stock solution were added to 970 ml of water and 8 g of agar. The mixture was then autoclaved at 121°C for 20 minutes and poured into plates. When cool, sufficient water was added to just cover the agar.

Spore germination

Ten replicate, sterile, 60 mm Petri dishes were used for each substrate (desmid agar, unburnt soil, recently burnt soil, partially decayed log, and trunk of *D. antarctica*). To test the influence of substrate texture on spore germination, prepared substrate was added to each Petri dish (except the agar plates) to a depth of 5 mm. Spores were applied directly and evenly to the surface of the substrate or agar using a fine-tipped brush for five replicates of each, while a wet piece of 45 μm Millipore filter paper was placed on top of the substrate or agar for the remaining five replicates, then spores spread on top of the filter paper using a brush. The filter paper eliminated the variability of texture between substrates without altering water availability. Each Petri dish was sealed using a fitted lid and wax paper to prevent drying out. No water was added during the experiment.

Spores were incubated in a growth cabinet (12 h of fluorescent light at 80 micromols/m^2/sec at 18°C; 12 h dark at 10°C). These conditions were chosen as representative of Tasmania's wet forests, despite evidence that fern spores prefer high light levels and long day lengths for germination (Garrett, cited in Neyland 1986). Two hundred spores were randomly examined each day from each plate. Germination was measured until 100% germination was reached. A spore was considered germinated when the first cells became visible.

Gametophyte development

Ten replicate Petri dishes for each substrate and agar were prepared as above, with spores added directly to the substrate or agar, sealed with wax and incubated as in the germination experiment for ten weeks. The stage of gametophyte development (for individual gametophytes) was then recorded as follows: 1 (< 5 cells), 2 (5–10 cells), 3 (10–20 cells) and 4 (> 20 cells) (Photo 2). Data were collected on 50 gametophytes for each replicate Petri dish and averaged.

Data analysis

The Kruskal-Wallis test (SPSSX 2003) was applied to test for significant differences among substrate medians. Variances of stage of gametophyte development were compared with the *F*-max test, with probabilities adjusted to allow for multiple comparisons using the Dunn-Sidak method (Sokal and Rohlf 1995).

Results

Spore germination

When spores were added directly to the substrate, the first spores to germinate

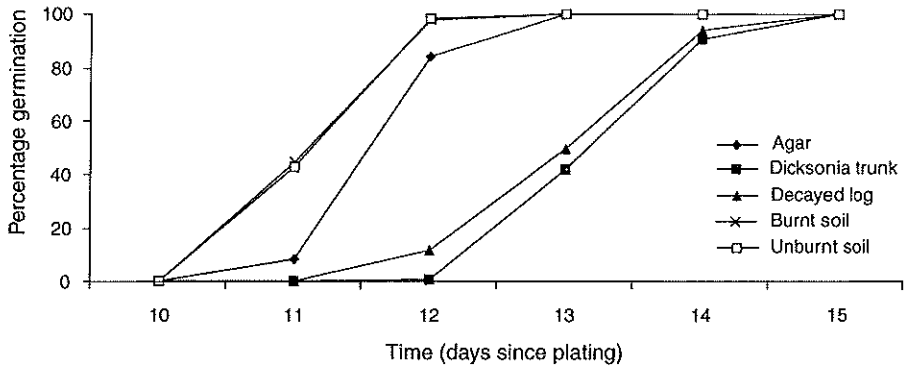

Figure 1. Percentage germination of D. antarctica *spores on different substrates for samples where spores were added directly to the substrate. Each point is a mean of five replicate Petri dishes. Standard errors are too small to appear on the graph.*

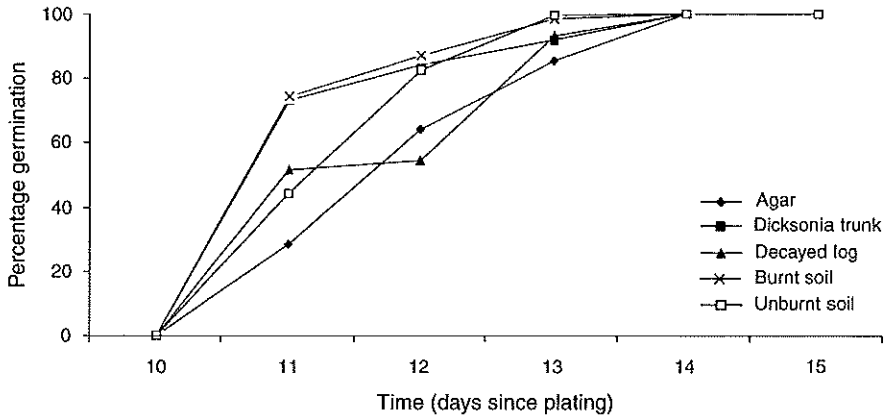

Figure 2. Percentage germination of D. antarctica *spores on different substrates for samples where spores were applied to filter paper laid over the substrate. Each point is a mean of five replicate Petri dishes. Standard errors are too small to appear on the graph.*

were observed on day 11 for agar and soil, day 12 for decayed log, and day 13 for *D. antarctica* trunk (Figure 1). By day 15, 100% germination of all spores had been recorded for all substrates (Figure 1).

When spores were placed on filter paper on top of each substrate, all substrates produced 100% germination by day 14 (Figure 2), one day earlier than without filter paper. Germination was most strongly affected by the use of filter paper on the *D. antarctica* trunk and decayed log, with germination on filter paper overlaying these substrates being significantly higher than without filter paper on days 11, 12 and 13. Spores germinated on filter paper overlaying the substrate therefore had more consistent germination across all substrates compared to those germinated directly on the substrates.

Gametophyte development

Gametophytes had developed to between one and over 20 cells after 10 weeks. Initial

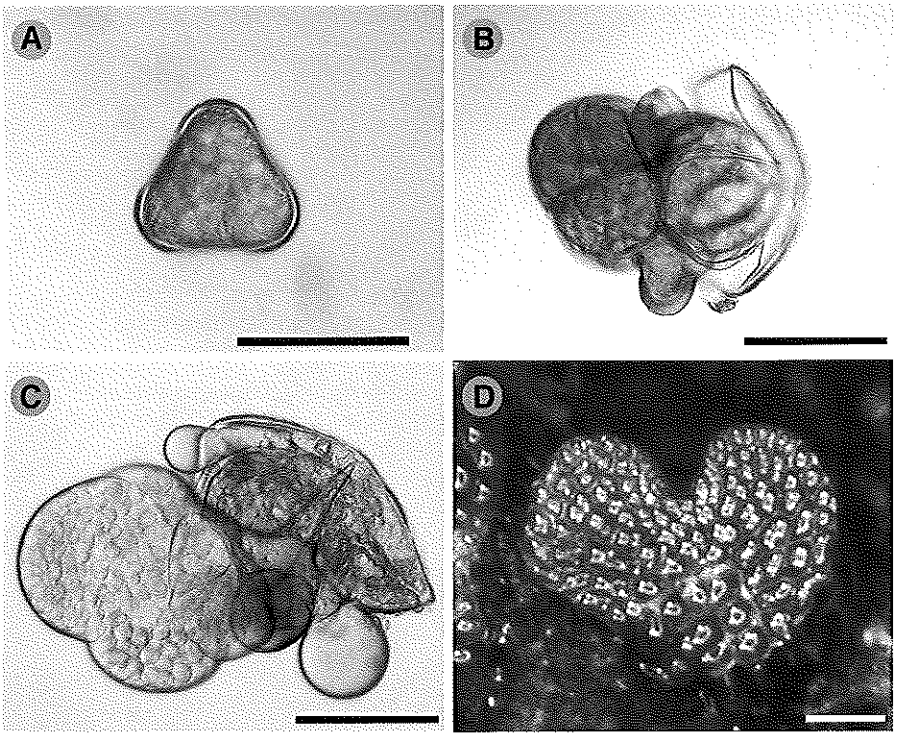

Photo 2. Four sequential stages in gametophyte development in Dicksonia antarctica. *A, spore before germination (score 1); B, early gametophyte development (score 2); C, later stages of gametophyte development (score 3); and D, heart-shaped prothallus (score 4). Scale bars for A–C represent 50 microns; scale bar for D represents 250 microns.*

germination (stage 1) and later stages of gametophyte development (stages 2–4) are shown in Photo 2. On both forms of soil, development reached the heart-shaped gametophyte stage (stage 4; Photo 2D) in each replicate, and the mean stage of development was significantly higher on both forms of soil compared to log and *Dicksonia* trunk (Figure 3). Development on the agar plates was between these extremes.

The extent of development after 10 weeks on *D. antarctica* trunk, decayed log and agar was significantly more variable than on either soil type (Figure 4). All cell stages (1–4) were recorded at 10 weeks on *Dicksonia* trunk. There was a highly significant difference in variability between development on *Dicksonia* trunk and on burnt or unburnt soil. Variability in development on the log substrate was also significantly different from that on burnt soil and unburnt soil. Cell stages 1–3 were recorded at 10 weeks on the log substrate. Development on the agar plates was significantly more variable than on burnt soil but not significantly different from that on the log substrate or *Dicksonia* trunk.

Discussion

Spore germination

This study demonstrates that substrate characteristics of Tasmanian wet forests are conducive to germination of *D. antarctica* spores, at least under laboratory conditions. Texture of the substrate did not affect the final extent of spore germination, with all substrates allowing 100% germination. However, the rate of germination

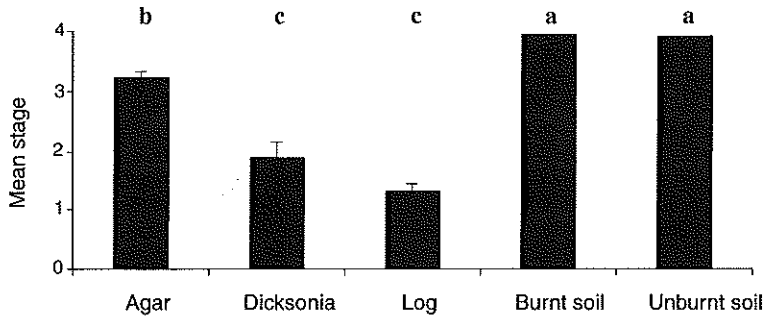

Figure 3. Mean stage of gametophyte development in D. antarctica *after ten weeks for each of five substrates. Developmental stages 1–4 are as described in the Methods. Presence of the same letter above columns represents no significant difference (P < 0.05) between the medians for those substrates; different letters represent a significant difference. Bars represent standard errors.*

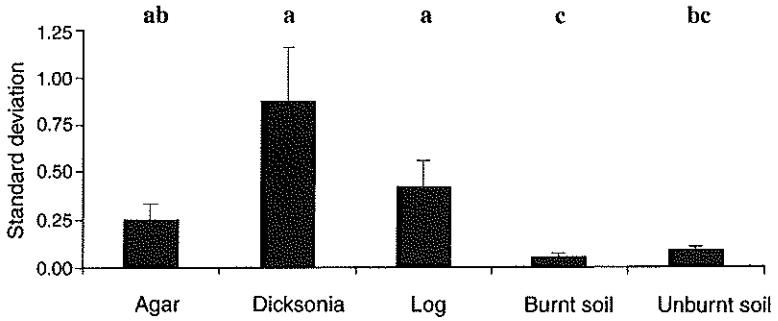

Figure 4. Variability in gametophyte development of D. antarctica *after ten weeks for each of five substrates. Presence of the same letter above the columns represents no significant difference (P < 0.05) between the variances for those substrates; different letters represent a significant difference. Bars represent standard errors. Variability was measured as standard deviation across 10 replicate Petri dishes for each substrate.*

(particularly on *D. antarctica* trunk and logs) did alter when filter paper was added. The higher variation between substrates without the overlaid filter paper may relate to the moisture-holding capacity of some substrates. *Dicksonia antarctica* trunk and decayed log had a coarser texture compared to both forms of soil. This affected the amount of water held on the surface of the substrate. As fern spores require water for germination (Page 2001), the reduced amount of water available on *D. antarctica* trunks and decayed logs may account for the slower rates of germination observed when spores were placed directly on these substrates. When substrates were covered with filter paper and the surface texture was no longer a variable, spores on all substrates experienced similar moisture availability, and germination rates were more consistent between substrate groups.

Gametophyte development

Garrett (cited in Neyland 1986) concluded that dehydration of the gametophyte of

D. antarctica arrested development of the prothallus. Page (2001) referred to the gametophyte phase as the 'Achilles' heel' of the fern life cycle because of its dependence on water for hydration and fertilisation. This is supported by our results as the two substrates with higher water-holding capacities, burnt and unburnt soil, supported the highest rates of gametophyte development. However, there was high variability between replicate Petri dishes of *Dicksonia* and decayed log at intermediate stages of gametophyte development. A plausible explanation for this is that each replicate Petri dish of these substrates varied sufficiently in microclimate to influence gametophyte development, with differences in moisture content between replicate Petri dishes of each substrate accounting for the variability. This would support previous observations by other authors (e.g. Garrett cited in Neyland 1986; Page 2001) that the gametophyte stage of the fern life cycle is dependent on water. Hence, the substrate itself is not a limiting factor with regard to the rate of gametophyte development but moisture availability is.

Conclusion

As clearance and conversion of native forest are phased out, the future wild-harvesting of *D. antarctica* will be in areas managed as native forest. Several authors have reported a significant loss of *D. antarctica* numbers after timber-harvesting operations (Ough 2001; Ough and Murphy 2004; Peacock and Duncan 1995). This suggests that the number of *D. antarctica* stems that survive an intensive logging disturbance, to be available for harvesting on subsequent rotations, may not be great enough to support future wild-harvesting in these areas.

In order to regulate this industry, the first *Tree Fern Management Plan for the Harvesting, Transporting or Trading of* Dicksonia antarctica *in Tasmania* (Forest Practices Board 2001) was introduced in 2002. Under this plan, *D. antarctica* could only be harvested on a salvage basis from native forest that would be cleared and converted to agriculture, infrastructure or plantation. As conversion of native forest in Tasmania is scaled down (ceased on State forest in 2007), the regulations for future wild-harvesting of this species needed to be changed. In 2007, the Forest Practices Authority released a revised *Tree Fern Management Plan for the Sustainable Harvesting, Transporting or Trading of* Dicksonia antarctica *in Tasmania* (Forest Practices Authority 2007) which allows wild-harvesting to occur in native forest which will be logged and regenerated to native forest.

Therefore, if tree fern harvesting is to occur in regrowth forest, it will be useful to understand the factors that influence the recruitment of *D. antarctica* individuals from spores. Knowledge of the substrates most utilised by *D. antarctica* and the microhabitat conditions required for spore development will give an indication of habitat availability in wet forest. In regrowth forest in southern Tasmania, decaying logs supported a high number of sporelings and had the highest water-holding capacity (Chuter 2003). Other authors have noted that *D. antarctica* requires water and light for growth (e.g. Barker 1988; Garrett cited in Neyland 1986). The study reported here demonstrated that the substrate itself is not a limiting factor in spore germination and early stages of gametophyte development, but that moisture availability associated with the substrate is important. The availability of moist site conditions in regrowth forest will therefore influence the establishment of *D. antarctica.*

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