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The long term survival of *Phytophthora cinnamomi* in mature *Banksia grandis* killed by the pathogen

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Summary

The ability of *Phytophthora cinnamomi* to survive long dry Mediterranean summers is the key to its persistence in the southwest of Western Australia. It has been proposed that dead *Banksia grandis* are a key long term reservoir for *P. cinnamomi* inoculum. To test this, 36 healthy *B. grandis* trees were inoculated in April 1999. The presence of viable inoculum in the trees' tissues was determined in two experiments from tissue 10 and 30 cm above and below ground: Experiment 1 from core samples 3-34 months after tree death; and Experiment 2, separating bark and wood in cross sections of trees 2-33 months after tree death. In the first summer (10 months after inoculation) 75% of the trees had died, with the remaining trees dead by the following summer. *P. cinnamomi* was more commonly recovered from bark than wood, except from those trees that died in the drier second summer where it was recovered more often from the wood than the bark. Recovery of *P. cinnamomi* from *B. grandis* cores declined over time from 60% at 3 months after death to 33% at 10 months, 5.5% at 12 months and 0.1% at 34 months after death. Two months after tree death, the recovery rate of *P. cinnamomi* was 87% from trunks and roots; this decreased to 0.5% by 33 months. *P. cinnamomi* was more commonly recovered from above- than below-ground tissue until 8 months after plant death, after which it was more frequent in below ground tissues. This study suggests that the pathogen does not behave as a competitive saprophyte in these plant tissues and *B. grandis* is unlikely to be a long term reservoir for *P. cinnamomi*. However, the manipulation of the frequency of *B. grandis* mid-storey of the jarrah forest areas and the use of fire to facilitate the breakdown of dead *Banksia* stems may reduce the effects of *P. cinnamomi*.

The long term survival of *Phytophthora cinnamomi* in mature *Banksia grandis* killed by the pathogen

1 Introduction

Banksia grandis is the dominant mid-storey species of the jarrah (*Eucalyptus marginata*) forest of south-western Australia. It is highly susceptible to *Phytophthora cinnamomi* and is one of the first species to die following introduction of the pathogen to previously healthy forest (SHEA 1979). Consequently, *B. grandis* is frequently used as an indicator species to visually determine which areas of the jarrah forest are infested with the disease (SHEA 1979). It is hypothesised that the root system of *B. grandis* trees killed by the pathogen could provide a large protected reservoir of inoculum for a long period of time (SHEA 1979; SHEA et al. 1980), and that when conditions are conducive for growth, *P. cinnamomi* is able to move from these inoculum sources to new hosts via zoospore release, mycelial growth or root to root contact (SHEARER and SHEA 1987; SHEARER et al. 2010).

In the jarrah forest, temporal and spatial soil inoculum dynamics of *P. cinnamomi* disease centres have been monitored (e.g. SHEARER and SHEA 1987; KINAL et al. 1993; SHEARER et al. 2010). SHEA (1979) and SCHILD (1995) concluded that *P. cinnamomi* could survive in the collar of dead *B. grandis* collar for more than 12 months. Viable inocula were isolated from soil around the collars of dead Banksia for 3 consecutive years (SHEARER et al. 2010). However, in all of these studies the time of tree death was based on estimates rather than actual dates of death, and no assessment was made of the distribution of the pathogen in the banksia tissues over an extended time period.

In order to effectively control *P. cinnamomi* it is important to be able to accurately estimate survival of the pathogen in tissues of highly susceptible species such as *B.*

grandis for their potential to act as a reservoir for the pathogen over extended periods of time. This will affect new containment and eradication programs (DUNSTAN et al. 2010) currently being trialled, and could help to determine the status of potential disease status of soils and gravels extracted from natural areas.

This study was designed to determine the *in planta P. cinnamomi* inoculum load persisting for 34 months after the death of mature *B. grandis* trees in the jarrah forest. In addition, this study examined the deterioration of *B. grandis* after death, enabling time since death to be estimated.

2 Materials and methods

2.1 Site selection and description

Jarrah forest with *B. grandis* as the dominant mid-storey species, approximately 30 x 150 m, and situated on North East Road, Dwellingup, Western Australia (32º39´S, 116º05´E) was selected for this study. This area has an annual rainfall for 1258 mm (average for last 70 years), and during the survey period an ambient temperature range of -3 to 35ºC, with a mean maximum temperature of 18.5 and minimum of 9.1ºC.

Phytophthora cinnamomi was not active in this area but the surrounding forest was infested by the pathogen. The site soil type was classified as Havel 'S' which is favourable to the lifecycle of *P. cinnamomi* (SHEARER and SHEA 1987).

2.2 Experimental design

Forty-two mature healthy *B. grandis* with a diameter at breast height of at least 10 cm were selected. Thirty-six mature *B. grandis* trees were underbark inoculated with *P. cinnamomi*, and six trees were inoculated with sterile discs as controls. After death, two experiments were carried out to assess the survival and distribution patterns of the pathogen within the plant tissues over time. The independent variables were; sample position on the tree, soil moisture and time since infection. The dependant variable was *P. cinnamomi* survival.

2.3 Inoculum preparation

Miracloth (Calbiochem, San Diego, USA) discs (5 cm dia.) were autoclaved in de-ionised water at 121ºC for 20 min over 3 consecutive days. The discs were placed on V8 agar (HUBERLI et al. 1997), inoculated with an actively growing culture of *P. cinnamomi* (isolate MU 97-16), and incubated in the dark for 6 days at 24ºC.

2.4 Tree inoculation

The trees were inoculated in April 1999, early autumn, when the warm wet conditions were favourable for *P. cinnamomi* and when the pathogen is believed to be most active in the jarrah forest (SHEA 1975; CAHILL et al. 2008). The trees were inoculated in the collar region approximately 10 cm below the soil line on the eastern side of each tree by cutting a hole (5 cm dia.), using a sharp knife, through the bark into the vascular cambium. The inoculum was placed over the exposed cambium tissue and covered with a 10 cm square plastic sheet to seal the wound. Soil was then replaced to the original soil line. All control trees were at least 7 m upslope or 10 m down slope from any inoculated tree.

2.5 Soil moisture and soil and air temperature

Average soil moisture content from six samples collected around the base of the trees was assessed periodically between 2000 and 2002. Three samples of 500 g, at 30 cm, for each tree were mixed and sieved (2 mm), weighed and dried at 70°C for 5 days, and again weighed.

Soil and plant tissue temperatures were monitored using data loggers (Model: Unidata Starlog 6003A). Temperature measurements were obtained from 5 and 30 cm below ground, ambient and inside plant tissues. For *in planta* data a hole was drilled into the eastern side of live trees, thermistor probes inserted and sealed with silicone. Due to periodic equipment failure, data are also presented from the West Australian Bureau of Meteorology, Dwellingup station (32.71^{o}S116.06^{o}E; 6 km from the trial site).

2.6 Experiment 1 - Periodic assessment for *P. cinnamomi* in dead *B. grandis* trees using tree cores

All trees were monitored after inoculation for symptoms of *P. cinnamomi* infection such as crown deterioration (shoot dieback) or death. Trees were determined to be dead when all leaves were brown and dry. After death, leaf retention was monitored to enable estimates of time since death for comparison with other forest studies.

Eighteen trees were sampled periodically after tree death to assess the distribution and survival of *P. cinnamomi*. For 11 trees which died within 10 months of inoculation, there were eight sample times (3, 6, 8, 10, 12, 15, 19, and 34 months) after death. The remaining seven trees died up to 12 months after the first 11 trees and were sampled at 2, 6 and 18 months after death. At each sample time, the inoculum discs were removed from 5 randomly chosen trees, placed on NARPH selective medium (HUBERLI et al. 2000), incubated for up to 7 days and assessed regularly for growth of *P. cinnamomi*, to ascertain if they were still a viable source of *P. cinnamomi* for infection of the inoculated trees.

At each sample time, 12 core samples were collected from the tap root; from 10 and 30 cm above and below the soil line at three points around the tree. These root samples were obtained using a petrol powered drill with a 'core removing attachment', which was cooled with deionised water. The samples consisted of bark and wood, and were approximately 5 to 8 cm long and 1 cm dia.

The root samples were cut into 7.5 mm thick discs (10 mm diam.) and plated onto NARPH to determine *P. cinnamomi* presence. As previous trials have indicated that *P. cinnamomi* is more commonly found in the bark and vascular cambium of plants (SHEA 1979), the samples were excised from the inner tissue toward the outer tissue to minimise cross contamination of samples with secateurs which were surface

sterilised in 70% ethanol between each sample. Bark was plated separately from wood.

2.7 Experiment 2 - Whole *B. grandis* tree harvests and assessment for *P. cinnamomi* survival

Trunks and roots of four trees were harvested at four time periods (2, 9, 12 and 33 months) after death and cut into sections to determine survival and distribution of *P. cinnamomi* in the tissues. Cross sections (1 cm thick) of trunks, tap roots, and major lateral roots were cut at 10 and 30 cm above and below the soil line. These sections were divided into 1 x 2 cm blocks and numbered according to a grid pattern, the blocks were then cut on a bandsaw with the blade sterilised between each section. Each block was then plated onto NARPH and monitored for the presence of the pathogen. A grid on paper, with *P. cinnamomi* isolations for each block marked, were assessed with APS Access - Image Analysis Software for Plant Disease Quantification (LAMARI 2002) to determine the percentage of *P. cinnamomi* recovery for each section.

2.8 Baiting methods for tissue samples that gave negative results when plated on NARPH

To test more stringently for the presence of the pathogen in the tissue samples, material that gave a negative result when plated on NARPH was baited. The methods evolved during the period of the trial as follows:

1. Single baiting method - For the harvests at 3 and 6 months of Experiment 1 and the first harvest of Experiment 2, the bark or wood samples were removed from the agar, placed in de-ionised water and baited with *Pimelia ferruginea* leaves.
2. Wet, dry and re-wet method - For harvests at 8, 10 and 12 months after death for Experiment 1, and second harvest 9 months after death for Experiment 2, baiting

method 1 was used first, and where this did not give a positive recovery of *P. cinnamomi,* the water in the baiting dishes was allowed to evaporate. After drying which usually took 5 - 7 days, more water was added and the baiting using *P. ferruginea* leaves was repeated in an attempt to stimulate any resting spores to break dormancy.

3. Rinsing method - For all later harvests for both Experiments 1 and 2, where the initial baiting did not give a positive recovery of *P. cinnamomi* the water in the baiting dishes was changed every 1 - 2 days for 5 days in order to remove any suppressive compounds such as phenolics. Rose petals instead of *P. ferruginea* leaves were used as baits because they are readily infected by *P. cinnamomi* (D Guest, *pers comm*). The petals were placed in the water at each interval and plated onto NARPH at each water change to assess presence of the pathogen.

For all baiting trials, positive controls were used to ensure that the baiting method was sound.

Due to the number of samples (50 to 200 samples per sampling time point for each depth), only 20% of all negative samples were baited. Plates for each sampling zone above and below ground were grouped, and negative samples were chosen randomly for baiting.

2.9 Statistical analysis

Data were analysed using the ANOVA module of Statistica (1999 edition, Statsoft Inc., USA). Percentages were arc-sin transformed for analysis. Data were assessed for homogeneity, variation of the mean from the variance and fit to a normal

distribution. Apparent mortality rate was calculated from the Logit of the proportion of plants dead against time in years.

3 Results

3.1 Environmental conditions during the trial

3.1.1 Rainfall and soil moisture

High soil moistures were recorded in winter (June-August) 2000 but were lower in winter 2001, due to low rainfall in that year (Fig. 1a). January, March and July 2000 had >50 mm above average rainfall. In July 2000 and September 2001, soil moistures were greater at 10 cm (Fig. 1b.) than 30 cm (Fig. 1c.) as sampling occurred during a rainfall event. Soil moisture varied with depth and according to season. In the middle of the study period (January 2001- summer) the lowest soil moisture levels were recorded; 3 and 4% moisture at 10 and 30 cm, respectively (Figs. 1b,c.).

<Figure 1a,b,c>

3.1.2 Soil and ambient temperatures

Ambient temperatures recorded at the trial site followed the same trend as those recorded by the Bureau of Meteorology but were usually 2.5ºC lower (Fig. 2.).

Belowground temperatures were more stable than ambient and tree temperatures, with minimum soil temperatures on average 7.4ºC higher, and maximum temperatures up to 8.2ºC cooler than ambient temperatures during the hotter months. The maximum temperatures belowground were very similar to ambient temperatures in the winter months (Fig. 2.).

3.1.3 *Temperature within the trunks of mature* B. grandis

Banksia grandis tissue temperatures were similar to soil temperatures, falling between the soil at 30 cm deep and ambient air temperatures, and remaining

consistently 4.5ºC higher than the minimum ambient temperature (Fig. 2.). Conversely, maximum temperatures of stem tissue closely followed the trends of ambient temperatures (Fig. 2.). Although the data loggers malfunctioned during the hotter periods, four days of readings obtained during February 2000 were within 1ºC of ambient temperatures suggesting that this trend of following ambient temperatures continued throughout the year.

<Figure 2>

3.2 Symptoms of *P. cinnamomi* infection in mature *B. grandis* trees over 29 months

Phytophthora cinnamomi was successfully recovered from the Miracloth discs removed from inoculation sites of the *B. grandis* trees 4 weeks after inoculation.

All *B. grandis* died by 22 months after inoculation with *P. cinnamomi*, with the apparent mortality rate of 3.4 trees/month (Fig. 3.). No symptoms were apparent in any of the trees during the autumn (April-May), winter (June-August) and spring (September-November) periods, with symptoms and deaths first occurring in late summer (February 2000) (10 months after inoculation). The rate of decline after death was variable between trees. Overall, 47% of *B. grandis* trees lost all leaves within 3 months, 94% had lost all leaves after 5 months, leaving only 6% (2 trees) that retained leaves for longer than 5 months after plant death (Table 1).

<Table 1>

Most *P. cinnamomi* inoculated trees showed the above progress of stages to death, but seven trees showed early symptoms in summer 2000 (8 months after inoculation) then recovered to produce some new growth as the season changed to autumn bringing cooler and moister conditions (Table 1). These trees were in an

area which was lower in elevation and close to a water course. All seven of these trees died during the next summer, and the trunk and root systems deteriorated quickly from termite damage.

By 22 months (February 2001), all control inoculated trees were still alive and had no marked leaf loss. Three *B. grandis* control trees died in the third summer (2002) or 30-33 months after inoculation of the trial and *P. cinnamomi* was recovered from the dead plants above and below the soil line. The trees were all from the same area, and although they were 10 m from any of the inoculated trees they had been exposed to *P. cinnamomi* infested water and soil during the harvest of the inoculated trees upslope.

3.3 Experiment 1 - Periodic assessment for *P. cinnamomi* in dead *B. grandis* trees using tree cores

Recovery of *P. cinnamomi* from *B. grandis* cores declined over time from 60% at 3 months to 0.1% at 34 months after death (Fig. 4a.). Between 10 and 12 months, late spring to summer, recovery of *P. cinnamomi* from the plant tissues was greatly reduced from 33 to 5.5% from all sampling points combined.

Phytophthora cinnamomi was more commonly recovered from above ground than below ground until 8 months after plant death (Fig. 4a.). After this time, recovery was higher from 10 and 30 cm below ground at 10 months and thereafter from 30 cm below ground. The drop in recovery from above ground tissue at 10 months coincided with the warmer, drier weather of late spring. There was no recovery of *P. cinnamomi* from above ground tissue after 18 months for trees that died in the summer of 2001 (Fig. 4b.).

<Figure 4>

Phytophthora cinnamomi was more commonly recovered from bark than wood in the core samples (Table 2a). Higher recoveries were obtained from the bark at all sampling positions in samples taken up to 10 months after plant death. *Phytophthora cinnamomi* recoveries were comparable from wood and bark at 10 months following tree death except at 30 cm above ground were no bark recoveries were made (Table 2a). At the final sampling time 34 months after plant death the only recovery from 755 samples was from woody tissue, and this from 30 cm below ground. In both wood and bark, more recoveries of the pathogen were made from above the soil line until 8 months but after this time below ground samples yielded the highest recoveries (Table 2a).

3.3.1 Deaths in the second summer of the trial

Recoveries were lower from trees that died during the second summer of the trial. This was considerably drier than the first summer (Fig. 1a.), and recoveries declined from an overall average of 14.6% at 2 months to 1.6% at 18 months after plant death (Table 2b). From these trees *P. cinnamomi* was recovered more commonly from wood compared to bark at 2 months after plant death, but recoveries were comparable between the tissue types thereafter (Table 2b). *Phytophthora cinnamomi* was not recovered from above ground tissue after 6 months. *Phytophthora cinnamomi* was recovered from both wood and bark 10 and 30 cm below ground at the final sampling 18 months after plant death (Table 2b).

<Table 2>

3.4 Experiment 2 - Whole *B. grandis* tree harvests and assessment for *P. cinnamomi* survival

The area of the cross-sections of trunks and roots from which *P. cinnamomi* was recovered decreased from 87% at 2 months after tree death to 0.5% at 33 months

after plant death (Table 3). Recoveries after 2 months were extremely high from samples both above and below ground. Recoveries were still high 9 months after tree death but declined sharply at 18 months after plant death when there was a maximum of 4% recovery from the cross-section tissue at 10 cm above the ground (Table 3). After 33 months *P. cinnamomi* was rarely isolated and only from below ground plant tissue.

Recovery of *P. cinnamomi* in *B. grandis* tissues was predicted using the equation: Isolation = 82.65 - 90.98 Year - 0.10 Position + 22.88 Year^2 ($r^2 = 0.98$).

Time since death (Year) had a significant ($P < 0.01$) effect on isolation, whilst position on the tree had no effect.

<Table 3>

Overall, bark samples had the highest recoveries ($P = 0.48$) and 9 months after death the pathogen was still isolated from 93% of the root samples at 30 cm below ground (Table 4). After 18 months, *P. cinnamomi* was recovered infrequently from both above and below ground. After 33 months, the few *P. cinnamomi* isolations made were from below ground bark (Table 4).

<Table 4>

3.5 Baiting for *P. cinnamomi* after initial negative results on NARPH

Baiting of the tissues after negative results on NARPH yielded 34 additional *P. cinnamomi* recoveries from ~700 tissue pieces (Experiment 1), with a similar number of recoveries from each of the three methods, but double the number of below ground samples providing positive results compared to above ground samples.

4 Discussion

Phytophthora cinnamomi was able to survive in *B. grandis* tissue for up to 34 months after tree death, but was recovered from less than 1% of samples after this period. Similarly, in the second experiment, there were few recoveries (0.5%) from the necrotic area 33 months after death. The inoculum potential of the pathogen drops rapidly in *B. grandis* with time. This supports the claim that few propagules of *P. cinnamomi* can survive for long periods of time (WESTE and VITHANAGE 1979; ZENTMYER 1980; SHEARER and SHEA 1987; SHEARER 1994; SHEARER and SMITH 2000) but does challenge the assertion that *B. grandis* tissue acts as an important long-term reservoir for *P. cinnamomi* in the jarrah forest (SHEA 1979; SHEA et al. 1980; SCHILD 1995). Banksia are also known to rapidly disappear from a site after death due to fire, termites and fungal decay (G Hardy, *pers comm*) and therefore *P. cinnamomi* may be out-competed in the plant tissues. However, SHEARER and SHEA (1987) and SHEARER et al. (2010) have shown that for at least 4 years, the amount of viable inocula in the soil at the collars of dead Banksia is greater than in random soil samples from the surrounding area. Stemflow of rainfall was attributed to be the cause of the higher soil moisture found around the collar of dead Banksia than nearby soil (SHEARER et al. 2010) which may also influence the survival of inoculum within the adjacent tissue.

The pattern of *P. cinnamomi* recovery showed that the pathogen extensively colonised the trees from the inoculation point up into the stem and down into the roots. The timing and position of the sampling points did not allow determination of whether or not the pathogen continued to colonise dead tissue after tree death, but the pattern of invasion does show that the pathogen moved readily into the stem and survived in this tissue for up to 12 months after the death of the plant. This pattern of

colonisation was similar to those recorded by DAVISON et al. (1994) and O'GARA et al. (1997) and in studies of *E. marginata* colonisation by *P. cinnamomi*.

Overall, more recoveries of *P. cinnamomi* were obtained from bark tissue (external to the vascular cambium) than wood, from areas both above and below the soil line. This finding is supported by SHEA (1979) where more *P. cinnamomi* was recovered from bark of *B. grandis* below the soil line. *Phytophthora cinnamomi* recoveries above ground declined more quickly in bark tissues than wood, possibly because bark could also be expected to dry out more rapidly than wood as it is more exposed to daily temperature variations. DAVISON et al. (1994) also found that *P. cinnamomi* recoveries tended to be higher from wood than bark for *E. marginata* stem tissue above the soil surface.

Soil moisture is also likely to have impacted upon *P. cinnamomi* survival in *B. grandis* plant tissues as it will affect the moisture content of dead root tissue. Low soil moisture in summer may have reduced *P. cinnamomi* survival in dead *B. grandis* tissues (SHEA 1975; SHEA et al. 1983; SHEARER and SHEA 1987). Soil moisture levels in this trial were comparable to those recorded for jarrah forest soils (SHEARER and SHEA 1987; SCHILD 1995) but there was extremely low rainfall during the winter of 2001 and the corresponding low soil moisture levels recorded in the trial may have caused the dead *Banksia* tissue to dry out faster than usual, thus influencing survival of *P. cinnamomi* in the plant tissues. In contrast, the high levels of *P. cinnamomi* in the plant tissues after death of the trees in the summer of 2000 may have been a consequence of un-seasonal rain in January where 100 mm was recorded over 8 days. This concurs with the assertion that rainfall, temperature and soil water content have a more distinct and consistent influence on inoculum viability than seasonal fluctuations (SHEARER et al. 2010).

In temporal soil studies in the jarrah forest there was significantly lower frequency of isolation of inoculum in the top 3 cm of soil and near-surface soil around the collar of dead *Banksia* trees, while in some of the Banksia woodlands on the Swan Coastal Plain the frequency of isolation of the pathogen was greatest from around dead *Banksia* collars compared to soil from 1 m or 3 cm deep (SHEARER et al. 2010). SHEARER et al. (2010) showed that the isolation of inoculum was greatest from infertile and least from relatively fertile biomes, prompting the need for further studies to be undertaken in the relatively infertile soils of the Banksia woodlands where there is less organic matter in the soil to support antagonistic microflora.

In disease centres in the jarrah forest, viable inoculum of *P. cinnamomi* was found in soil at 2.5 m below the soil surface (KINAL et al. 1993) and in roots of *E. marginata*, at least 1.25 m below the soil surface (SHEA et al. 1982). In disease centres in *Banksia* woodlands, viable inoculum was found in groundwater 5 m below the soil surface (SHEARER et al. 2010) and from taproots of *B. attenuata* 2 m below the soil surface (HILL et al. 1994, 1995). The depth at which viable inoculum of *P. cinnamomi* occurred imposes limitations on management options available for control (SHEARER et al 2010). A study of the survival of *P. cinnamomi* in *B. grandis* roots over a longer time period, and at greater depths, would enhance our knowledge of its long term fecundity and give a better insight of the importance of root tissues as a protective barrier from environmental variations.

Phytophthora cinnamomi did not exhibit high levels of dormancy and saprophytic ability in this trial. Above ground, *P. cinnamomi* was not recovered from any tissue 18 months after plant death. The pathogen survived for the duration of this trial in *B. grandis* roots only in the more protected soil conditions, albeit in low concentration. If saprophtism was a strong feature of the pathogen, it could have been expected to

colonise dead *B. grandis* tissues when conditions were favourable and survive for extended periods of time. Harvests carried out in winter and spring when conditions were favourable for *P. cinnamomi* yielded very low recovery of the pathogen. The cooler, wetter temperatures of the latter harvests would suggest conditions were available to break dormancy if dormant propagules were present and allow saprophytic ability to prevail but this is not evident by the recoveries obtained. Baiting methods were very thorough and became more intensive as the trial progressed. If *P. cinnamomi* dormancy was operating it could have been expected to be broken with the wetting and drying procedures used. However, due to the lack of recoveries after baiting, drying and wetting and re-baiting, it appears unlikely that long term dormancy structures are being produced. .

The ability of *P. cinnamomi* to survive the long dry Mediterranean summers is the key to its persistence in the region, however there has been very little research on the difficult subject of dormancy and dormancy breakage under natural conditions (McCarren et al. 2005). The relative importance of survival through parasitism of tolerant plant species compared with susceptible ones has not been adequately addressed.

All *B. grandis* trees inoculated with *P. cinnamomi* died during the experiment, which supports the designation of this species as highly susceptible (Shearer 1994) and its use as an indicator species for *P. cinnamomi* presence in the jarrah forest (Shea 1979; Shea 1988; Shearer and Smith 2000). The extended survival of some trees may indicate different levels of resistance within *B. grandis.* However, it is more likely that the range in survival times was linked to proximity to a water course, with the closer trees experiencing less water stress in the first summer.

Despite extensive baiting, very few additional positive *P. cinnamomi* recoveries were detected over those recovered using NARPH. This suggests that the methods applied did provide an accurate assessment of the distribution of *P. cinnamomi* in the plant tissues.

Rapid leaf loss observed after death in the current study show that assumptions on time since death were over estimated by SHEA (1979) and SCHILD (1995). They assumed that trees with a few leaves had died 12 months previously, whilst our data shows there was a 94% chance that these trees would have been dead for only 3-5 months. In SHEA'S (1979) study, trees with stems partially retained and no leaves present were deemed to have been dead for more than 1 year, but we conclude they may have been dead for as little as 5 months. Adjusting for these differences, the findings of the present study concur closely with SHEA (1979) for the percentage of *P. cinnamomi* survival in *B. grandis.*

The results of this trial have important implications for management of *P. cinnamomi* in the northern jarrah forest. Understanding inoculum dynamics is fundamental for the development of integrated disease management in order to identify environments favourable to disease development. While *B. grandis* has been singled out as a key reservoir for *P. cinnamomi* inoculum, this study has shown that tissue of trees that died from infection with *P. cinnamomi* are unlikely to perform this function in the long term. . The persistence of any inoculum in dead *B. grandis* can possibly be further reduced by manipulating the frequency of *B. grandis* mid-storey of the jarrah forest (SHEA 1975; MURRAY 1987) in areas where the pathogen is present or likely to spread to, to reduce the effects of *P. cinnamomi.* Fire will also help facilitate the breakdown of dead *Banksia* stems.

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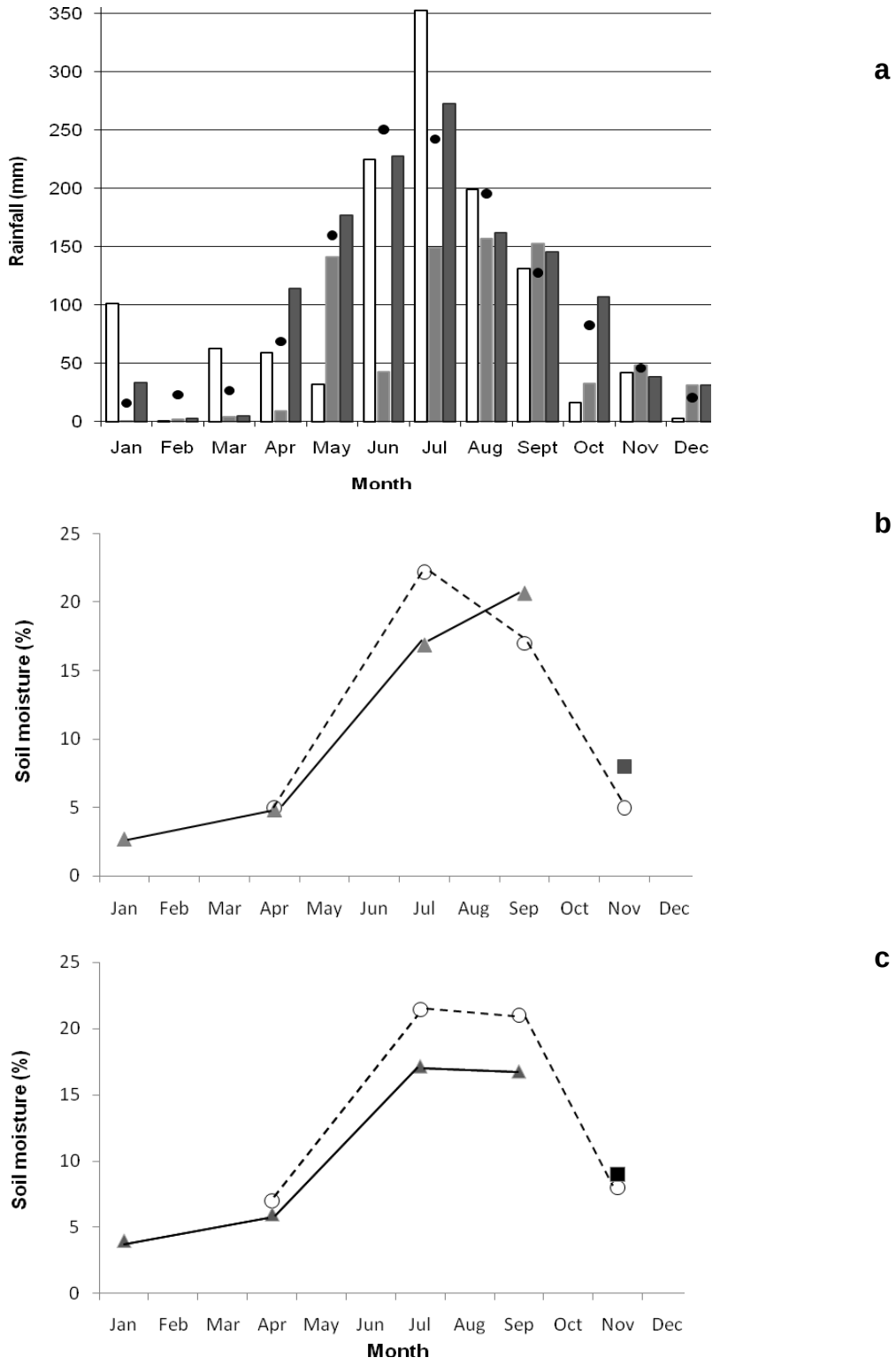


Fig. 1. a) Monthly rainfall for the years 2000 (□), 2001 (■), 2002 (■) and the average for previous 70 years (●) recorded by the Western Australian Bureau of Meteorology Dwellingup Station (009538), S32.7103 E116.0594. b) Soil moisture at 10 cm and c) at 30 cm, in soils surrounding *Banksia grandis* trees in 2000 (○), 2001 (▲) and 2002 (■).

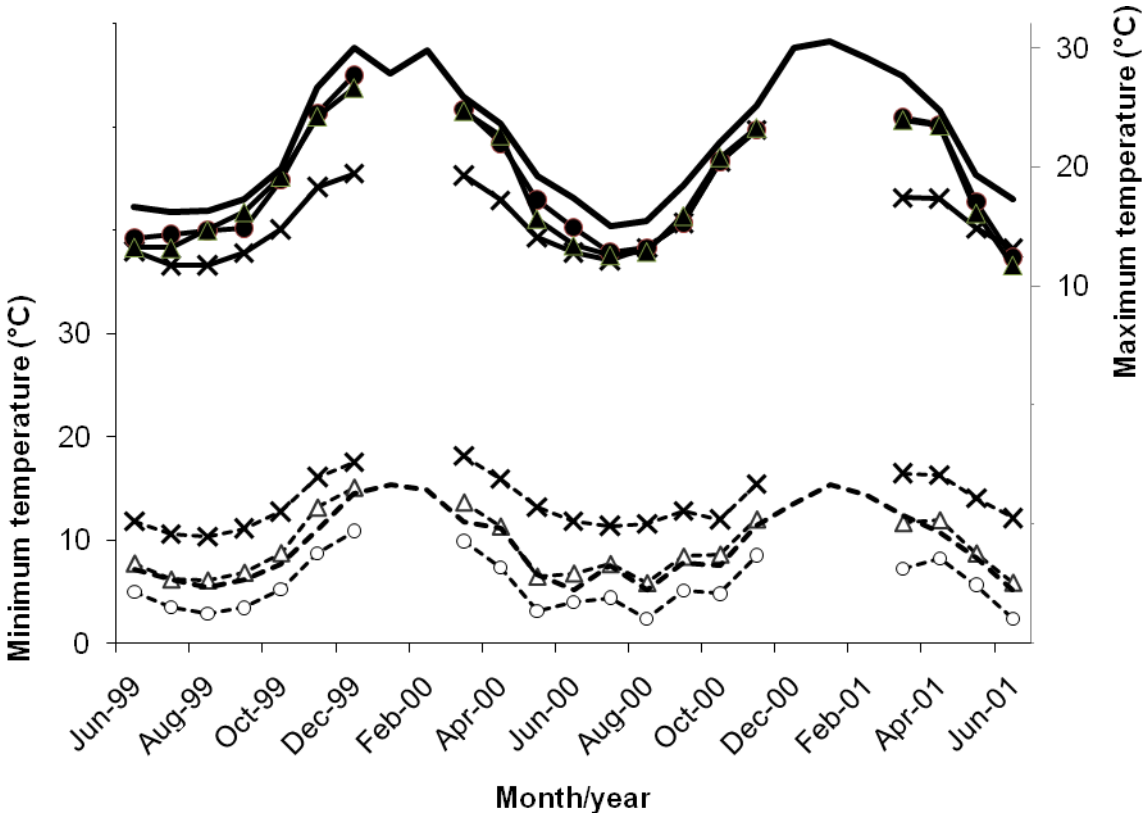


Fig. 2. Minimum (dashed lines) and maximum (solid lines) temperatures measured at Dwellingup study site (ambient, **O**; *Banksia grandis* trunk tissue, △; and soil at 30 cm, **x**) and air temperatures from Bureau of Meteorology Dwellingup station (complete data sets).

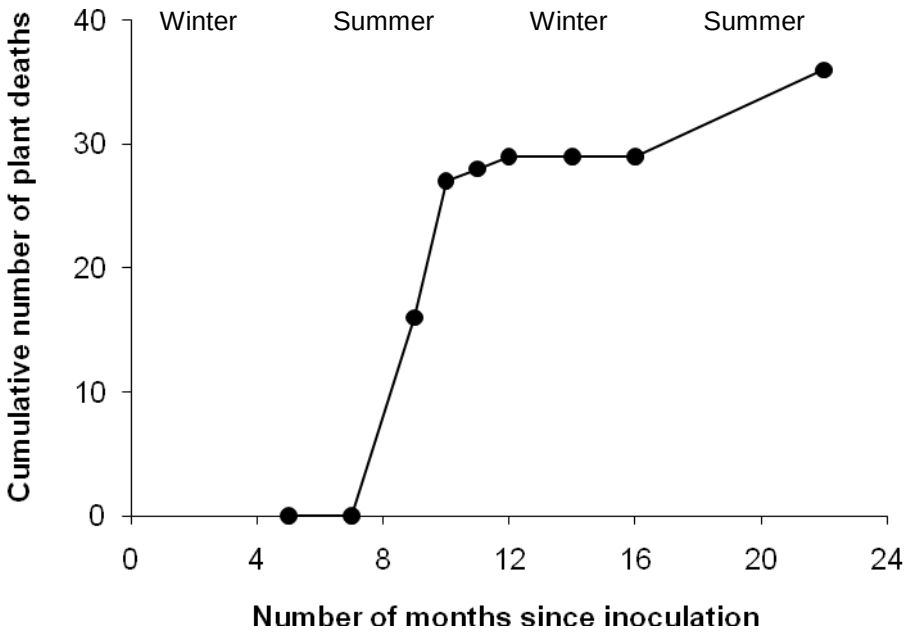
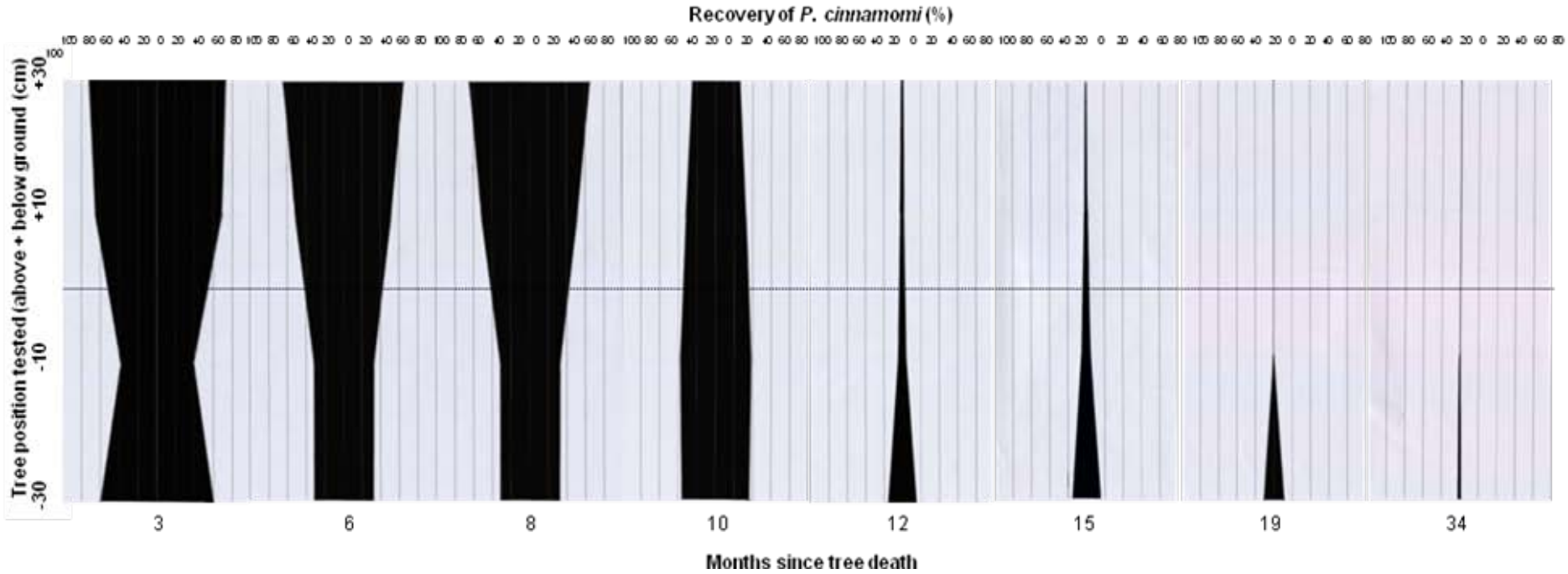


Fig. 3. Mortality curve for 36 *Banksia grandis* trees inoculated with *Phytophthora cinnamomi* in Autumn 1999.

a


Recovery of P. cinnamomi (%)
Tree position tested (above + below ground (cm)
+30
+10
-10
-30
3
6
8
10
12
15
19
34
Months since tree death

b

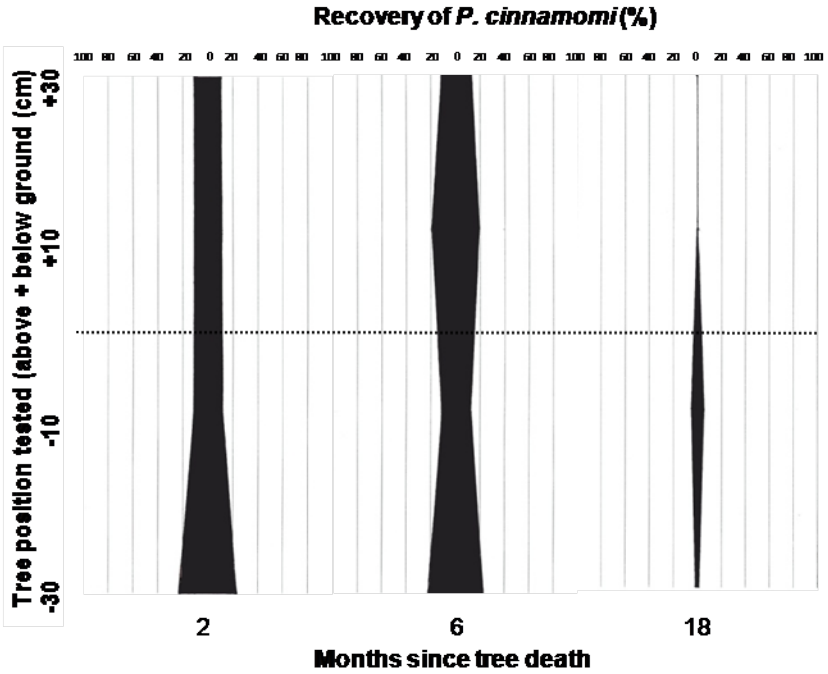


Fig. 4. Percentage recovery of *Phytophthora cinnamomi* from *Banksia grandis* trees over 34 months from core samples at 10 and 30 cm above and below the soil line. Data shows recovery from bark and wood combined for trees that died in a) summer of 2000 or b) summer of 2001.

Table 1. Progress of symptoms and leaf loss in 36 *Banksia grandis* trees inoculated with *Phytophthora cinnamomi* in April 1999. At 12, 23 and 30 months after inoculation, 11.2% of trees were harvested for Experiment 2, prior to this assessment.

		Plant alive		Plants dead			
Months since inoculation	**Season**	**Asymptomatic (%)**	**Symptomatic (%)**	**all leaves intact (%)**	**<50% leaves lost (%)**	**>50% leaves lost (%)**	**all leaves lost (%)**
8	Summer	66.6	33.4				
10	Summer	19.4	36.2	8.3	27.8	8.3	
10.6	Summer	19.4	5.6	36.2	27.8	11.2	
12	Autumn	19.4		25.0	25.0	19.4	
13	Autumn	19.4		19.4	16.6	33.4	
14.5	Winter	16.6	2.8			8.3	61.1
23	Autumn			2.9	13.8		61.1
24.5	Autumn			5.5	5.6	5.6	61.1
30	Spring					2.7	63.9

Table 2. Percentage recovery of *Phytophthora cinnamomi* from the bark and wood of *Banksia grandis* tree cores harvested up to 34 months after tree death, from 10 and 30 cm above (+) and below (-) ground level. Data show recoveries from bark and wood from trees that died in a) summer 2000 and b) summer 2001 (each record represents the average of 3 cores from 11 trees).

Months since tree death	Sample position above and below the soil line									
	Wood					Bark				
	-30cm	-10cm	+10cm	+30cm	Wood average	-30cm	-10cm	+10cm	+30cm	Bark average
a) death in summer of 2000										
6	26.7	27.3	52.2	63.3	42.9	46.9	50.0	55.1	66.7	55.7
8	21.2	10.9	30.9	49.1	28.9	37.9	34.1	55.3	70.3	49.3
10	34.5	37.1	30.7	31.0	33.0	40.0	38.5	31.4	10.8	30.9
12	13.0	2.6	1.2	0.7	3.2	22.2	10.0	7.9	0.0	8.3
15	10.1	3.9	0.6	0.0	2.0	27.8	7.4	0.0	0.0	6.0
19	9.5	0.0	0.0	0.0	2.3	20.0	0.0	0.0	0.0	3.4
34	0.6	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
b) death in summer of 2001										
2	19.7	12.1	21	13.1	16.3	20.6	8.8	15.8	5.9	12.9
9	20.6	11.8	9.3	12.1	13.4	29.4	6.7	15.6	2.9	13.7
18	0.8	4.8	0.0	0.0	1.5	3.7	3.2	0.0	0.0	1.9

Table 3. Average distribution (±SE) of *Phytophthora cinnamomi* in dead *Banksia grandis* trees 2 – 33 months after tree death. The percentage of cross section area colonised was estimated from the percentage of samples from which *P. cinnamomi* was recovered. Three trees were harvested at each time period.

Months since tree death	Position of cross-section above or below the soil line							
	-30cm		-10cm		+10cm		+30cm	
	Mean cross section area (cm²)	Area colonised (%)	Mean cross section area (cm²)	Area colonised (%)	Mean cross section area (cm²)	Area colonised (%)	Mean cross section area (cm²)	Area colonised (%)
2	8969.8 ± 1951.5	86.9	17025.6 ± 2637.6	68.0	14082.9 ± 3026.9	77.6	11771.9 ± 2555.8	72.0
9	6182.7 ± 1765.0	70.2	14326.3 ± 2234.8	42.1	19360.2 ± 1208.5	31.5	15704.9 ± 4143.3	21.1
18	9904.8 ± 3365.8	1.2	23171.2 ± 2071.1	1.7	16553.1 ± 1890.4	3.8	13671.3 ± 2281.3	0.0
33	8220.6 ± 1892.7	0.4	13828.7 ± 2118.8	0.5	15188.9 ± 3455.0	0.0	8752.9 ± 2661.4	0.0

Table 4. Percentage recovery of *Phytophthora cinnamomi* from the bark and wood of four *Banksia grandis* tree cross sections cut into 1 x 2 cm blocks and harvested 2 – 33 months after tree death. Samples were taken at 10 and 30 cm above (+) and below (-) ground level.

Months since tree death	**Recovery of *Phytophthora cinnamomi* (%)**									
	Wood					**Bark**				
	-30cm	**-10cm**	**+10cm**	**+30cm**	**Average wood**	**-30cm**	**-10cm**	**+10cm**	**+30cm**	**Average bark**
2	87	62	77	70	74	71	73	59	70	68.3
9	66	32	30	23	37.7	93	53	38	15	50
18	1	1	3	0	1.3	0	4	3	0	1.8
33	0	0	0	0	0	1	2	0	0	0.8