May 2008

Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia

Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite?

Tender Number 19/2005
Sub project 19.2.3

Murdoch LINK

Australian Government
Department of the Environment and Heritage

CPSM
Centre for *Phytophthora* Science and Management

Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia.

Tender Number: 19/2005DEH Sub Project 19.2.3 30 May 2008

Project Period and Period Covered by this Report
25 May 2005 – 30 May 2008

Project Manager Details
Associate Professor Giles Hardy
Forest Pathology
Rm 3.037 Biological Sciences Building
Murdoch University, South Street
MURDOCH WA 6150
Ph: 08 0360 6272
Email: G.Hardy@murdoch.edu.au

Report compiled by:
Dr Kay Howard
Email: K.Howard@murdoch.edu.au

Sub-Project Manager Details
Dr Daniel Huberli
Research Associate
Rm 3.039 Biological Sciences Building
Murdoch University
South Street
Murdoch WA 6150
Ph: 08 0360 7537
E-mail: D.Huberli@murdoch.edu.au

Other Sub-Project Staff
Sub-Project Co-ordinators (CPSM) - Dr Emer O'Gara– (2005-2007), Dr Kay Howard (2008)
Project Advisors: Associate Professor Bernie Dell (CPSM), Emeritus Professor Jen McComb (CPSM), Dr Barbara Bowen (Murdoch University), Bryan Shearer (DEC), Colin Crane (DEC), Sarah Barrett (DEC), Greg Freebury (DEC)
Statistician: Associate Professor Mike Calver (Murdoch University)
Dr Trudy Paap - Graduate Research Assistant (2007-2008)
Ms Nicole Moore – Graduate Research Assistant (2005-2007)
Dr Madeleine Wouterlood - Research Associate (2006)
Ted Spadek (Western Australian Chemistry Centre)

Ackowledgements
Dr William Dunstan, Janet Box (CPSM)
Karyn Gower, Nathalie Long, Bryony Palmer (Murdoch University)
P Barua, J Ellery, P Hollick, A Illfield, O Fry, S Jackson, T Johnson, J O'Brien, T Phillips, G Scott, P Scott, T Scott, M Sommeechai, R Swift, D White (Murdoch University)

How to cite this report:
Huberli D, Shearer BL, Calver MC, Paap T, Moore NA, Barrett S, Freebury G, Howard K, O'Gara E, Dunstan WA, Bowen B, Gower K, Palmer B, Long N, Crane C, Spadek T, Dell B, O'Brien P, McComb JA, Hardy GEStJ (2008). Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia. Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite? Prepared by the Centre for Phytophthora Science and Management for the Australian Government Department of the Environment, Water, Heritage and the Arts.

Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia.

Tender Number: 19/2005DEH Sub Project 19.2.3 30 May 2008

Contents

Sub Project 19.2.3 Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite?

EXECUTIVE SUMMARY

Phosphite is of major importance in controlling root disease caused by *Phytophthora cinnamomi.* It acts both directly and indirectly on the pathogen. In order to maximise the efficacy of phosphite we need to understand how the physiological status of the plant at the time of phosphite application affects control. The physiological status of plants is not constant but varies over time depending on developmental gene expression (e.g. leaf phenology, flowering/fruiting and senescence) and interactions with the environment (e.g. temperature, moisture, light, fire, nutrients and other biota). In Mediterranean environments in particular, plants experience stresses due to extremes in water availability and the incidence of wild fire is high. Furthermore, individuals and species of plants are not in synchrony due to differences in recruitment, ontogeny, longevity and rest periods. Therefore, from a management perspective when considering all of these stresses native plant communities are subjected to, it is critical to know when to apply phosphite to ensure optimal disease control.

We examined each of the key environmental stresses (water excess, water deficit, fire and flowering) independently, on the efficacy of phosphite to control disease.

WATERLOGGING

In Australia, *P. cinnamomi* is more prevalent in regions with rainfall greater than 600 mm per annum. This includes much of the Mediterranean-climate areas, especially in the southwest of Western Australia. These areas are subject to waterlogging events both during autumn/winter and in summer. Summer cyclonic rainfall events are predicted to occur more frequently in the southwest of Western Australia as a result of climate change. Such events are ideal for *P. cinnamomi* which is at its most active under warm, wet conditions. Consequently, it is important that we understand how (i) our native plant species respond to waterlogging events, and (ii) the efficacy of phosphite in controlling *P. cinnamomi* might change if applied before or after such waterlogging events. This was examined in two separate experiments in growth chambers and in the glasshouse.

The first experiment examined the physiological responses of *Banksia attenuata* and *B. baxteri* seedlings waterlogged for 8 or 21 days in a growth chamber. *B. attenuata* was more sensitive to waterlogging than *B. baxteri* as photochemical yield, water potential, transpiration, photosynthesis and leaf stomatal conductance declined rapidly and some deaths occurred. In a second trial, *B. baxteri, B. grandis* and *B. littoralis* were waterlogged for 3 or 21 days. Waterlogging reduced stomatal conductance, photosynthesis and transpiration rates, and shoot and root growth of *B. baxteri* and *B. grandis*, and leaf water potentials indicated severe water stress after 21 days. Lack of water stress, continuance of some photosynthesis and low mortality indicate these two species could survive and recover from short-term waterlogging, but are intolerant of extended waterlogging periods. Growth of *B. littoralis* was unaffected, and this species had higher rates of gas exchange and net photosynthesis that were also unaffected by waterlogging. Differences in plant stress responses to waterlogging needs to be considered when flooded or hypoxic conditions occur, or whilst screening containerised seedlings for resistance to *Phytophthora.*

Sub Project 19.2.3 Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite?

The second experiment examined the efficacy of phosphite, applied before and after waterlogging events, to effectively contain *P. cinnamomi.* Comparisons of host physiological processes under waterlogged or non-waterlogged conditions were also tested to help understand how waterlogging might impact on phosphite *in planta. Banksia baxteri* was more tolerant to waterlogging than *B. attenuata* based on transpiration rates, stomatal conductance and net photosynthesis. In contrast, *B. baxteri* was able to maintain stomatal aperture and gas exchange under waterlogging conditions. There were no differences in colonisation of *P. cinnamomi* in the stems of non-phosphite treated plants between waterlogged or non-waterlogged plants. Waterlogging did not appear to adversely affect phosphite uptake and distribution when applied prior to waterlogging. It also did not affect uptake when applied after waterlogging as by 27 weeks post–waterlogging, *P. cinnamomi* colonisation was as effectively contained as in non-waterlogged plants. In contrast, colonisation was more extensive in *B. attenuata* two to 5 weeks post-waterlogging than in the non-waterlogged controls, indicating at least for this waterlogging intolerant species that its physiology was still impaired after waterlogging, reducing the effectiveness of the phosphite induced host response.

This is the first study to demonstrate that waterlogging does not have long-term detrimental effects on the ability of phosphite to induce host defense responses irrespective of whether phosphite was applied before or after the waterlogging event. Therefore, managers of native ecosystems do not need to consider any negative impacts of waterlogging on *P. cinnamomi* management with regards to the application of phosphite.

Future work is required to ascertain the length of time required to allow plants to recover from a waterlogging event before applying phosphite and know it will be effective in the plant species being treated. The effect of multiple stresses on phosphite efficacy also warrants further research; in particular, drought followed by flood which may occur from time to time in Mediterranean-type ecosystems.

RECOMMENDATIONS

Scientific:

S1. For an accurate assessment of the efficacy of phosphite treatments, disease should be measured as total colonisation and not visible lesions only. *B. baxteri* had larger extensions beyond the lesion than *B. attenuata*, in most cases.

S2. Whether phosphite application offers protection to plants that have been predisposed to flooding, disease development needs to be assessed by soil inoculations. We assessed whether uptake of phosphite was effective before and after a flooding event and assessed disease to monitor phosphite efficacy. We did not assess how phosphite may protect flooded plants predisposed to *P. cinnamomi*. Also, we did not explore whether short-term waterlogging increases the risk of infection due to greater attraction of zoospores to roots or alterations to infection sites.

S3. Further research should investigate whether partial flooding of the root system can elicit changes in the plant's defense response in non-flooded

roots and whether phosphite longevity and efficacy remains unaltered. This can be studied by using a split root system and flooding one compartment.

S4. We did not investigate multiple stress events either with the same stress type or two or more stresses. Two that are worthy of consideration because of their occurrence from time to time in Mediterranean-type ecosystems are drought followed by flood, and drought followed by fire. With predicted changes in climate, especially in the southwest of Western Australia, these stresses are likely to become more frequent events. This is particularly true in relation to wild fire. Therefore, future studies should combine drought and fire when asking questions about the use of phosphite to control *P. cinnamomi* in key areas with threatened species and/or communities.

S5. Future work is required to ascertain the time required to allow plants to recover from a waterlogging event before applying phosphite and ensure its effectiveness in the plant species being treated.

Management:

M1. It is recommended that phosphite be applied after a flooding event where there is a high risk of disease by *P. cinnamomi* and plants have not been protected by phosphite application previously. However, it should not be applied until at least one week after the flooding event to allow plants to recover from water stress otherwise phosphite effectiveness may be reduced. (Although, as indicated above, more research is required to determine how long we need to wait after a waterlogging event before phosphite can be applied to ensure plants will respond effectively).

M2. It is advisable to protect plant communities susceptible to *P. cinnamomi* by being proactive and applying phosphite prior to high risk flooding events.

M3. The rate of application of phosphite does not need to be altered with the level of plant stress.

WATER DEFICIT

Water deficit due to prolonged drought, seasonal dry soil, or air saturation deficit is an annual feature of Australian vegetation in Mediterranean-type regions. These conditions normally follow or precede a wet season where soil moisture and temperature are suitable for *P. cinnamomi* to reproduce, infect plants and to be spread as spores in free water, soil movement or anthropogenically.

To determine how water stress at the time of phosphite application influences the efficacy of phosphite to control the pathogen, three field sites were investigated: Cape Riche on the south coast of Western Australia, Jandakot Airport close to Murdoch University, and Whiteman Park northeast of Perth. Field experiments could not be implemented because of unseasonal dry-season rainfall events. Therefore, two glasshouse experiments were undertaken.

The first glasshouse trial showed that *Banksia attenuata* is drought (water deficit stress) sensitive. Seedlings were droughted and then maintained at five levels of stress for 21 days. Water levels below 40% of container capacity severely reduced photosynthesis and

growth. Even when plants were rewatered to 60 and 80% of container water capacity after wilting, they were only able to restore photosynthesis, transpiration and stomatal conductance to rates about half those of unstressed plants.

The second glasshouse trial examined the effect of 5 days of drought on the efficacy of phosphite in *Banksia attenuata* and *B. baxteri*. Phosphite was applied 1 week before drought, during the drought or 1 week after the drought. Plants sprayed before drought had higher phosphite concentrations than those sprayed after drought and concentrations were generally higher in *B. attenuata* than *B. baxteri*. Phosphite reduced stem colonisation by *P. cinnamomi* inoculated 4 weeks after phosphite was applied in all drought treatments. Phosphite reduced colonisation the most when applied before or during the drought.

This is the first study to demonstrate that short-term drought does not impair the uptake and translocation of phosphite in two *Banksia* species. This study also confirms the effectiveness of phosphite to contain *P. cinnamomi* in susceptible *Banksia* species. Recommendations for research and management are provided.

RECOMMENDATIONS

Scientific:

S6. Evaluate phosphite uptake and translocation in long-term water stress of young and mature plants in the field. In the current study, we show that phosphite applications after water deficit stress were a little less effective compared to pre- and during-water stress applications. We do not know whether under severe and prolonged water deficit stress if phosphite uptake, translocation and efficacy will be impacted on.

S7. Determine the effect of water deficit stress (especially long-term deficits) on phosphite longevity and mobilisation within the plant and efficacy in containing *P. cinnamomi* colonisation.

S8. Measure phosphite translocation over time after application to determine if translocation or uptake is affected by water deficit stress. In the current study we assessed phosphite efficacy four weeks after spraying. It is possible that assessments closer to the time of phosphite application would determine if uptake and subsequent translocation are affected by water stressed plants compared to non-stressed control plants. This is potentially relevant to managing infection occurring after the breaking of drought in autumn.

Management:

M4. As phosphite was effective in reducing colonisation of all *Banksia* treatments, phosphite levels in susceptible flora in drought-prone areas should be maintained during extended periods of drought even though the *P. cinnamomi* may not be active at that time.

M5. As it was not possible to assess the impact of long-term drought on the efficacy of phosphite uptake, phosphite application in the field should be timed so that the level of drought stress in the plant is minimal at the time of spraying. This is to ensure that sufficient phosphite uptake occurs to be

effective. Vegetation condition and rainfall data can be used to define these periods.

M6. This study only examined two *Banksia* species, and recommendations need to be applied with caution to flora that respond differently to drought stress.

FIRE

Fire is a frequent event in the Australian landscape both from prescribed burns to prevent excessive wildfire events in bushland adjacent to farmland and urban centres and due to wildfires. It is also predicted that climate change will increase the frequency of wildfires. The Mediterranean-climate in Australia tends to be in areas that have high human activity due to farming activities and to towns and larger urban centres. It is in the Mediterranean regions where *Phytophthora cinnamomi* is most prevalent, and also where there are key plant communities, such as in the 'biodiversity hotspot' in the southwest of Western Australia. Increasingly, phosphite is used to protect these key plant species and communities from the impact and severity of *P. cinnamomi.* Consequently, it is important to understand the role of fire on the efficacy of phosphite in native plant communities when it is applied (i) prior to fire, or (ii) post-fire.

Two separate experiments were conducted to determine the following: (i) if the application of phosphite pre-fire (Experiment 1), or (ii) post-fire (Experiment 2) will adversely affect the efficacy and persistence of phosphite's ability to contain *P. cinnamomi.* The experiments were conducted in the Stirling Range National Park (NP) in the southwest of Western Australia that had not been burnt for approximately 10 years. Three species were studied in detail; *Banksia attenuata* and *Adenanthos cuneatus* (both resprouter species) and *B. baueri* (a reseeder species).

Fire did not appear to have a large impact on the physiological processes of the three plant species studied in detail, although, photosynthetic rates did increase after fire in *B. attenuata* and this was likely due to the resprouting foliage having a greater photosynthetic capacity than the foliage pre-fire. Phosphite application also did not appear to affect the three species adversely in terms of physiological functions, although in *B. attenuata* it did depress transpiration of plants which were not burnt. That there was not a concomitant change in stomatal aperture suggests that the change may have been due to the water uptake and supply pathway being modulated by phosphite. However, this did not influence phosphite uptake and efficacy. Therefore, regardless of the differences in physiology all three plant species took up phosphite and distributed it throughout the plant.

For *B. attenuata*, the results clearly indicate for phosphite to be effective it should be applied at least 2 months before a burn (Experiment 1). This will allow phosphite to be effectively taken up by the plants and distributed throughout the tissues of this resprouting species. The plants are able to recover post-fire and retain sufficient phosphite *in planta* to respond effectively to challenge by *P. cinnamomi.* In contrast, the results clearly indicate that when phosphite is applied 11 months post-fire (Experiment 2) it is not taken up in sufficient quantities to effectively contain *P. cinnamomi* when challenged.

For *A. cuneatu*s, another resprouter species, phosphite was not able to control *P. cinnamomi* irrespective of whether it was applied before or after a fire (Experiments 1 and

2). This was true even with high phosphite concentrations being present in the plant tissues (even higher than in *B. attenuata*). Fire apparently inactivates the ability of phosphite to induce plant defense responses in *A. cuneatus* when challenged by *P. cinnamomi.* This response occurs beyond 11 months post-fire.

Therefore, our data show conflicting results for the two resprouter species. One is not affected by fire, whilst the other is, irrespective of whether phosphite is applied before or after fire. Consequently, it is important to study a greater range of resprouter species under similar conditions to determine how much variation occurs between resprouter species in terms of fire-phosphite interactions and their ability to contain *P. cinnamomi.* Irrespective, managers should apply phosphite to plant communities which are under threat from *P. cinnamomi* and due to be burnt at least two months prior to the burn. This should 'capture' and protect those species that respond to phosphite after fire. With regards to those species that take up phosphite but do not respond to its presence when challenged by *P. cinnamomi*, further research is required to determine how long after the burn phosphite applications become effective.

For *B. baueri*, the reseeder species phosphite was not able to control *P. cinnamomi* at both phosphite application times in the unburnt plots despite phosphite being present in all tissues. *B. baueri* is a susceptible plant species that appears not to respond to phosphite.

Fire kills reseeder species such as *B. baueri* used in the present study. Further work is required to determine how soon after germinating reseeders can be sprayed to effectively protect them from *P. cinnamomi* for approximately three years. Reapplication of phosphite every three to 5 years is the time period currently considered optimal for effective and sustained control of *P. cinnamomi* in plant communities.

RECOMMENDATIONS

Scientific:

S9. Phosphite applications at different times after fire need to be investigated to determine when phosphite becomes effective after fire. The current study showed that phosphite applied 11 months post-fire was not effective in controlling colonisation of *B. attenuata* and *A. cuneatus* stems by *P. cinnamomi*.

S10. Determine if intensity of fire affects phosphite uptake. Our prescribed burn was relatively patchy and varied largely in intensity. A wildfire and/or burning the plots with higher fuel loads would have produced a more intense fire.

S11. Measurement of both lesion length and colonisation are crucial in disease assessment of field studies. If only lesions were measured we would not have got any results for *B. baueri* where lesions were not visible and inaccurate results for unburnt *A. cuneatus* and *B. attenuata* where lesions were difficult to interpret. Plating out stem material onto *Phytophthora* selective agar beyond the point of inoculation or the lesion margin in these instances, allowed us to accurately assess colonisation.

S12. A larger range of susceptible reseeding and resprouting plant species from different genera and families need to be assessed for efficacy of phosphite

applications before and after fire. These should be selected based on their susceptibility and rarity.

S13. Further research should determine why phosphite did not contain *P. cinnamomi* colonisation in *A. cuneatus* post-fire, despite having higher phosphite concentrations in the stems than prior to fire.

S14. Further work is required on *Banksia baueri* to determine why phosphite does not appear to induce plant defenses when challenged by *P. cinnamomi.*

S15. The likely redistribution of phosphite from below ground to above ground parts of the plant after fire should be studied as if concentrations of phosphite fall below a critical threshold level in roots, this might reduce the ability of phosphite to control *P. cinnamomi*.

S16. The effective half-life of phosphite in a plant could be reduced by fire in resprouter species and needs to be investigated.

S17. The application of phosphite to reseeder species after fire needs to be studied further. After fire, reseeding species grow rapidly and it would be expected that phosphite will be accumulated in the stems and leaves which will be acting as a photosynthate sink during rapid growth. Consequently, it is expected that rapidly growing reseeder species will need more regular applications of phosphite than resprouter species. This needs to be confirmed.

Management:

M7. Sites that require a prescribed burn and need to be protected from *P. cinnamomi* should be sprayed at least 2 months prior to a prescribed burn to offer post-fire protection when the pathogen may be more active at the site (Moore, 2005).

M8. Accurate records must be maintained with regards to fire regimes and the presence of *P. cinnamomi* on sites to ensure that sites are managed strategically with regards to fire when *P. cinnamomi* is present on or adjacent to a site.

M9. Vegetation consisting of species where pre-fire phosphite applications remain effective after a fire, e.g. *B. attenuata*, does not require reapplication of phosphite immediately after a fire event. For high valued conservation species, the responsiveness of phosphite after a fire needs to be investigated to determine if applications pre-fire will be effective after the fire. For species, such as *A. cuneatus*, pre-fire phosphite applications are ineffective post-fire; therefore, species such as *A. cuneatus* will need to be resprayed at some time after fire to ensure they are protected from *P. cinnamomi*. However, actual timing of when they can be effectively sprayed to ensure phosphite induces a defense response still needs to be determined (see S13).

M10. Post-fire, it is the susceptible reseeders that will determine when phosphite should be applied, or reapplied, to a plant community. This is because reseeding species will have no phosphite present in their tissues, consequently they will need to be sprayed at a time post-germination when sufficient phosphite is taken up to allow for adequate protection whilst they are rapidly growing.

Sub Project 19.2.3 Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite?

FLOWERING

In a number of Australian plant species such as in *Banksia* species, flowering and seed cones can be substantial in size and represent a large sink for photosynthates and as a result potentially accumulate phosphite in these tissues. The question has to be asked "does phosphite accumulate in flowers and seed cones at flowering and seed development at the expense of phosphite in the roots, making plants more susceptible to challenge by *P. cinnamomi*?" This study aimed to determine whether phosphite application at the time of flowering influences the efficacy of phosphite to control *P. cinnamomi*.

The study was conducted on *Banksia attenuata* and *B. menziesii,* the former starts its flowering in spring and ends in autumn, whilst the latter starts to flower in autumn and ends in spring. Selected plants of both species were either sprayed with 0.5% phosphite plus 130 µL/L BS 1000® alcohol alkoxylate surfactant in spring or autumn and the stems of plants were underbark inoculated with *P. cinnamomi* for weeks after each of the two spray treatments. Stems were then harvested nine days after inoculation to determine if phosphite had contained colonisation by *P. cinnamomi.* The results clearly showed that the stage of flowering and fruiting had no impact on phosphite efficacy, as both *Banksia* species, despite flowering in opposite seasons, responded similarly to the two spray and inoculation events. Therefore, phosphite can be safely applied to plants during flowering with no reduction in its ability to induce host defense responses that control *P. cinnamomi.*

RECOMMENDATIONS

Scientific:

S18. The application of phosphite during flowering to a wider range of *P. cinnamomi* susceptible species is required to further confirm that flowering does not reduce the efficacy of phosphite in containing the pathogen.

Management:

M11. Phosphite can be applied to plants during flowering without reducing its efficacy to control *P. cinnamomi.*

We did not investigate multiple stress events either with the same stress type or two or more stresses. Two that are worthy of consideration because of their occurrence from time to time in Mediterranean-type ecosystems are drought followed by flood, and drought followed by fire. With predicted changes in climate, especially in the southwest of Western Australia, these stress events are likely to become more frequent. This is particularly true in relation to wild fire. Therefore, future studies should combine drought and fire when asking questions about the use of phosphite to control *P. cinnamomi* in key areas with threatened species and/or communities.

Sub Project 19.2.3 Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite?

INTRODUCTION

A major factor limiting our understanding of the control of *Phytophthora cinnamomi* with phosphite is our lack of knowledge on how the physiological status of the plant at the time of phosphite application affects the efficacy of this treatment to control disease. In this sub-project we explore the key seasonal stresses of excess water, water deficit and fire on plants, and the subsequent efficacy of phosphite on *P. cinnamomi in planta*.

The physiological status of plants is not constant but varies over time depending on developmental gene expression (e.g. leaf phenology, flowering/fruiting and senescence) and interactions with the environment (e.g. temperature, moisture, light, nutrients and other biota). In Mediterranean environments in particular, plants experience stresses due to extremes in water availability, and the incidence of wild fire is high. Furthermore, individuals and species of plants are not in synchrony due to differences in recruitment, ontogeny, longevity and rest periods. From a management point of view, where aerial application of phosphite may be restricted by climatic conditions, this presents uncertainty as to what are the most effective times for applying phosphite.

For each of the key stresses we will measure plant water potential, and gas exchange in the leaf including transpiration, leaf stomatal conductance, vapour pressure deficits and the internal CO_2 concentration. There is little information in the literature on physiology of Australian native plant species of interest, so a series of preliminary experiments were undertaken to determine the optimum times to undertake physiological measurements. In addition, there is a lack of information on the effect of stress in *Banksia*, or other native species, so a preliminary waterlogging experiment was undertaken to determine time to death, and the time at which photosynthetic activity was reduced to half of normal values. The information obtained in these preliminary studies was used to establish the more comprehensive and detailed main experiments.

Physiological measurements

Water Potential

The plant water potential signifies the demand for water within a plant. This is influenced by the soil moisture tension in the rooting zone, the resistance to water movement within the plant and the demand for transpiration imposed by the environment. Measurements of water potential were taken using a pressure chamber (Model 1000; PMS Instrument Company, Oregon, USA; Figure 1).

The pre-dawn leaf or stem water potential (Ψmax) was used to estimate changes in minimum levels of water stress (Scholander *et al*. 1965). Predawn measurements were completed before sunrise when the plant water potential is most stable and usually at the minimum for the day. This value is close to equilibrium with the soil moisture (under most conditions). The soil water potential is usually between 0 to -2 MPa during the main rainfall season (Lambers *et al*. 1998), however, if the plant is stressed this value will be more negative.

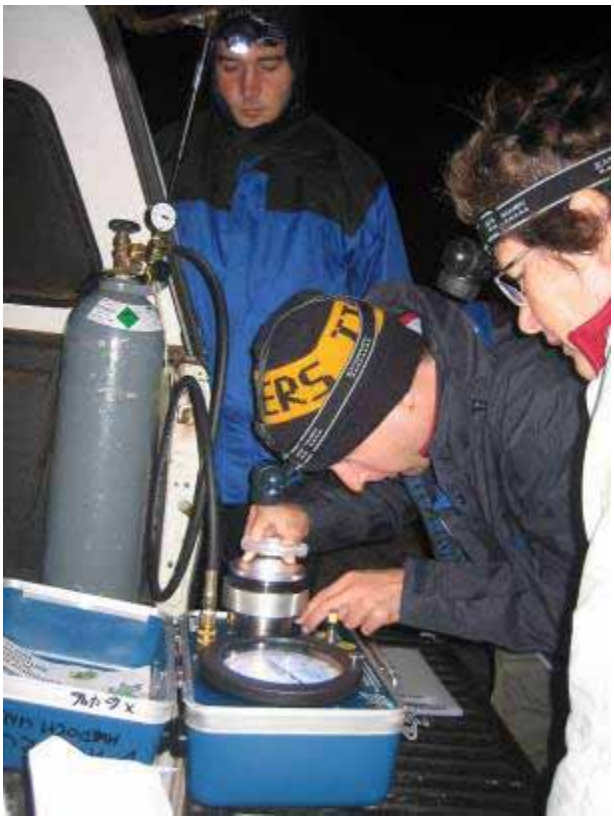
Midday leaf water potential (Ψ) readings were taken during the 2 - 3 h peak midday period which can vary for plant species and areas.

On each tree, a minimum of two leaves (or shoot tips with several leaves in the case of plants with small leaves such as *Adenanthos cuneatus*) were selected from main branches near the trunk. Leaves were removed and measurements immediately made on one leaf by inserting the leaf into the chamber and applying increasing nitrogen gas until the xylem fluids were released from the freshly cut petiole or shoot stem.

Figure 1

Water potential measurements taken pre-dawn in the field ➢

(Photos: M Sommeechai)



The pressure chamber used (Model 1000; PMS Instrument Company, Oregon, USA) ➢

Gas Exchange

A CIRAS-2 (PP Systems, Massachusetts, USA) was used to measure gas exchange in the leaf. In addition, CIRAS-2 provides an assessment of the ambient light intensity by measuring photosynthetically active radiation (PAR, µmol $m^{-2}s^{-1}$). The narrow chamber was always used (Figure 2). Where leaves were too big to be fully enclosed in the chamber the gas exchange values were determined by accounting for the proportion of leaf surface measured as a proportion of the total leaf surface.

Figure 2
The CIRAS-2 (PP Systems, Massachusetts, USA) being used to measure gas exchange in a leaf of *Banksia baxteri*

(Photo: N Long)

Transpiration
Transpiration is a passive process that is largely controlled by the atmospheric humidity and the moisture content of the soil. It enables mass flow of mineral nutrients from the roots to the shoots. This is caused by the decrease in water pressure in the foliage due to diffusion of water out of stomata. The rate of transpiration (mmol $m^{-2}s^{-1}$) is directly related to the degree to which the stomata are open or closed.

Photosynthesis
Photosynthesis is the conversion of light energy into chemical energy. The rate of photosynthesis (measured as µmol $m^{-2}s^{-1}$) is affected by CO_2 concentration, light intensity and temperature. In the mesophyll cells of leaves (and sometimes stems) water is split in the light reaction of photosynthesis generating O_2.

Leaf Stomatal Conductance
The stomata play a key role in minimising the loss of water from plants relative to CO_2 uptake (Jones 1998). Stomata close in response to a decrease in leaf water potential, relative water content or turgor pressure and allow osmotic adjustment to occur within the leaf. In wheat and lupin there is a close relationship between stomatal conductance (µmol $m^{-2}s^{-1}$) and soil water status (Henson *et al*. 1989).

Vapour Pressure Deficits
Leaf water potential readings can vary from day to day due to differences in daily vapour pressure deficits (VPD). McCutchan and Shackel (1992) have shown that midday stem water potential is negatively correlated with vapour pressure deficit (VPD), which can be expressed as a function of temperature and humidity. The CIRAS-2 was used to calculate the VPD from the average of the recorded minimum and maximum temperature values during leaf water potential measurements and is presented as kPa.

Internal CO_2 Concentration
The internal CO_2 concentration of the leaf provides the primary substrate for photosynthesis, and balances the biochemical and diffusion pathways used by photosynthesis. These measurements are presented as μmol^{-1}.

Phosphite
Phosphite is systemic and is transported in the xylem and phloem (Cohen and Coffey 1986). It can be applied as a root drench, stem injection or foliar spray and is transported

to the root tissue, the primary site of infection by *P. cinnamomi* (Wilkinson *et al*. 2001). It has been shown to act directly on the pathogen by decreasing the production of zoospores by *P. cinnamomi in planta* (Wilkinson *et al*. 2001) and indirectly by protecting roots from infection by *P. cinnamomi* (Jackson *et al*. 2000). Glasshouse tests on *B. grandis* show that spray inoculation of phosphite significantly reduces the production of zoospores within seedlings (Wilkinson *et al*. 2001).

The effect of the plant's physiological status on the efficacy of phosphite in controlling P. cinnamomi

The physiological status of the plant at the time of phosphite application could impact on the efficacy of phosphite by:

1 reducing the uptake of phosphite applied to the shoot;
2 reducing the transport of phosphite from the shoot to the root;
3 reducing the persistence of phosphite within the plant;
4 reducing the remobilisation of phosphite to new susceptible organs as they form;
5 reducing the effectiveness of phosphite on containing *P. cinnamomi* by dampening its mode of action; and
6 causing leaf-drop and loss of phosphite in abscised leaves consequently diluting phosphite out of the plant.

Plants can respond to specific types of stress, and each of these responses can influence the efficacy of phosphite *in planta* (Table 1).

Table 1 Possible impacts of stress on uptake and transport of phosphite to roots.

Stress		**Type of plant response**	**Likely impact on efficacy of phosphite**
Drought	Dehydration avoidance	Deep root systems	Low if stomata remain open and carbon (C) is being transported to roots
	Dehydration tolerance	Osmotic adjustment, stomatal closure, leaf shedding	High if stomata close restricting uptake, if there is restricted C transport to roots, and if phosphite is lost in shed parts
Waterlogging		Transpiration shuts down	High if stomata close restricting uptake
		Carbon redirected to shoot	High if there is restricted C transport to roots
Fire		Reseeders	Low if seedlings are actively growing
		Resprouters	Recent new growth should not restrict uptake but there may be less transport to roots if C is preferentially directed to new shoot growth
Reproductive demand		Intense flowering/fruiting	High if there is restricted C transport to roots

Sub Project 19.2.3 Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite?

Phosphite application and analysis

In all experiments, phosphite (Agrifos 600; Agrichem, Loganholme, Qld, Australia) was applied as a 40% solution by spraying with a Microfit low-volume fine mist applicator (Micron Sprayers Ltd., Herefordshire, UK) at 24 kg/ha. This is the standard rate of aerial application of phosphite used by the Department of Environment and Conservation, Western Australia and others to control *P. cinnamomi* in natural ecosystems. It should be noted the 24 kg/ha rate we used is not comparable to the 0.5% application rate used by others in backpack (high volume) applications to run-off (Colin Crane, *pers. comm*.). To the phosphite solution, 0.2% (v/v) of the adjuvant BS1000® (100% alcohol alkoxylate; Crop Care Australasia, Murarrie, QLD) was added and agitated prior to spraying. The Microfit applicator (Figure 3) simulates as closely as possible the aerial broad scale treatment of natural ecosystems with phosphite.

The phosphite concentration of the leaf, stem and root were determined for the three replicate plants (randomly selected) for which physiological measurements were also recorded. Leaf and stem samples were washed in 1% Deconex 15E (phosphate free detergent; In Vitro Technologies, Noble Park North, Vic) solution and rinsed twice in DI water. All samples were dried at 60°C for 4 days, ground to 1 mm and sent to the WA Chemistry Centre (Perth) for phosphite analysis. To 0.5 g ground sample, 5 mL of 0.1 M sulphuric acid was added and extractions occurred overnight on a roller-shaker. Following 20 min centrifugation at 6970 g, 100 µL of the clear acid extract was added to 1 mL of 50 µg/mL methyl phosphonic acid in methanol (internal standard solution). A phosphite standard curve was prepared by adding 100 µL of solutions containing from 0.05 to 100 µg mL^{-1} phosphite to 11 tubes containing 1 mL of internal standard solution. The solutions were mixed and diazomethane was added to 400 µL of the samples in excess until a persistent yellow colour was observed. Excess diazomethane was neutralised with a few drops of 2% acetic acid, then the dimethyl phosphite content was determined by gas chromatography. A splitless injection with a D.B-Wax column (J & W Scientific, Salsam, CA) and a phosphorous-specific flame photometric detector (Hewlett Packard, USA) were used. The limit of quantitation was 0.5 µg g^{-1} dry weight. A replicate sample was taken every ten samples to provide a control during the analysis. Two control samples of known phosphite content were included in each batch of 40 samples analysed.

Figure 3

➢ Microfit low-volume fine mist applicator (Micron Sprayers Ltd., Herefordshire, UK)

Using the applicator in the field ➢

(Photos: T Paap)

Phytophthora cinnamomi

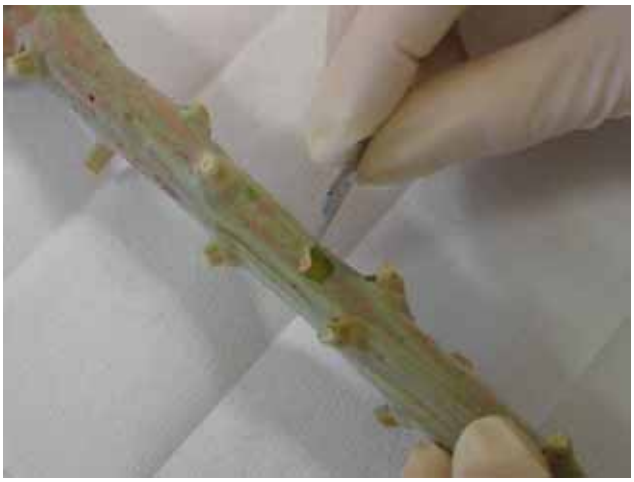
Inoculation

Stems of plants were inoculated in the glasshouse and field trials using the under bark method (Figure 4) as described in Hüberli *et al*. (2001) with one isolate of *P. cinnamomi* recovered from the Stirling Ranges National Park, adjacent to the experimental fire plots. To avoid the introduction of a foreign isolate to the Stirling Ranges National Park a local isolate SR2 was chosen. The isolate was selected on the basis of a preliminary inoculation experiment comparing four Stirling Range isolates with MP94-48, a highly pathogenic isolate (Hüberli 1995). Two inoculation experiments were undertaken over two weeks. In the first inoculation experiment, detached *B. grandis* stems were inoculated under bark and incubated at 28°C in the laboratory. A second experiment used *B. grandis* seedlings and these were inoculated under bark on the stem and then incubated in the glasshouse.

Figure 4

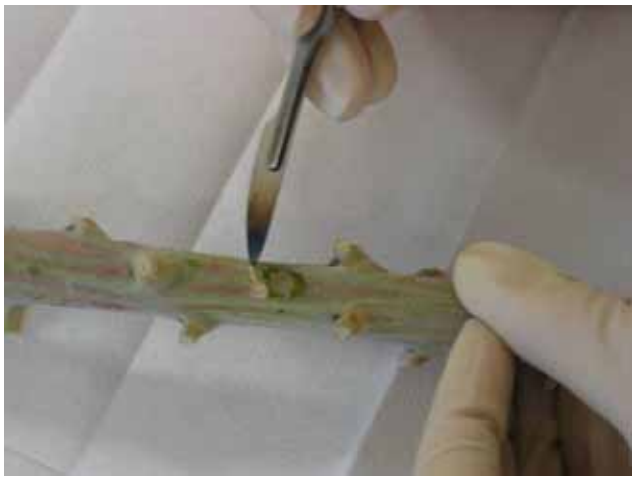
Under bark inoculation of a stem with *Phytophthora cinnamomi.*

➢ Cutting a flap in the bark of the stem avoiding damage to the cambium

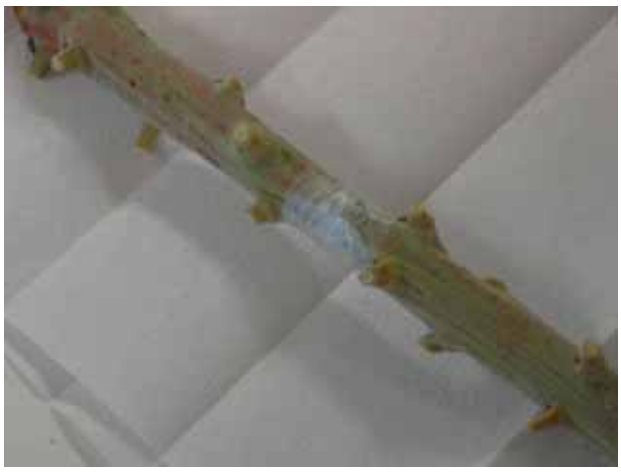


➢ The flap is lifted

(Photos: D Hüberli)



➢ The inoculum is inserted, then the flap is closed



The site of inoculation is wrapped to retain moisture and hold the inoculum in place ➢

Measurement of infection

At harvest, 2 - 4 weeks after inoculation, the inoculated side branches were removed at the trunk and returned to the laboratory. The outer bark was carefully scraped back to uncover the lesion which was measured (Figure 5). To determine the extent of spread of *P. cinnamomi* in the stem, five 1 cm sections of the stem from the lesion front and subsequent 1 cm section up to 5 cm beyond the lesion front were plated onto NARPH, a *Phytophthora* selective agar medium (Hüberli *et al.* 2000).

Figure 5

The outer bark has been stripped from this branch to expose the lesion (marked by red arrows) which has extended from the point of inoculation (blue arrow).

(Photo: D Hüberli)

Sub Project 19.2.3 Does the physiological status of the plant at the time of spraying affect the efficacy of phosphite?

SUMMARY OF EXPERIMENTS

The effect of the four key stresses on the efficacy of phosphite, and preliminary experiments are presented in the following five sections.

Preliminary Measurements

Preliminary field and glasshouse experiments determined the optimal time to take physiological measurements, the physiological variation within and between species, and the time it took to halve the photosynthetic activity of *Banksia* under waterlogged conditions. The information obtained in the preliminary studies was then used to establish the main experiments.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-waterlogging

These glasshouse experiments examined the physiological responses of two *Banksia* species to short term waterlogging, and recovery after waterlogging. Plant health (shoot height and weight, root weight, and physiological measurements), disease progression (symptoms, lesions and pathogen extension *in planta*) and translocation of phosphite by High Performance Ion Chromatography (HPIC) of tissue samples were measured.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-water deficit

These experiments examined the effect of water deficit on *Banksia attenuata* and *B. baxteri* in both the glasshouse and in the field. Plant health (shoot height and weight, root weight, physiological measurements), disease progression (lesions) and translocation of phosphite (HPIC of tissue samples) were measured.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-fire

This experiment examined the effect of fire in the field on two species of *Banksia* and *Adenanthos cuneatus*. Plant health (recovery and physiological measurements), disease progression (lesions) and translocation of phosphite (HPIC of tissue samples) were measured.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated during flowering

This experiment examined how reproductive growth at the time of phosphite application affects the efficacy of phosphite to control the pathogen.

AIMS

To provide managers with improved operational guidelines for the use of phosphite to control *P. cinnamomi* in the conservation estate by obtaining information on how:

1. abiotic stresses, such as drought and waterlogging at the time of phosphite application, influence the efficacy of phosphite to control the pathogen; and
2. the fire history at the time of phosphite application influences the efficacy of phosphite to control the pathogen.
3. reproductive growth at the time of phosphite application influences the efficacy of phosphite to control the pathogen.

INTRODUCTION

Preliminary field and glasshouse experiments determined where stomata were located, the optimal time to take physiological measurements, the physiological variation within and between species, and the time it took to half the photosynthetic activity of *Banksia* under waterlogged conditions. The information obtained in these preliminary studies has been used to establish the three main experiments; the effect of waterlogging, drought and fire on efficacy of phosphite.

METHODS

Preliminary experiment 1: Physiological variation within species and stomata

Gas exchange and water potential measurements were taken in order to compare the health of plants provided by two nurseries to determine if there was a photosynthetic difference between the populations (Nindethana Seed Service, Albany WA) that may compromise the experiment.

The Plants

Banksia attenuata *Banksia baxteri* *Banksia grandis* *Banksia littoralis*

Seedlings of *B. attenuata, B. baxteri, B. grandis* and *B. littoralis* were purchased from Australian Native Nurseries (Oakford, WA) and Muchea Tree Farm (Muchea, WA). For each species, plants of a similar size were selected. They were re-potted into free draining 100 mm polyethylene pots containing composted pine bark, coarse river sand and cocopeat fibre (2:2:1; Richgro Garden Products, Canning Vale, WA) with added basal fertiliser (O'Gara *et al*. 1996) and a low phosphate fertiliser (Osmocote Plus Native Gardens, Scotts Australia, NSW). They were kept in a temperature-controlled glasshouse (6 to 23°C) where they were watered daily.

Stomata Morphology

Leaf sections were fixed in formal-acetic-alcohol and embedding in paraffin wax. Thin sections were viewed with an Olympus UV filter set U-MWU2, which consists of an Excitation Filter at 330 - 385 nm, a Dichromatic Mirror at 400 nm and an Emission (barrier) Filter at 420 nm.

Physiological Measurements

Details of the apparatus used for the physiological measurements are given in the introduction. One set of each measurements were taken at midday for three replicate plants of each species, with ten measurements per plant.

Preliminary experiment 2: Variation between species and determination of optimal time to take physiological measurements

A preliminary study on four *Banksia* species (*Banksia hookeriana, B. media, B. petiolaris* and *B. grandis*) was conducted on trees on the Murdoch University campus to determine the optimum time to take field measurements of photosynthesis, leaf transpiration, leaf stomatal conductance, leaf internal CO_2 concentration and leaf vapour pressure deficit. These were taken at approximately 45 min intervals between 9.30 am and 4.00 pm on 22 June 2006 totalling ten measurements per plant for each physiological feature.

Measurements were repeated on 26 June 2006 to confirm the results from the previous day, again using trees on Murdoch University campus. In this study, only *B. media* and *B. petiolaris* were measured. There were three replicate trees per species and measurements were taken between 8.30 and 11.15 am, with five measurements per plant.

Preliminary experiment 3: Photosynthetic activity under waterlogged conditions

Seedlings of *B. attenuata* and *B. baxteri*, also purchased from Australian Native Nurseries and maintained in the glasshouse (as above), were subjected to waterlogged conditions to determine the time it took for the photosynthetic rate to drop to half of the normal peak and "the time to death" of each species. The predawn water potential, photosynthetic rate, gas exchange and fluorescence were measured in five plants of each species every two days for two weeks, and then at 21 days.

Growth chamber

Plants were randomised in trays in a growth chamber (Figure 6) with a photoperiod of 12 h with six sodium lamps (each light approximately 1000 W) providing a photosynthetically active radiation (PAR) of 500 ± 250 µmol $m^{-2}s^{-1}$. Plants were placed so their tips were 1 m from the light source. The temperature was 22 ± 1°C during the "light period" and 18 ± 1°C during the "dark period", and the plants were watered daily to field capacity. The level of water for the waterlogged plants was maintained at 1 cm above the soil surface. The relative humidity and the temperature within the growth chamber were measured every 15 min by a data logger during the experiment. The experiment began after one week of acclimation in the growth chamber.

(Photo: N Long)

Figure 6 *Banksia attenuata* and *B. baxteri* in trays in growth chamber.

Waterlogging

The waterlogging treatment was achieved by placing a plastic bag over the bottom of the pot and flooding the pot. Water was then maintained about 1 cm above the soil surface. All non-waterlogged plants were free draining and watered daily to container capacity, while those that were waterlogged had their water levels topped up to maintain water 1 cm

above the soil surface. The *Banksia* were either kept under waterlogged conditions for 21 days (WL21); waterlogged until the photosynthetic rate dropped to 50% of the T_0 measurement – which occurred at day 8 (WL8); or free draining (C).

Physiological Measurements
For each of the three treatments (C, WL8 and WL21) there were four, four and three replicate plants for measuring gas exchange, fluorescence and water potential, respectively.

Prior to waterlogging and every second day between 1 - 3 pm, the time of peak photosynthesis, gas exchange measurements were taken from four replicates (total of 12 plants for the three treatments, Control, WL8 and WL21). The same leaf on each plant was measured for the duration of the experiment. When photosynthesis dropped to 50% of the peak rate at T_0 the measurement intervals were increased to biweekly or weekly. The water potential measurements were taken on three replicate plants at T_0 and then at weekly intervals (T_7, T_{14} and T_{21}). Plants were only used once for water potential measurements as leaves needed to be sacrificed at each measurement and excessive leaf removal may alter plant physiology. Twelve plants was the maximum number from which it was possible to take physiological measurements at the optimum time of photosynthesis.

Photosynthesis/Fluorescence
The Photosystem II is considered to be the most vulnerable part of the photosynthetic apparatus to light-induced damage. Photosynthesis, heat dissipation and fluorescence are the three pathways possible for a photon when light energy is absorbed by a chlorophyll molecule. These three processes occur in competition.

A pulse amplitude modulated fluorometer (a Diving PAM; Walz, Effeltrich, Germany; Figure 7) was used to measure the fluorescence produced by the PSII to provide information on photosynthetic activity of the plant. This instrument is specialised for quick and reliable assessment of the effective quantum yield of photochemical energy conversion. It applies pulse modulated measuring light for selective detection of the yield of chlorophyll fluorescence (Fv/Fm).

Figure 7

A pulse amplitude modulated fluorometer (Diving PAM; Walz, Effeltrich, Germany) was used to measure fluorescence. Here a leaf of *Banksia attenuata* is being measured.

(Photo: N Long)

Growth
A photographic record was taken to show the effect of waterlogging on the roots of the two species.

Statistical analyses
Data were analysed by STUDENT and ANOVA tests, using the software "R" version 2.2.0 (GNU project, Boston, MA, USA). Results were reported as significant where $p < 0.05$.

RESULTS

Preliminary experiment 1: Variation within species

There was no significant difference in any of the physiological measurements taken in the *B. attenuata, B. baxteri* and *B. grandis* plants sourced from the two nurseries so these species were used in the third preliminary experiment. However, there was a significant difference in several of the measurements made on *B. littoralis,* which was attributed to a difference in age between the two groups of plants, so they were not used (data not included).

All species had stomata on the underside of the leaves and none were found on the upper surface (Figure 8). In *B. baxteri* the stomata were located within leaf pits.

Preliminary experiment 2: Variation between species and determination of optimal time to take measurements

Variation between species
While there were differences between *Banksia* species, especially in the leaf transpiration measurements, the photosynthesis, leaf stomatal conductance, leaf vapour pressure and leaf CO_2 concentration measurements were similar between species (Figure 9). *B. hookeriana* displayed the least amount of change during the day in most measurements, but had a similar internal CO_2 concentration to the other three species.

Regardless of species, measurements of transpiration, photosynthesis and stomatal conductance were found to be highest between 10 am to 12 noon (Figure 9). After this time period, as a result of partial stomatal closure, both transpiration and photosynthesis declined. Stomata appeared to be opening again at about 3 pm with associated increases in photosynthesis and transpiration.

Determination of optimal time to take measurements
Both species responded similarly (Figure 10). A large increase in VPD after 9 am appears to have had a direct inverse effect on stomatal conductance for both species (Figure 10). This resulted in lower than anticipated values for transpiration and photosynthesis. On this particular day of measurement, the peak photosynthetic activity had occurred at suboptimal levels between 9 and 10 am. The time period of optimal level was shorter by about 1 h compared to the previous measurement (Figures 9 and 10).

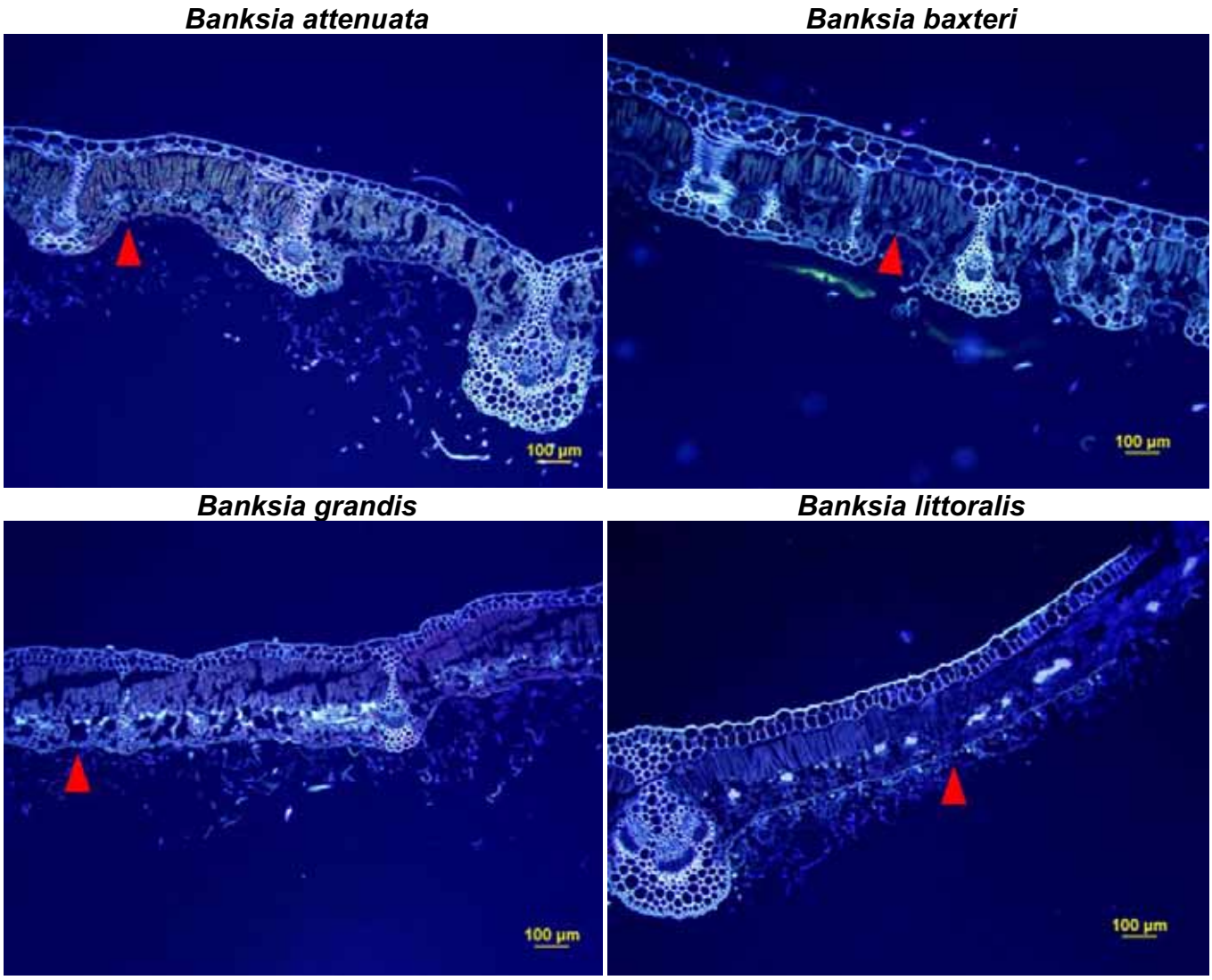


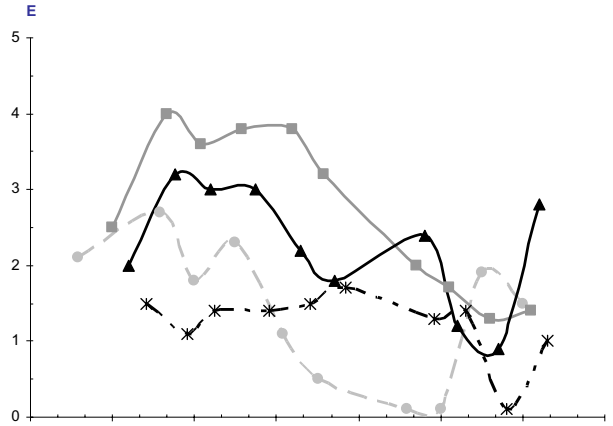
Figure 8 Transverse sections of leaves of *Banksia attenuata*, *B. baxteri*, *B. grandis* and *B. littoralis* under UV radiation. Red arrow shows stomata, which only occur on the abaxial surface of the leaf. (Photos: N Long)

Preliminary experiment 3: Photosynthetic activity under waterlogged conditions

Under waterlogged conditions, the photosynthetic activity of *B. attenuata* halved by day 8 (Figure 11), while the activity of *B. baxteri* was not affected during predawn measurements, and decreased only slightly during midday measurements. Photosynthetic activity for the WL8 treatment improved six days after the treatment ended for *B. attenuata* in both predawn and midday measurements. This indicates there is a lag period prior to recovery of photosynthetic activity after free-draining. For *B. baxteri*, no difference was observed during midday measurements for the WL8 treatment.

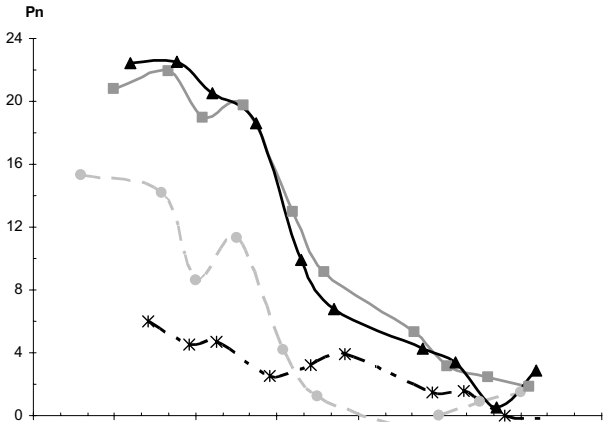
TRANSPIRATION
E= transpiration rate (mmol $m^{-2}s^{-1}$)

Leaf transpiration peaked around 11 am for all species except *B. hookeriana* where there was little change throughout the day.



PHOTOSYNTHESIS
Pn = net photosynthetic rate (µmol $m^{-2}s^{-1}$)

Maximum photosynthetic activity was usually reached before midday, between 9 and 11 am. While *B. petiolaris* and *B. hookeriana* had lower Pn values than the other two species, the maximum peaked at the same time of day as the other two species.



LEAF STOMATAL CONDUCTANCE
GS = stomatal conductance (µmol $m^{-2}s^{-1}$)

By 11am the leaf stomatal conductance and internal CO_2 concentration was declining for all species except for *B. hookeriana*.

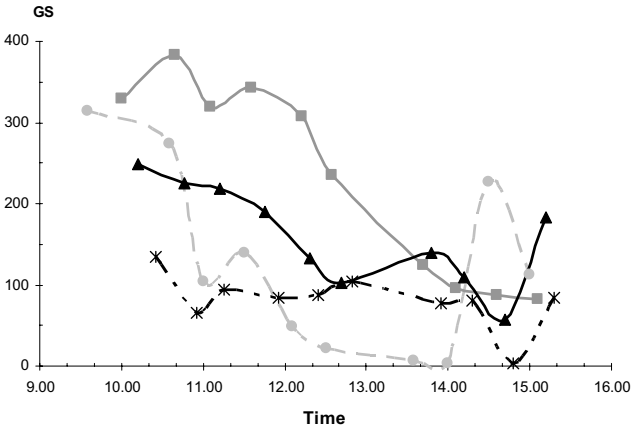
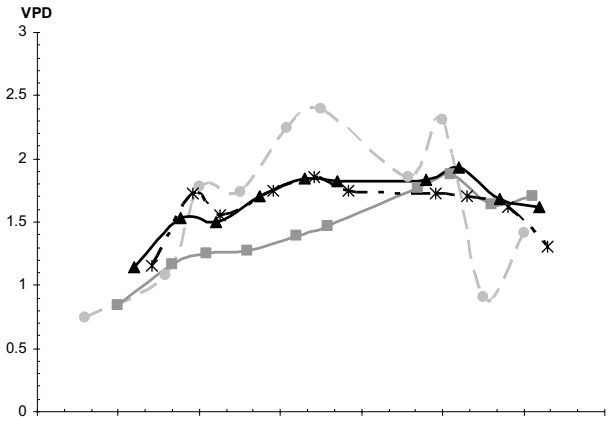


Figure 9 Physiological measurements taken on: *Banksia grandis* ( ), *B. hookeriana* ( ), *B. media* ( ) and *B. petiolaris* ( ) on Murdoch University campus to identify the peak period of photosynthetic activity. Data are means of one leaf from three replicate plants.

LEAF VAPOUR PRESSURE DEFICIT
VPD = kPa

The VPD peaked around midday for all species except for *B. grandis* which peaked at 2pm. *B. hookeriana* and *B. media* maintained a relatively constant pressure, while *B. petiolaris* was more variable.



LEAF CO_2 CONCENTRATION
Ci = Internal CO_2 concentration (PAR, µmol $m^{-2}s^{-1}$)

The internal CO_2 concentration of all species fell during the morning and then levelled off during the afternoon, with the exception of *B. petiolaris*, where the concentration rose after midday then continued to drop steadily.

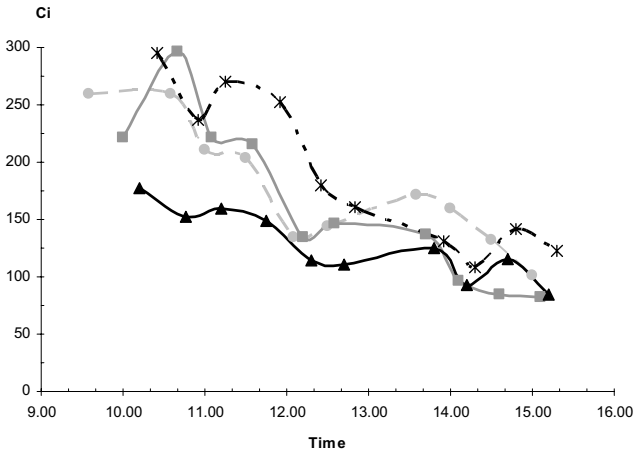
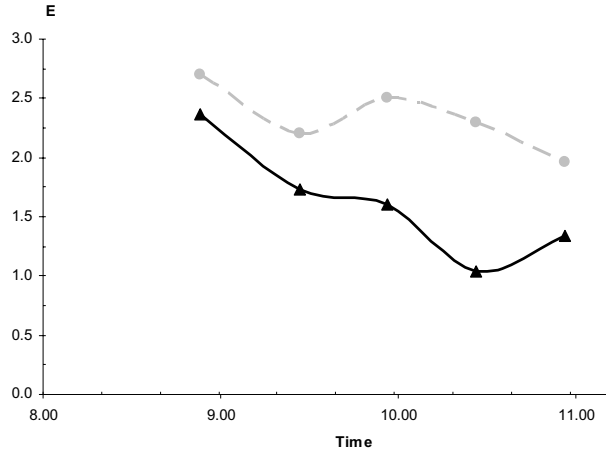


Figure 9 cont. Physiological measurements taken on: *Banksia grandis* (—■—), *B. hookeriana* (—✳—), *B. media* (—▲—) and *B. petiolaris* (—●—) on Murdoch University campus to identify the peak period of photosynthetic activity. Data are means of one leaf from three replicate plants.

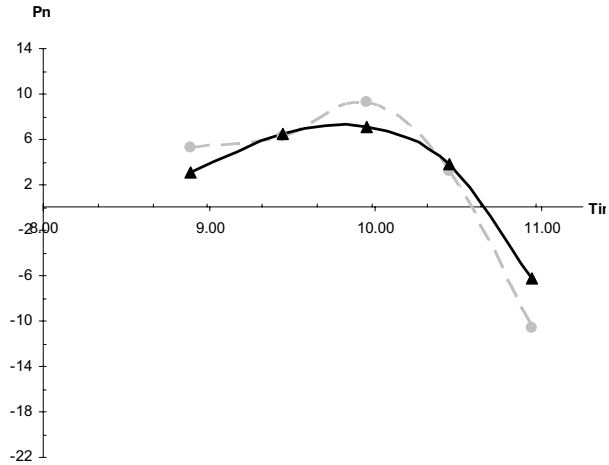
TRANSPIRATION
E= transpiration rate (mmol $m^{-2}s^{-1}$)

The transpiration rate declines between 9 and 11 am with some fluctuation.



PHOTOSYNTHESIS
Pn = net photosynthetic rate (µmol $m^{-2}s^{-1}$)

Maximum photosynthetic activity occurred at around 10 am.



LEAF STOMATAL CONDUCTANCE
GS = stomatal conductance (mmol $m^{-2}s^{-1}$)

By 10 am the decline of the leaf stomatal conductance halted and was relatively stable for the following hour.

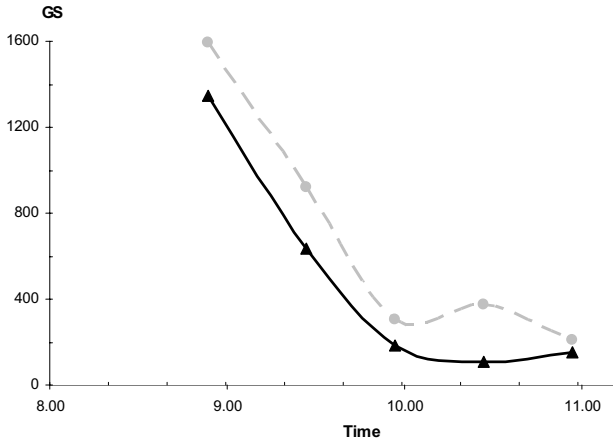
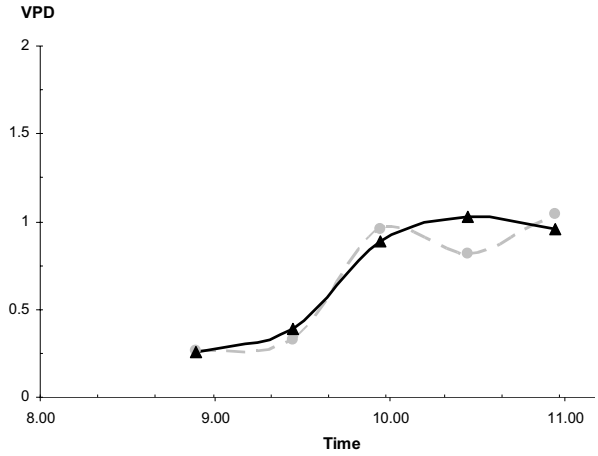


Figure 10 Mean physiological measurements taken on *Banksia media* (▲) and *B. petiolaris* (●) on Murdoch University campus to identify the peak period of photosynthetic activity. Data are means of one leaf from three replicate plants.

LEAF VAPOUR PRESSURE DEFICIT
VPD = kPa

The VPD peaked around 10.30 am in *B. media* while *B. petiolaris* was again more variable.



LEAF CO_2 CONCENTRATION
Ci = Internal CO_2 concentration (μmol $m^{-2}s^{-1}$)

The internal CO_2 concentration was lowest around 10 am for both species.

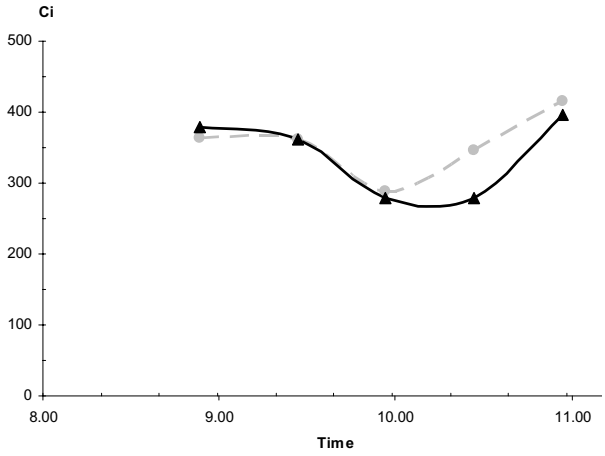


Figure 10 cont. Mean physiological measurements taken on *Banksia media* (▲) and *B. petiolaris* (●) on Murdoch University campus to identify the peak period of photosynthetic activity. Data are means of one leaf of three replicate plants.

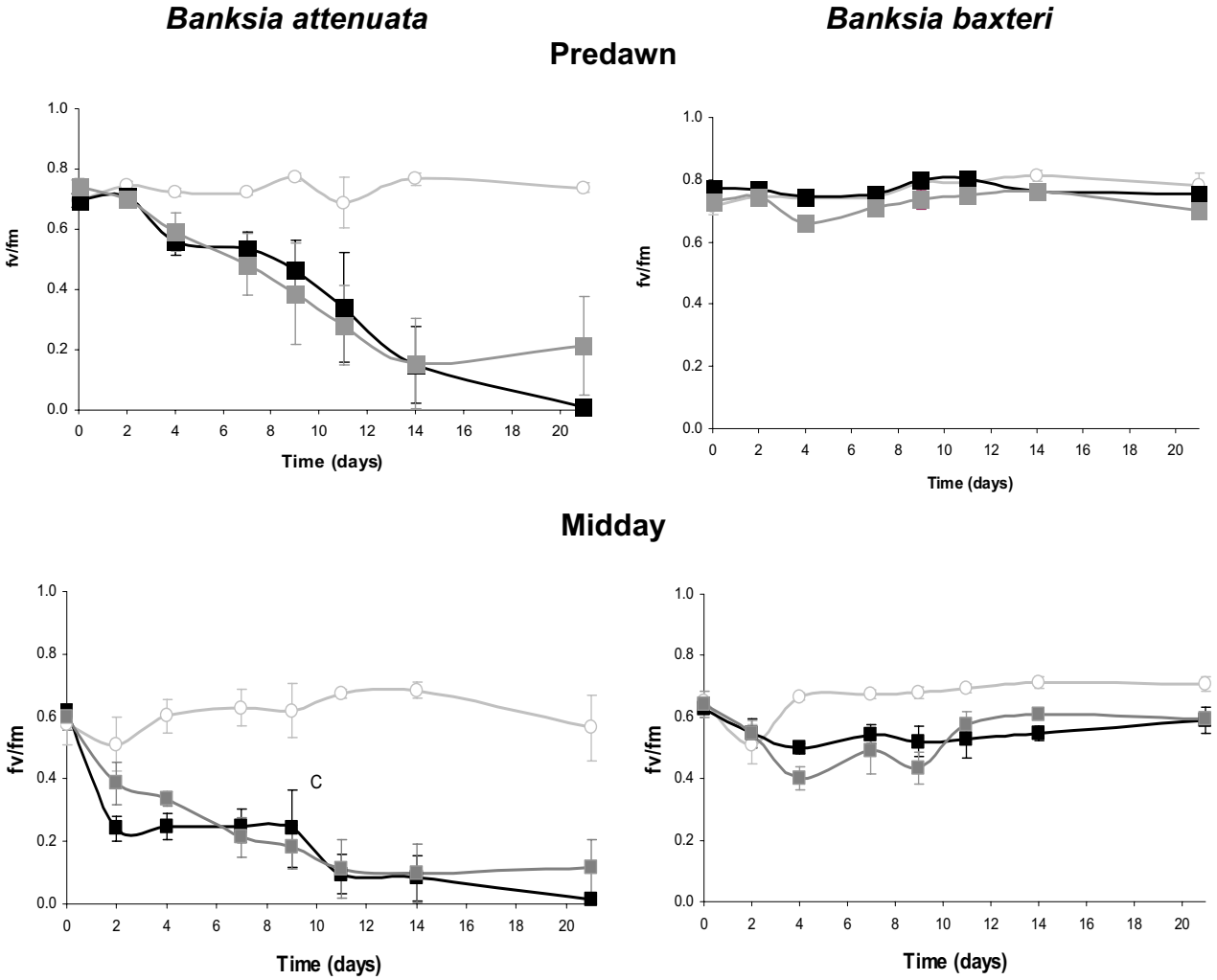


Figure 11 Photochemical yield (F_v/F_m) in leaves of *Banksia attenuata and B. baxteri* under waterlogged conditions in the glasshouse. Measurements were taken on three replicate plants, with three treatments: Control, no treatment (—○—); WL8, waterlogged for 8 days and then free draining (—■—); and WL21, waterlogged for the duration of the experiment (—■—).

There was no evidence of any recovery of *B. attenuata* when returned to free-draining conditions after 8 days of waterlogging (Figure 12). *B. baxteri* was more tolerant than *B. attenuata* to waterlogging. There were no differences in water-potential for this species when waterlogged compared to the control. Reduction of photosynthesis, transpiration and stomatal conductance took four days to plateau before stabilising for *B. baxteri* Additionally, after waterlogging for 8 days plants showed evidence of recovery by 14 - 21 days, although gas exchange measurements did not return to control levels. Recovery of *B. baxteri, B. grandis* and *B. littoralis* is examined further in the waterlogging Experiment 1.

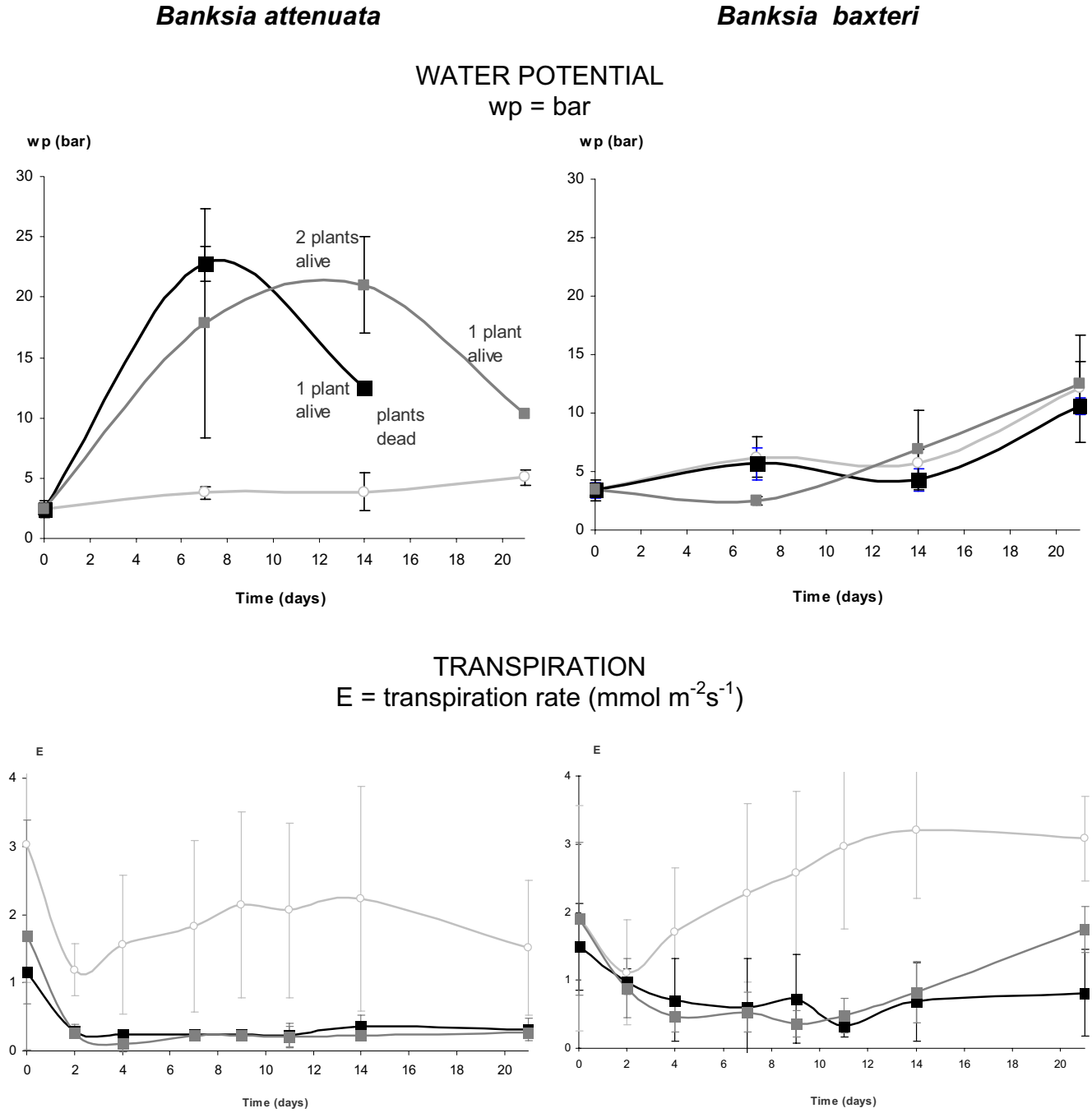
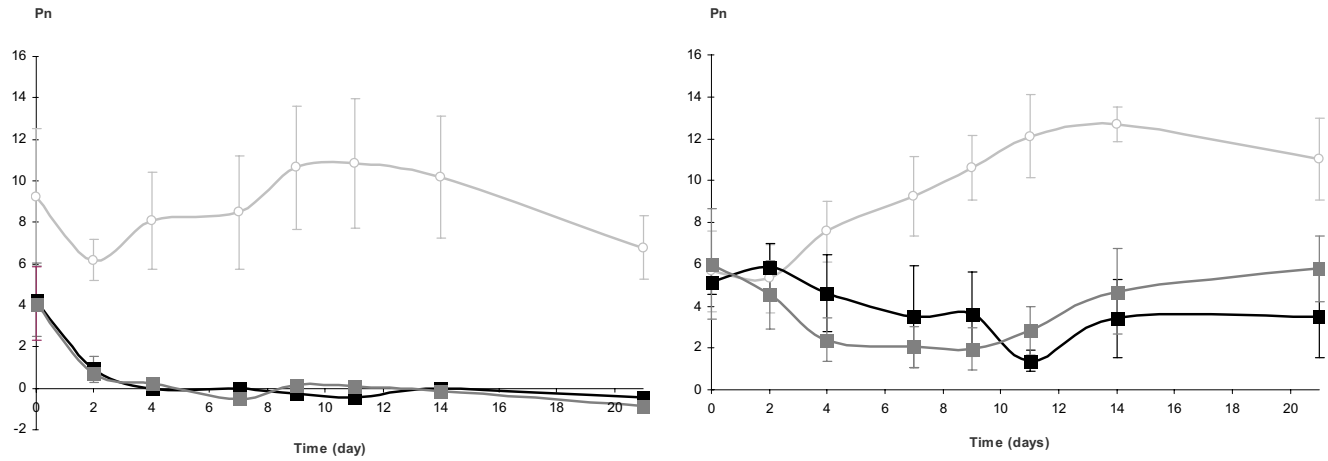


Figure 12 Mean physiological measurements taken on *Banksia attenuata and B. baxteri* under waterlogged conditions in the glasshouse. Measurements were taken on four replicate plants, with three treatments: Control, no treatment (—○—); WL8, waterlogged for 8 days and then free draining (—■—); and WL21, waterlogged for the duration of the experiment (—■—).

Banksia attenuata ***Banksia baxteri***

PHOTOSYNTHESIS
Pn = net photosynthetic rate (µmol $m^{-2}s^{-1}$)



LEAF STOMATAL CONDUCTANCE
GS = stomatal conductance (µmol $m^{-2}s^{-1}$)

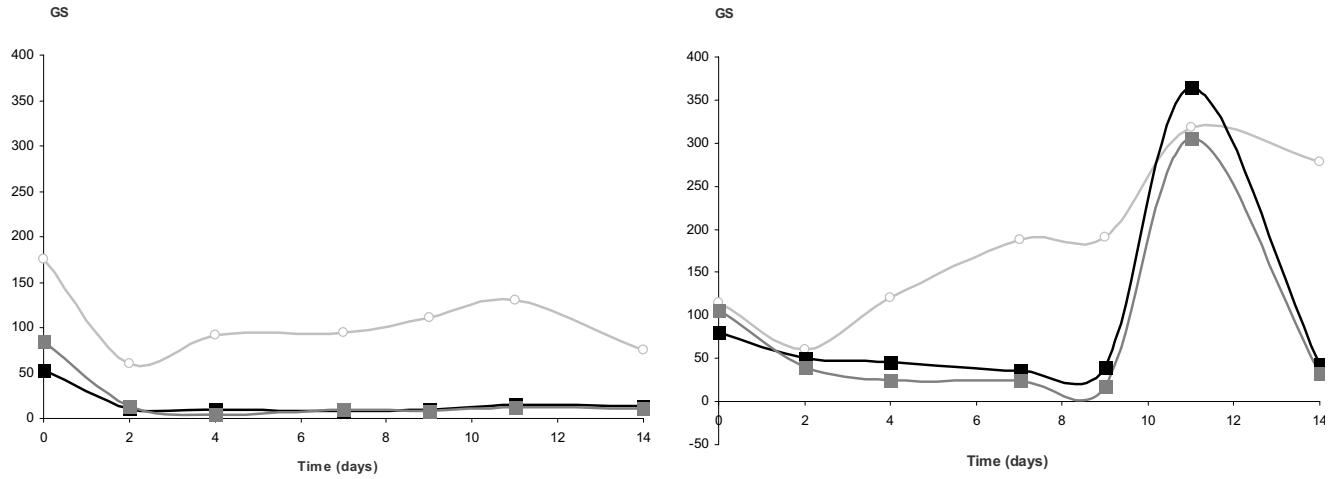


Figure 12 Mean physiological measurements taken on *Banksia attenuata and B. baxteri* under waterlogged conditions in the glasshouse. Measurements were taken on four replicate plants, with three treatments: Control, no treatment (—○—); WL8, waterlogged for 8 days and then free draining (—■—); and WL21, waterlogged for the duration of the experiment (—■—).

Banksia attenuata ***Banksia baxteri***

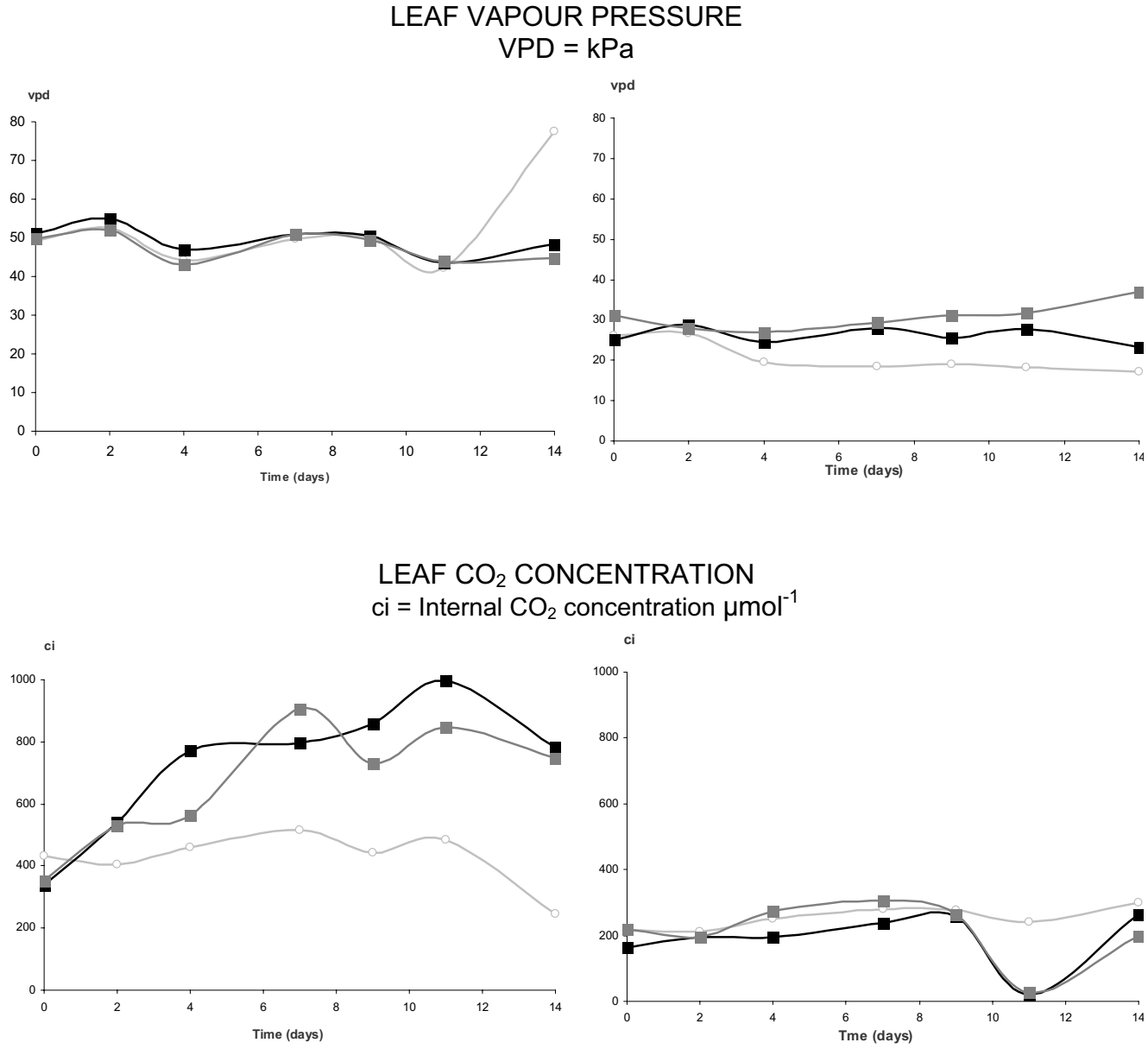


Figure 12 cont. Mean physiological measurements taken on *Banksia attenuata and B. baxteri* under waterlogged conditions in the glasshouse. Measurements were taken on four replicate plants, with three treatments: Control, no treatment (—○—); WL8, waterlogged for 8 days and then free draining (—■—); and WL21, waterlogged for the duration of the experiment (—■—).

Plant Growth and Survival
Under waterlogged conditions, *B. attenuata* plants died within 21 days, while *B. baxteri* showed little response (Figure 13a). At the end of the experiment the control plants of *B. attenuata* had more root mass than the waterlogged ones, as growth continued through the experiment (not measured). In *B. baxteri*, the waterlogged root systems of WL8 and WL21 were necrotic and slightly smaller than those of the control plants (Figure 13b).

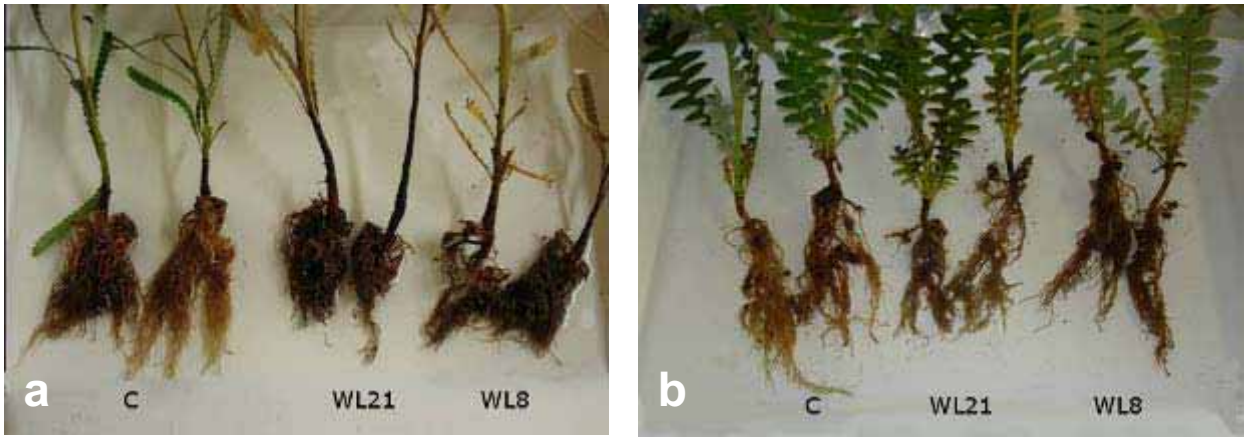


Figure 13 Roots and shoots of **a**) *Banksia attenuata* and **b**) *B. baxteri* after waterlogging. Treatments were; control (C), waterlogged for 21 days (WL21), and waterlogged for 8 days then free draining (WL8). (Photos: N Long)

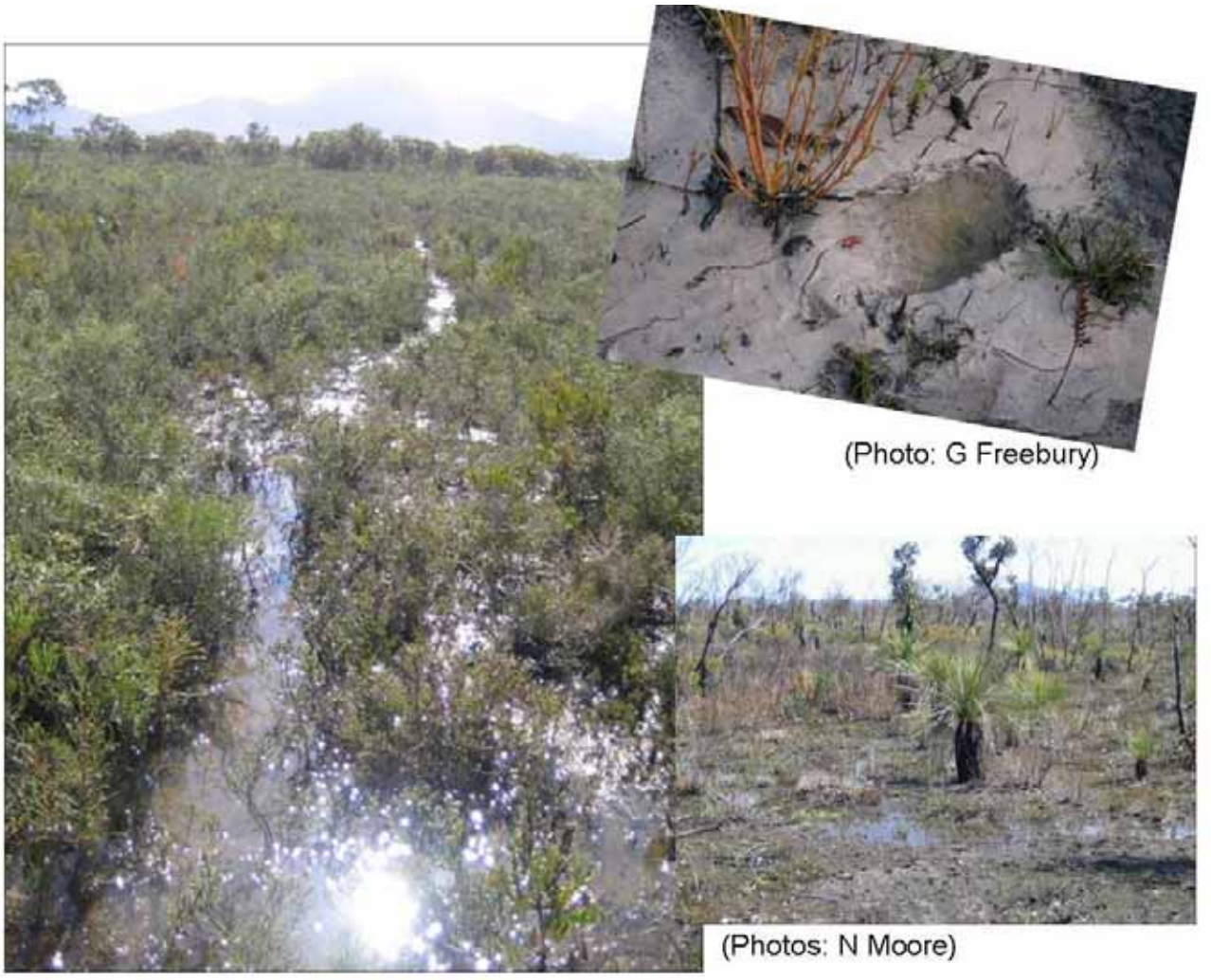
CONCLUSION

The results of these preliminary experiments have provided a wealth of information on the physiology of *Banksia*, and have allowed us to incorporate the findings into the experimental design of the main experiments for the waterlogging, drought and fire studies. In addition, a lot of technical complications and logistical problems were realised and rectified during these experiments.

Transverse sections of *Banksia* leaves revealed that all species studied have stomata on the abaxial side of the leaf. As only one surface contains stomata this needs to be taken into account in CIRAS-2 measurements. CIRAS-2 provided information on inter- and intra-species variation in gas exchange levels and also the optimal time to take physiological measurements in the growth chamber.

The main preliminary experiment indicated that the two *Banksia* species varied in sensitivity to the waterlogging treatment, with *B. attenuata* more sensitive to these conditions than *B. baxteri*. This means that a waterlogging time of 8 - 21 days is too long for the highest waterlogging treatment as *B. attenuata* started to die after 8 days of waterlogging. Hence it was decided to use a short term waterlogging of 2 - 3 days and a long term waterlogging of 6 - 8 days. Recovery after the waterlogging treatments is assessed in the waterlogging Experiment 1. This will provide information on how long it takes for plants to recover to levels pre-waterlogging.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-waterlogging



(Photo: G Freebury)

(Photos: N Moore)

INTRODUCTION

Temporary soil inundation is a seasonal feature of many parts of the south coast of Australia even where soils are sandy in texture. Inundation can occur in summer and winter. Soil inundation induces multiple physiological dysfunctions in plants (Kozlowski 1997) but it is unknown whether waterlogging alters the uptake, distribution and efficacy of phosphite.

The effect of waterlogging on plants

In waterlogged soil, the diffusion of gases through soil pores is strongly inhibited by their water content. A slowing of oxygen influx is the principal cause of injury to roots and the shoots they support (Vartapetian and Jackson 1997). These anaerobic conditions impede the diffusive escape and/or oxidative breakdown of gases such as ethylene and carbon dioxide that are produced by roots and soil microorganisms. This leads to an accumulation of the gases that can influence root growth and function (Rhodes and Nadolska-Orczyk 2001). The disappearance of molecular O_2 also triggers a sequence of changes in the physico-chemical properties of the soil, including accumulation of reduced metal ions, organic acids and volatiles, which are potentially harmful to the roots (Drew 1992). These anaerobic conditions limit the uptake of water and nutrients by the plant roots, and plants intolerant of waterlogging have reduced leaf water potential (Else *et al*. 2001) and stomatal conductance (Bradford and Hsiao 1982, Vartapetian and Jackson 1997). They generally exhibit lower water potentials and reduced rates of gas exchange when exposed to waterlogging (Drew 1992).

Starch is used by plants as a way to store excess glucose. The accumulation of starch in leaves of waterlogged plants is attributed to a reduced rate of translocation of carbohydrates from leaves to roots. However, starch reserves in roots are considered to be easily mobilised during flooding and can readily provide sugars for anaerobic metabolism in roots of waterlogged plants (Liao and Lin 2001). Flood-tolerant species have a continued production and translocation of assimilates, which is important for the maintenance of high root starch concentrations (Gravatt and Kirby 1998).

There is a tendency of waterlogged plants to wilt under high light intensity. This is the outcome of lowered conductivity to water uptake in oxygen-deficient roots. Root function is reduced as hypoxic or anoxic conditions require a switch to anaerobic metabolism (Rhodes and Nadolska-Orczyk 2001). Translocation of photosynthate from the shoot to where it is required in the roots is also impaired under anaerobic conditions (Kozlowski 1997). Irreversible dehydration of the leaves is slowed by rapid signalling from roots to shoots that results in a reduction of water loss from the foliage. This is achieved by the prompt decrease of stomatal apertures and leaf expansion.

Many plants respond to anaerobic conditions by closing their stomata to restrict water loss (Bradford and Hsaio 1982), however, the mechanism of stomatal closure is unclear. Veneklaas and Poot (2003) proposed that in the *Banksia* woodland, shallow rooted species close their stomata in response to lower leaf water potentials, while in deeper-rooted canopy species, stomatal closure operates to prevent a drop in water potentials. Waterlogging-sensitive species respond to waterlogging through rapid stomatal closure that remains for the duration of waterlogging (Kozlowski and Pallardy 1979, Beckman *et al*.

1992). High root-porosity of waterlogging-tolerant species enables them to re-open their stomata soon after initial exposure to waterlogging (Tang and Kozlowski 1984).

Species intolerant of waterlogging generally do not exhibit morphological changes and show no recovery of gas exchange during exposure to the waterlogged condition (Tang and Kozlowski 1982). Species tolerant of waterlogging often have reduced stomatal conductance and transpiration but tend to recover immediately after adventitious roots are formed (Sena Gomes and Kozlowski 1980). In tolerant plants the formation of adventitious roots, hypertrophied lenticels and aerenchyma improve oxygen uptake and aeration of the root. Adventitious roots compensate for reduced function in the old root system, providing increased surface area for water and mineral uptake. Hypertrophied lenticels develop on the submerged stems of some woody species, enhancing gas exchange and allowing removal of toxic by-products of anaerobic metabolism (Sena Gomes and Kozlowski 1980, Kozlowski 1997).

Plant reactions to waterlogging vary with the duration, season, and tolerance to the stress (Florentine and Fox 2002). Long-term effects of waterlogging can include reduced plant growth and a shift in resource allocation from above- to below-ground biomass (Naidoo and Naidoo 1992). Under waterlogged conditions, fine roots of fir and spruce had considerably greater dead than live biomass.

The effect of waterlogging on plant pathogen interactions

Any environmental disturbance which stresses or damages the plant, or upsets the microbial balance in the soil can either promote or reduce disease. Flooding may increase the incidence of soil-borne fungal diseases (Yanar *et al*. 1997) such as *Phytophthora* (wilting), *Pythium* (damping-off) and anaerobic bacteria (e.g. *Pseudomonas putida*). In the case of *P. cinnamomi*, flooded soils are assumed to increase disease severity by increasing the mobility of zoospores and by adversely affecting host physiology, resulting in predisposition or poor regeneration of damaged roots (O'Gara *et al*. 1996).

Lesion development was least for roots exposed to waterlogging and greatest for roots exposed to anoxia suggesting increased resistance of *E. marginata* to *P. cinnamomi* following waterlogging (Burgess *et al*. 1998). Root extension during waterlogging was greatly reduced. In contrast, stems of *E. marginata* inoculated with *P. cinnamomi* showed greater colonisation by *P. cinnamomi* in waterlogged plants than control plants and seemed less able to recognise the pathogen and switch on rapid defense responses (Burgess *et al*. 1999).

Davison and Tay (1987) observed reduced lesion frequency in jarrah when the roots were inoculated with zoospores of *P. cinnamomi* either post waterlogging or at day three of a four-day waterlogging treatment. Waterlogging and associated hypoxia had a direct effect on *P. cinnamomi* by reducing mycelial growth and sporangium production (Davison and Tay, 1986).

The effect of waterlogging on the uptake and efficacy of phosphite

Flooding generally does not cause leaf water deficits (Pezeshki and Chambers 1985). Much of the early reduction in the rate of photosynthesis is correlated with stomatal closure, resulting in decreased CO_2 absorption by leaves (Pezeshki *et al*. 1996). In

Eucalyptus marginata, conductance increased in plants whose roots had been exposed to hypoxia (2 mg O_2/L) and this effect lasted for at least two weeks after the resumption of normal oxygen conditions (Burgess *et al*. 1999). Thus, waterlogging in this species is not likely to impair entry of phosphite into the leaf. This could mean that plant tissues might be slower in responding to phosphite in some susceptible species. The recovery time for stomatal conductance to return to pre-flood values also varies between plants (Kozlowski 1997). Flooding often causes a change in allocation of photosynthate in plants (Kozlowski 1997). Thus, inundation affects not only the synthesis of carbohydrates but also their transport to meristematic sinks and their utilisation. The transport of phosphite to roots following leaf uptake is likely to be impaired if carbohydrates are directed away from roots to sinks in the shoot.

Preliminary experiments (see page 12) in the glasshouse and field determined the optimal time to take physiological measurements, the physiological variation within and between species, and the time it took to halve the photosynthetic activity of *Banksia* under waterlogged conditions. This information was then used to establish the methods for Experiments 1 and 2.

Experiment 1 - The physiological responses to waterlogging and recovery after waterlogging

This study examined the physiological and growth responses of four *Banksia* species subjected to short- (3 day) and long-term (21 day) waterlogging. The chosen species were from southwestern Australia: *B. littoralis* R. Brown is confined to winter-wet depressions and near watercourses on the coastal plain and Darling Range; *B. attenuata* R. Brown and *B. grandis* Willdenow are widely distributed keystone species on well-drained profiles. Lastly, *B. baxteri* R. Brown occurs on sandplains or consolidated dunes near the south coast of Western Australia (George 1984, Marchant *et al.* 1987). Gas exchange, water relations and mortality rates were monitored to determine their ability to recover from varying periods of inundation. Shoot growth and root regeneration were also examined to assess the degree of tolerance to waterlogging.

Experiment 2 – Effect of waterlogging on the efficacy of phosphite

In a two part experiment we examined the effect of waterlogging:

- before a phosphite treatment on phosphite redistribution in the plant and the ability to reduce disease caused by *P. cinnamomi* in *Banksia* species; and
- after a phosphite treatment on phosphite redistribution in the plant and the ability to reduce disease caused by *P. cinnamomi* in *Banksia* species.

In this experiment, we reduced the short term waterlogging period from 8 to 3 days, in response to the mortality that occurred in Experiment 1.

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-waterlogging

Experiment 1 The physiological responses to waterlogging and recovery after waterlogging

AIMS

The aims of this study were to determine:

- the physiological responses post waterlogging, and
- the recovery period after waterlogging by measuring plant water stress and stomatal behaviour in *Banksia* species.

OUTCOMES

This experiment will provide information for land managers on the best time for spraying phosphite in natural ecosystems in relation to waterlogging. By studying the physiological responses during waterlogging and the recovery of plants after waterlogging we will gain an understanding of how inundation stress may impact on phosphite efficacy during plant stress.

METHODS

Experimental species

Banksia baxteri is a non-lignotuberous species occurring in dry, sandy habitats on the south coast. *Banksia grandis* is also found in dry habitats on sand or laterite and has a lignotuber (Marchant *et al*. 1987). *Banksia littoralis,* the swamp banksia, grows on peaty sand and is usually associated with watercourses and seasonally wet depressions (Marchant *et al*. 1987). Under extreme weather conditions, these sites can become temporally waterlogged. This temporary waterlogging can be conducive to *Phytophthora* and inhibitory to plant growth.

Plant material and growth conditions

Fifteen-month-old *B. baxteri, B. grandis* and *B. littoralis* seedlings were raised under glasshouse conditions in 100 mm free-draining plastic pots containing composted pine bark, coarse river sand and cocopeat fibre (2:2:1; Richgro Garden Products, Canning Vale, WA) with added basal fertiliser (O'Gara *et al*. 1996). They were then treated with an additional 15 g of a low phosphate slow release fertiliser (Osmocote Plus Native Gardens, Scotts Australia, NSW) and placed into a growth chamber for a 7 day acclimatisation period before experimental treatments were imposed

In the growth chamber, seedlings were exposed to a photoperiod of 12 h with light being provided by 8 x 1000 W Son-T-Agro sodium lamps. Light intensity at a distance of 90 cm from the lamps was measured at ~3 lux using the CIRAS-2. The daily minimum and maximum average temperatures were 20.3 and 21.8°C. The relative humidity averaged 63%.

Waterlogging treatments were for 0, 3 or 21 days. Waterlogging was achieved by placing potted plants in larger plastic pots lined with polythene bags and adding de-ionised water until the water level was ~1 cm above the soil surface. This level was maintained by daily replenishment with deionised water to replace water lost by evapotranspiration. Free draining plants (0 days) were watered daily to container capacity with deionised water. Commencement of waterlogging was staggered over 2 days (two species on the first day

and the third species on the next) to facilitate completion of gas exchange measurements within the time of maximum photosynthesis. For each treatment and species there were five replicates arranged in a randomised block design.

On cessation of waterlogging, seedlings were re-potted into 150 mm deep pots with coarse river sand and 15 g Osmocote/10 L sand. All plants were then grown under free-draining conditions in the growth chamber for a recovery period of 21 days.

Gas exchange measurements
Leaf gas exchange was measured using an LCpro+ portable photosynthesis system (ADC Bioscientific, Herts, UK). The time taken for the photosynthetic rate to drop to half of the normal peak was determined, as well as the "time to death" of each species. One set of each physiological measurement was taken for three replicate plants of each species, with ten measurements per plant. Measurements were made on the third or fourth youngest fully expanded leaf at least 2 h after lights had been turned on. This was the period of maximum photosynthetic activity and was determined by plotting the photosynthetic rate of three plants per species at 90 min intervals over the course of one photoperiod at the end of the 7-day acclimatisation period.

Gas exchange measurements were recorded every two to three days during the 21 day waterlogging period and during the 21 day recovery period. The same leaf was used throughout the experiment. Leaf area was determined using image analysis software (Assess, Version 1.01; American Phytopathological Society Press, St. Paul, MN, USA) from scanned tracings of leaf visible in the exposed window of the LCpor+.

Pressure chamber measurements
Leaf water potential (ψ) was measured with a pressure chamber (Model 1000; PMS Instrument Company, Oregon, USA). The fourth or fifth (and subsequent) youngest fully expanded leaves were measured 1 - 2 h prior to lights being turned on (predawn period) immediately before drainage, one week later and again at the end of the 21 day recovery period.

Shoot and root growth
The stem height was recorded at the end of the acclimatisation period, on drainage and at the end of the recovery period. At harvest, new roots which had emerged from the original root ball and grown into the coarse river sand mix were separated by washing over a 1 mm sieve. Root samples were dried for 2 - 3 days at 37°C. Root dry weight, number and length were recorded.

Starch allocation
Prior to the waterlogging treatments and at the end of the recovery period, a hand cross-section was taken from the woody roots, main stem and the leaves. The sections were stained in iodine, examined immediately under the microscope at 100× magnification and rated on a scale of 1 (no starch) to 5 (most starch).

Nitrogen analysis
Leaves were placed in a drying oven for three days at 60°C. Samples were analysed (CSBP Soil and Plant Laboratory, Bibra Lake, WA) for percentage nitrogen content.

Statistical analyses
Single ANOVAs were performed on gas exchange data, water potentials, new root growth, starch storage and leaf nitrogen content, while shoot growth data were analysed using repeated measures ANOVAs using Statistica version 5 (StatSoft Inc., Tulsa, OK, USA). Data were log transformed before analysis. Root growth and starch data were square root transformed.

RESULTS

Plant Growth and Survival
There was a significant interaction on shoot growth between species and treatment ($p < 0.002$). Shoot growth was depressed by short and long-term waterlogging in *B. baxteri*, but much more so by the WL21 treatment (Figure 14). Only the WL21 treatment significantly decreased shoot growth in *B. grandis* where growth was more affected during the recovery period than during inundation (data not shown). Waterlogging had no effect on shoot growth of *B. littoralis* ($p = 0.586$).

Root growth was unaffected by short-term waterlogging but WL21 treatments severely impacted root recovery of both *B. baxteri* and *B. grandis* (Figure 15). As with shoot growth, waterlogging did not affect new root growth of *B. littoralis*.

Four of the five WL21 plants of *B. baxteri* and *B. grandis* died (Figure 16) as did one/five plants for *B. grandis* in the WL3 treatment (day 18). No *B. littoralis* died. At the commencement of waterlogging all seedlings had a flush of new growth which was monitored for signs of water stress. All WL21 *B. baxteri* and *B. grandis* developed some degree of wilting of their new growth. Deaths were preceded by severe wilting, whilst those seedlings that survived wilted only slightly and within a week (while still waterlogged) had regained turgidity. Some plants wilted, then recovered, but later developed severe wilting and died. All deaths occurred during the recovery period. White patches were observed on the submerged portion of stems of *B. littoralis* after 17 days of waterlogging, disappearing shortly after drainage. These appeared to be hypertrophied lenticels as they were similar in appearance to those described by Sena Gomes and Kozlowski (1980). Some leaf chlorosis was observed in young leaves of all three species but was most pronounced in *B. littoralis*, appearing after 17 days of WL21 treatment. This chlorosis improved towards the end of the recovery period.

Starch
Inspection of sections of woody roots prior to treatments revealed little or no starch in. *baxteri*, intermediate amounts in *B. littoralis* (occurring mainly in ray tissue) and larger amounts in *B. grandis* present in ray tissue and around the pericycle. After 21 days of waterlogging, starch content was reversed, with *B. littoralis* having a larger proportion of starch than *B. grandis*.

Lower stem starch, immediately after extended waterlogging, was greatest in *B. grandis* and this was present in rays and cortex (Figure 17). Starch was present to a lesser extent in *B. littoralis* and located in the rays only. No starch was observed in stem tissue or woody roots of *B. baxteri.*

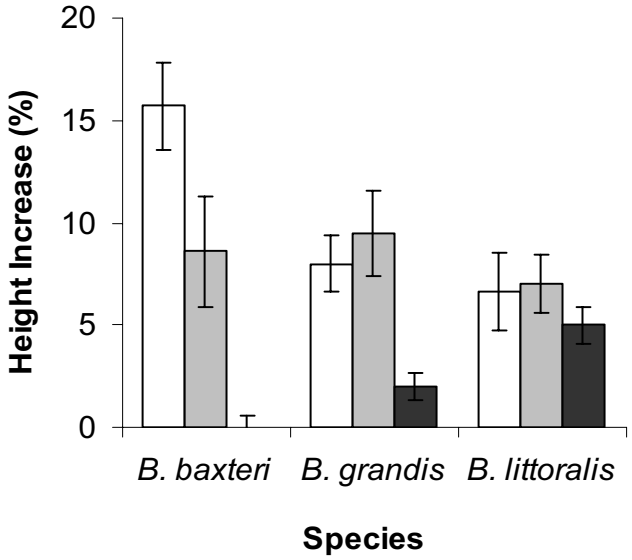


Figure 14

Shoot growth (percentage of height increase) of *Banksia baxteri, B. grandis* and *B. littoralis* plants over 42 days which included a 21 day recovery period after waterlogging treatment. Treatments were: free draining control (□); WL3, waterlogged for 3 days and then free draining (▒); and WL21, waterlogged for 21 days (■). *n* = 5. Vertical bars represent two standard errors of the mean.

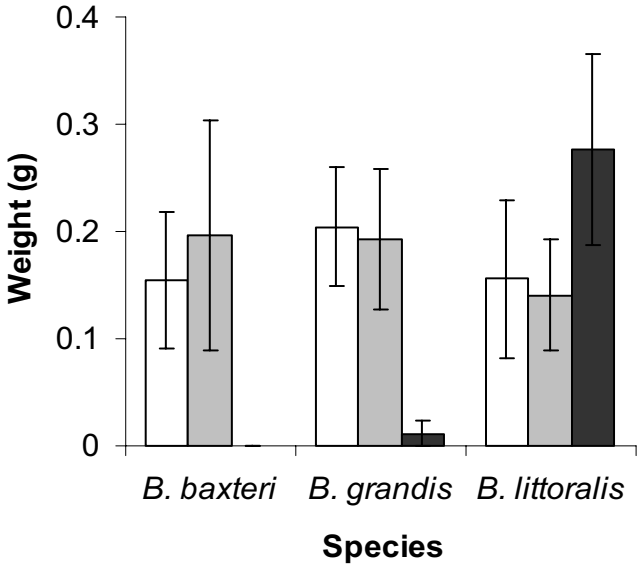
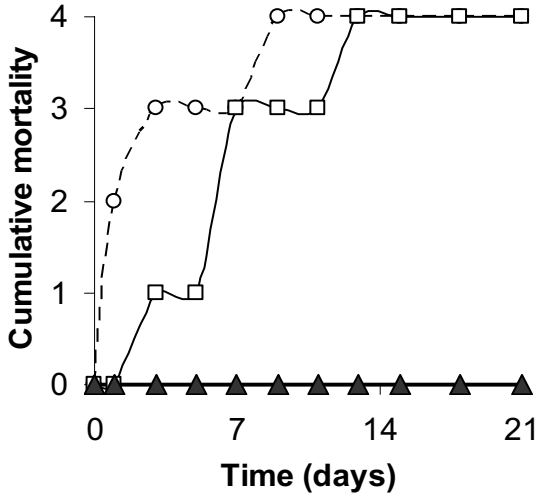


Figure 15

Mean dry weight of new roots of *Banksia baxteri, B. grandis* and *B. littoralis* 21 days after the conclusion of waterlogging treatments. Treatments were; free draining controls (□), waterlogged for 3 days (▒) or 21 days (■). *n* = 4. Vertical bars represent two standard errors of the mean.

Figure 16

Cumulative mortality of *Banksia baxteri* (○), *B. grandis* (□) *and B. littoralis* (▲) 21 days after the conclusion of a 21 day waterlogging treatment. *n*= 5.

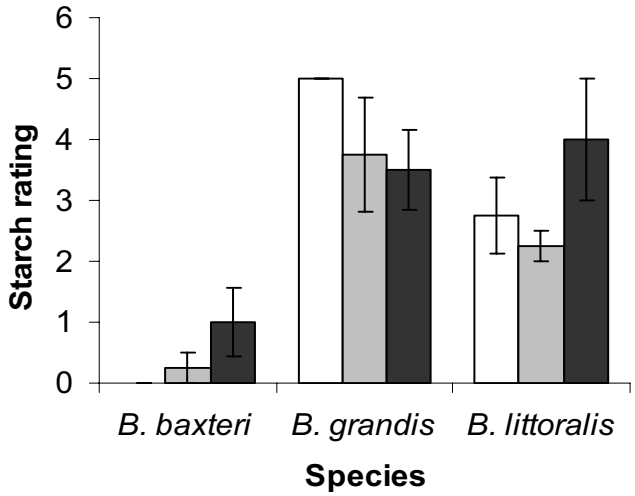


Although ANOVA analysis at the end of the recovery period showed no significant difference between the treatments, mean starch ratings indicate a possible trend towards reduced starch in *B. grandis* in both root and stem tissue (Figure 17). Conversely, *B. littoralis* demonstrated a possible increase in starch levels after the treatment and recovery periods. Starch levels were higher in *B. littoralis* plants subject to extended waterlogging than *B. grandis*, but not significantly so.

Starch storage was significantly ($p < 0.0001$) lower for *B. baxteri* in both root and stem tissue than in the other two species (Figure 17). This was supported by the low starch levels in stem tissue and absence of starch in woody roots.

Figure 17

Mean starch rating of lower stem sections of *Banksia baxteri, B. grandis* and *B. littoralis* 21 days after the conclusion of waterlogging treatments. Treatments were; free draining controls (□), waterlogged for 3 days (▒) or 21 days (■). Sections were rated on a scale from 0 - 5, with nil starch being scored "0" and maximum starch "5". *n* = 4. Vertical bars represent two standard errors of the mean.



Nitrogen Concentration

A significant interaction between waterlogging treatment and leaf nitrogen concentration was observed ($p < 0.01$) in young leaves of *B. grandis* and *B. littoralis* (Figure 18). Percentage leaf nitrogen was reduced in *B. grandis* by both waterlogging treatments but extended waterlogging was required to induce a similar response in *B. littoralis*. Extended waterlogging also reduced the N levels in the mature leaves of *B. littoralis*.

Nitrogen levels in young and mature leaves were unchanged by waterlogging in *B. baxteri* (Figure 18a and b). A species difference was also observed, where both young and mature *B. baxteri* leaves had significantly lower nitrogen levels than *B. littoralis* and *B. grandis* ($p = 0.01$ and $p = 0.03$, respectively).

Leaf Water Potential

Extended waterlogging induced severe water stress in both *B. baxteri* (< -3.0 MPa) and *B. grandis* (approx. -2.5 MPa) and this was still evident one week after drainage (Figure 19). Water potentials (approx. -0.5 MPa) of WL21 *B. littoralis* on days 0 and 7 were significantly ($p = 0.014$) higher than the other two species. There was a significant interaction between species and treatment ($p < 0.003$) in this period. By the end of the recovery period, water potentials of the sole surviving *B. grandis* had returned to control levels. In addition, due to

the allocation of a value of -4.0 MPa to all measurements equal to or lower than -4.0 MPa, water stress may be understated in the WL21 treatment (*B. baxteri* and *B. grandis* only). Leaf water potentials in the WL3 treatment were similar to controls in all species (> 1.0 MPa) indicating water stress did not develop as a result of short-term flooding (Figure 19).

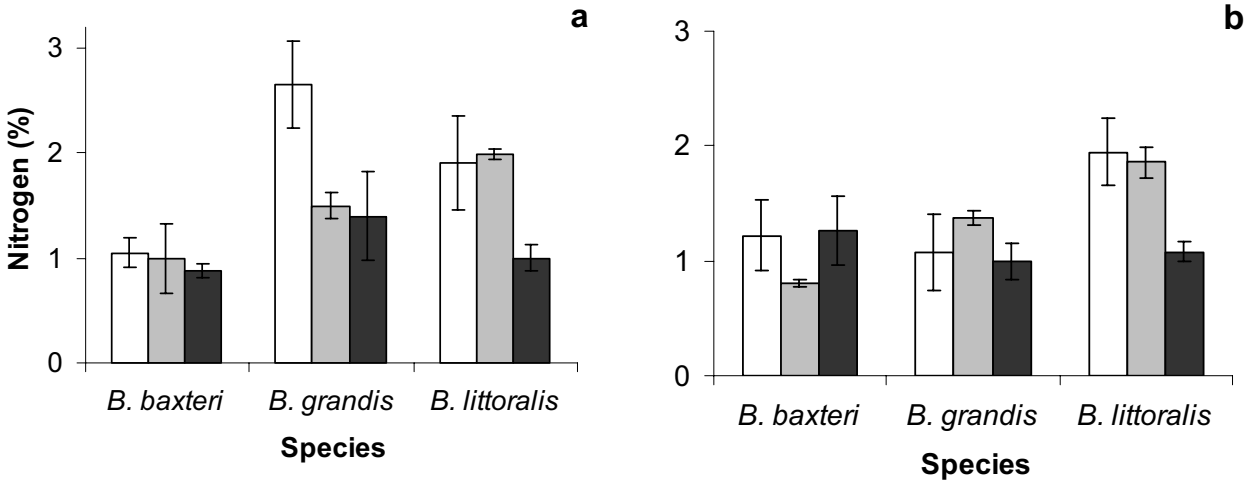


Figure 18 Mean % nitrogen concentration of **a**) youngest fully expanded leaves and **b**) mature leaves of *Banksia baxteri, B. grandis* and *B. littoralis* 21 days after the conclusion of waterlogging treatments. Treatments were; free draining controls (□), waterlogged for 3 (▒) or 21 days (■). *n* = 2 (each sample is a combination of two replicates). Vertical bars represent two standard errors of the mean.

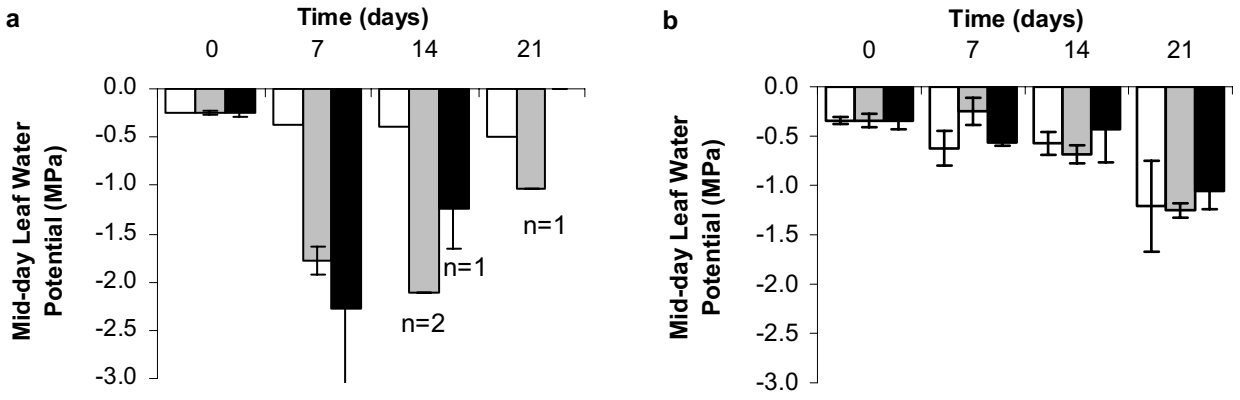


Figure 19 Mean water potentials of **a**) *Banksia baxteri* and **b**) *B. grandis* during recovery from waterlogging. Water potential was measured prior to start of the photoperiod (light cycles) in a controlled growth chamber. Treatments were free-draining controls (□), waterlogged for 3 (▒) or 21 days (■). Day 0 is at drainage. *n* = 3, except as otherwise indicated. Vertical bars represent two standard errors of the mean.

Photosynthetic rate

The photosynthetic rate of WL21 *B. baxteri* and *B. grandis* dropped rapidly to 15 and 22% of controls, respectively, within the first week of treatment and remained low before commencing recovery one week after drainage (Figure 20). Photosynthetic levels of WL21 plants approached those of controls by 15 days after drainage, and they were fully recovered by the end of the three-week recovery period. Photosynthesis in WL3 plants also fell but not to the same extent as in WL21, being 54 and 69% of controls in *B. baxteri* and *B. grandis*, respectively. Recovery followed a similar pattern to the WL21 treatment, commencing at 7 - 9 days and reaching similar levels to controls in the last week of the recovery period. *B. littoralis* had a higher ($p < 0.001$) rate of photosynthesis than the other species and rates were unchanged by either waterlogging treatment.

Stomatal conductance

Rapid stomatal closure occurred during the first week of WL21 treatment in both *B. baxteri* and *B. grandis* (Figure 21). Stomata remained only marginally open before reopening commenced in the recovery period at 7 (*B. grandis*) and 9 days (*B. baxteri*). Aperture subsequently increased but had not reached control levels by the end of the three-week recovery period. Short-term waterlogging of these species also resulted in rapid stomatal closure but to a lesser extent than the WL21 treatment. Stomata remained partially open (~40% of control plants), before aperture increased at 7 days for *B. grandis* and 9 days for *B. baxteri*. By the end of the recovery period, conductance had returned to control levels.

B. littoralis had significantly ($p < 0.001$) higher rates of conductance than either of the dry habitat species and these rates were unaffected by WL3 or WL21 treatments (Figure 21).

Transpiration

Transpiration rates in both WL21 *B. baxteri* and *B. grandis* also dropped rapidly during the first week to 11 and 18% of controls, respectively. They remained at very low rates before recovery began on day 7 for *B. grandis* and day 9 for *B. baxteri* (Figure 22). At the end of the recovery period, *B. grandis* was transpiring at a similar level to controls while rates for *B. baxteri* were not fully recovered. Responses to 3 days of waterlogging were similar in *B. grandis* and *B. baxteri*, with transpiration being suppressed but not to the same extent as the 21 day treatment (53 and 59% of controls), and increasing at 7 and 9 days, respectively. Both returned to control levels by day 21. Transpiration rates of *B. littoralis* were significantly ($p < 0.001$) higher than for the two other species and were unaffected by either the WL3 or WL21 treatments (Figure 22).

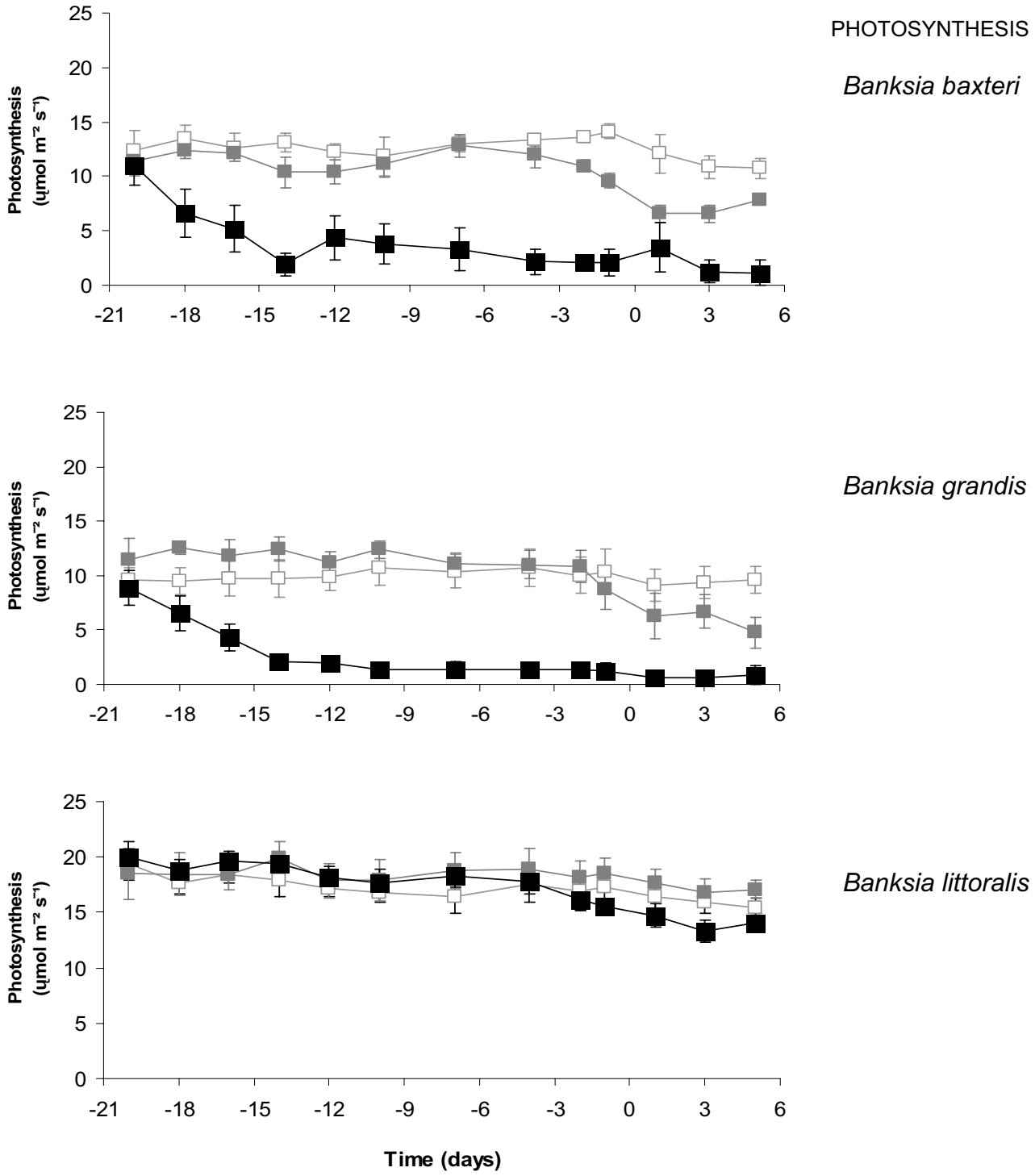


Figure 20 Mean photosynthesis of *Banksia baxteri, B. grandis* and *B. littoralis* under waterlogged conditions. A 21 day treatment of waterlogging (WL21) commenced on day -21, and a 3 day waterlogging (WL3) commenced on day -3. Plants were returned to free-draining conditions on day 0. Controls (C) were free draining for the duration of the experiment. *n* = 5, except for *B. grandis* where *n* = 4 in WL21 treatment and for *B. baxteri* WL21 treatment *n* = 2 due to deaths during the experiment. Vertical bars represent two standard errors of the mean.

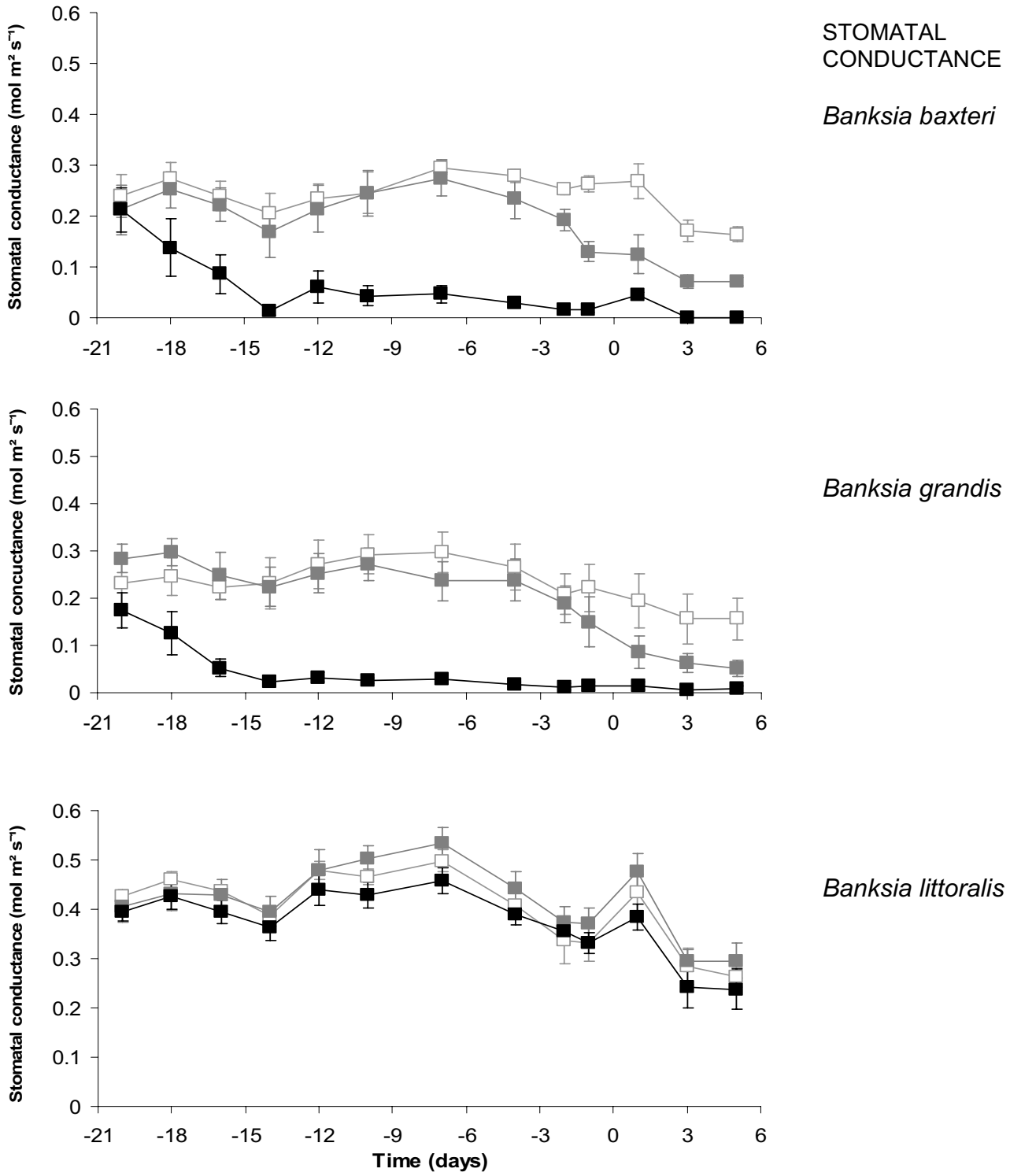


Figure 21 Mean stomatal conductance of *Banksia baxteri, B. grandis* and *B. littoralis* under waterlogged conditions. A 21 day waterlogging (WL21) commenced on day -21, and 3 day waterlogging (WL3) commenced on day -3. Plants were returned to free-draining conditions on day 0. Controls (C) were free draining for the duration of the experiment. *n* = 5 except, *B. grandis n* = 4 WL21, *B. baxteri* WL21 *n* = 2. Vertical bars represent two standard errors of the mean.

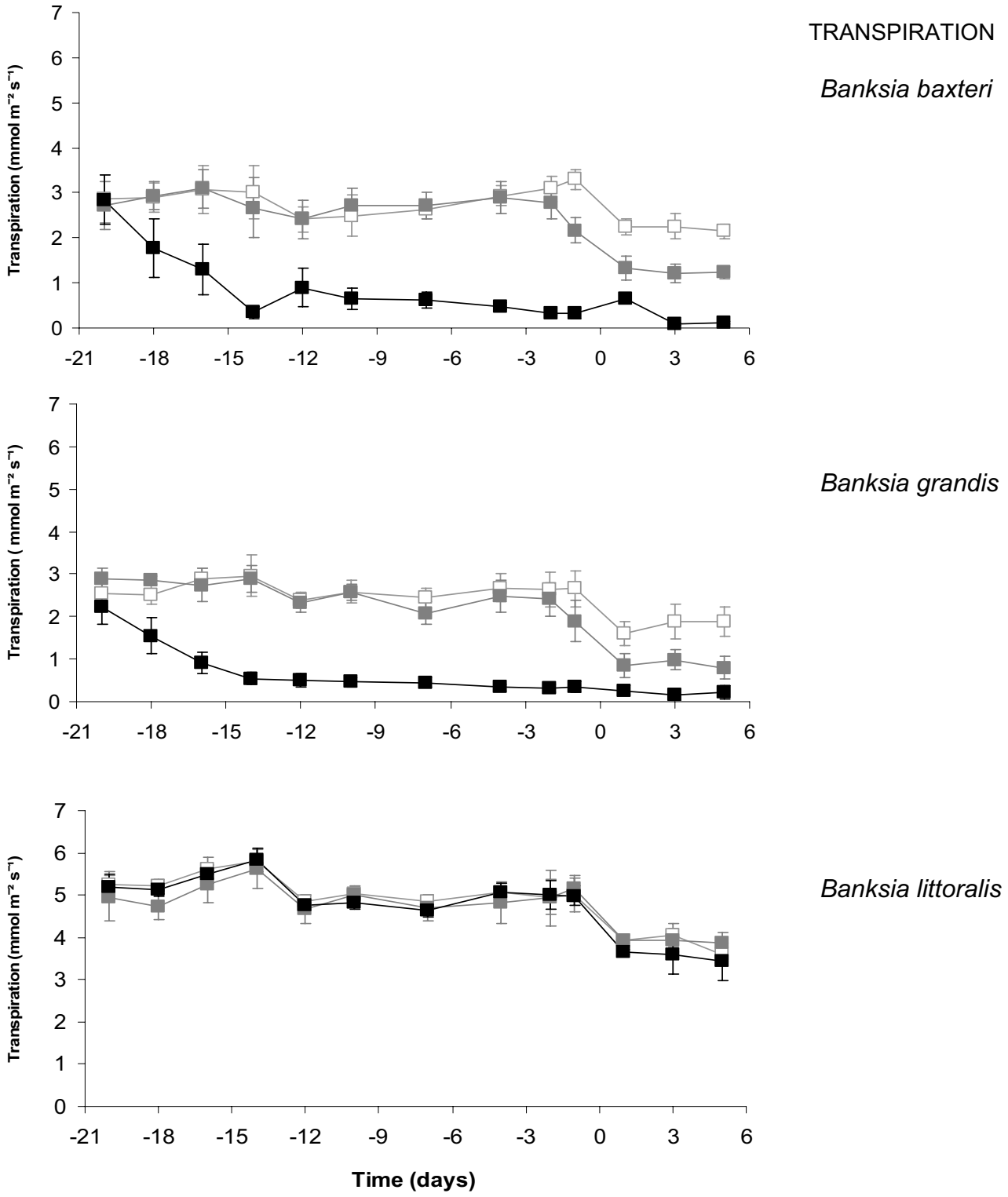


Figure 22 Mean transpiration of *Banksia baxteri, B. grandis* and *B. littoralis* under waterlogged conditions. A 21 day treatment of waterlogging (WL21) commenced on day -21, and 3 day waterlogging (WL3) commenced on day -3. Plants were returned to free-draining conditions on day 0. Controls (C) were free draining for the duration of the experiment. *n* = 5 except, *B. grandis n* = 4 WL21, *B. baxteri* WL21 *n* = 2. Vertical bars represent two standard errors of the mean.

DISCUSSION

The *Banksia* species had varying responses to waterlogging which correlated to the habitat in which the species occur. In coastal Western Australia, *B. baxteri* and *B. grandis* grow predominantly on sandplains where waterlogging conditions are rarely encountered. Marginal rates of photosynthesis, very low water potential and high mortality rates indicate that these species are similar in their intolerance of extended waterlogging. However, high water potential, continuance of a degree of carbon reduction and low mortality rates show these species can recover from short-term waterlogging. *B. littoralis* is highly adapted to wet habitats, as evidenced by its high water potential and high rates of photosynthesis, transpiration and stomatal conductance, which remained unchanged by waterlogging.

B. baxteri and *B. grandis* responded to waterlogging by rapidly closing their stomata, a response to leaf water deficits, caused by reduced root conductivity or to root/shoot signalling as a result of water stress in the root (Drew 1983, Veneklaas and Poot 2003). In glasshouse trials with *B. prionotes*, another dry habitat species, Groom (2004) recorded a 69% drop in pre-flood conductance from 140 to 40 µmol $m^{-2}s^{-1}$ after 17 days of waterlogging. Results were more pronounced in the present study where *B. baxteri* and *B. grandis* had an 86 and 89% drop in conductance, respectively, after 17 days of waterlogging. However, the stomata of *B. littoralis* did not close in response to either WL3 or WL21 treatments, which is in contrast to work by Groom (2004), who found a significant decrease in stomatal conductance (56%) in *B. littoralis* after 17 days of waterlogging, albeit not as low as in *B. prionotes*. Notwithstanding, Groom (2004) considers conductance rates of *B. littoralis* to be relatively high (50 - 75% of controls over the 104 day trial) and attributes the maintenance of shoot growth in flooded seedlings to the ability to maintain sufficient stomatal aperture to support photosynthesis which allowed growth similar to plants that were not waterlogged.

After drainage, stomatal conductance declined and then recovered in control treatments of all species and this may be due to a change in conditions resulting from the temporary removal from the chamber for re-potting. Effects on *B. littoralis* were more pronounced and this is possibly due to a greater sensitivity to dryness in this wet habitat species. The effects of these stresses are less evident in the photosynthesis curves, suggesting they do not override the stress of waterlogging.

The photosynthetic rate of *B. baxteri* and *B. grandis* were closely aligned with stomatal behaviour. Photosynthesis dropped to very marginal rates in the WL21, but in the WL3 treatment dropped to around 60% of controls, and this was associated with partial stomatal closure. Partial closure has the advantage of lowering transpiration and therefore water stress, while some diffusion of CO_2 is still possible, allowing a degree of photosynthesis to continue. Duration of waterlogging is therefore important for these species in terms of maintaining photosynthesis, and thus physiological function, and may impact on their ability to recover from inundation.

The significantly higher photosynthetic rate of *B. littoralis* remained relatively constant throughout the study period in all treatments reflecting its adaption to wet habitats. Results in this study are in contrast with the work of Groom (2004) who found a significant reduction in the photosynthetic performance of *B. littoralis*, i.e. a reduction of 57% after 17 days of waterlogging. Observed differences may be due to the difference in conditions i.e.

glasshouse versus growth chamber, plant age, season and fewer measurement days in the Groom (2004) study.

Low water potential seldom develops in *Banksia* species, as they are generally able to access groundwater as soil moisture levels fall (Dodd and Bell 1993). In *Banksia* woodlands, pre-dawn summer water potential between -1.0 and 0 MPa have been recorded for *B. attenuata*, *B. menziesii* and *B. ilicifolia,* a species occurring in similar habitats to *B. littoralis* (Dodd and Bell 1993, Zencich *et al*. 2002, Froend and Drake 2006) and in the jarrah forest, the lowest water potential for *B. grandis* were -0.55 MPa (Crombie *et al.* 1988). These values are consistent with the control values of all species in the present study, which were around -0.5 MPa. A critical water potential of -2.55 MPa in *B. attenuata* and *B. menziesii* were recorded at a site where groundwater was inaccessible, beyond this point the plants cannot recover and death ensues (Froend and Drake 2006). After 21 days, mean water potential of *B. grandis* were close to -2.55 MPa and potentials of *B. baxteri* were below this value, suggesting they had reached this critical point and this is supported by the 4/5 mortality rates in both these groups. Severe wilting of new growth which preceded deaths provided visible confirmation of considerable water stress while lack of wilting in *B. littoralis* was evidence of absence of water stress in this species.

Short-term waterlogging did not produce a drop in water potential in the drier habitat species but stomatal aperture was decreasing. This does not dismiss the involvement of water potential in stomatal closure, but does indicate root-shoot signalling may be involved at least initially in the process. Normal water potential, after three days of waterlogging, also suggest that longer waterlogging periods are required for *B. baxteri* and *B. grandis* to develop the degree of water stress beyond which they cannot recover.

Extended waterlogging of *B. littoralis* did not result in any change in water potential. Lack of water stress in combination with a high rate of transpiration implies *B. littoralis* was able to maintain root function despite anaerobic conditions in the root zone. Tolerance of these conditions suggests the presence of adaptations which improve oxygenation of the root and rhizosphere. Development of hypertrophied lenticels were not observed until after 17 days of waterlogging so it is likely other adaptations are present.

Recovery after flooding is dependant on the ability of the potentially damaged root systems to adequately replenish transpiration losses. In this study, root regeneration of WL21 *B. littoralis* was not significantly different to controls but was approximately twice that of the other two species, indicating a possible stimulatory effect of flooding. Factors which assist in maintaining root function in *B. littoralis* could be the formation of aerenchyma tissue, adventitious roots or hypertrophied lenticels. A stimulatory effect might be achieved by up-regulation of genes coding for auxin production, for example, but further investigation is required to provide specific knowledge for *Banksia* species in this area.

Shoot growth reductions are usually associated with rapid decreases in photosynthesis (Poot and Lambers 2003). This is reflected in the current study as photosynthesis fell rapidly in *B. baxteri* and *B. grandis* in response to WL3, more so in the latter species. Greater reductions in photosynthesis associated with extended waterlogging would explain the more severe growth reductions in the WL21 as compared with the WL3 treatment. In addition, relatively lower photosynthetic rates in *B. baxteri* relate well to lower growth in

this species compared to *B. grandis*. The photosynthetic rate in *B. littoralis* did not fall in either treatment and this was reflected in shoot growth rates that were similar across all treatments before and after drainage.

Root recovery was severely impacted by the WL21 treatment in the dry habitat species. Groom (2004) found a sizeable, although not significant, reduction in root dry weight of *B. prionotes* compared to controls after 72 days of flooding. Interestingly, root growth in *B. littoralis* in the Groom (2004) study was significantly lower than controls but this was also accompanied by a significant reduction in photosynthesis (57% lower than pre-flood values after 17 days) which was not experienced in the current study. In addition, root status was measured at drainage, rather than after a period in which regrowth could occur.

After fire, resprouters regenerate from subterranean tissues or epicormic buds on stems (Pate and McComb 1981) and overall they store more starch in their roots than seeder species (Bowen 1991). Large starch stores allow them to quickly refoliate and exploit the post-fire environment (Pate *et al*. 1990). *B. littoralis* and *B. grandis* have starch levels among the highest of the resprouting *Banksia* spp. (Bowen 1991) while *B. baxteri* employs the seeder fire response strategy (George 1984).

The storage of starch by the resprouter species may confer other advantages by providing resprouters with substrates for anaerobic metabolism during waterlogging or enabling rapid recovery upon oxygen re-entry (Poot and Lambers 2003). In the current study, the observed trend towards depletion of starch stores in *B. grandis* may reflect use of reserves to compensate for reduced photosynthesis, inhibited translocation and/or the switch to less efficient anaerobic metabolism. On the other hand, maintenance of high root starch levels in *B. littoralis* might be due to sustained high photosynthetic levels, mechanisms to maintain phloem transport, and/or mechanisms which aerate the root and allow aerobic respiration to continue, using proportionately less of available carbohydrate. Lower starch levels in *B. baxteri* may have contributed to the relatively lower pre-drainage shoot growth rates.

The reduction in leaf nitrogen concentration in *B. grandis* is consistent with the findings of Kreuzwieser *et al*. (2002) who demonstrated dramatic reduction in N uptake in the flood-sensitive species *Fagus sylvatica* (beech). Nitrogen uptake in the highly flood-tolerant hybrid *Populus tremula* x *P. alba* (poplar) was not affected by waterlogging. However, in the current study, nitrogen depletion was indicated in mature leaves and young leaves of the tolerant species, *B. littoralis*. Growth was sustained during WL21, and this may have placed a heavier demand for N on plants, resulting in lower leaf N content. In addition, growth may have been slightly lower, although not significantly so, in the WL21 treatment, indicating some loss of root function/integrity, which may have impacted on N uptake. Alternatively, denitrification, which reduces nitrate levels in flooded soil (Davies *et al*. 2000), may have occurred in the waterlogging solution, reducing available N. The dynamics of N supply, uptake and utilisation is complex as it depends on the form of inorganic N in the soil solution. Unlike for nitrate, ammonium must be assimilated in the roots and this requires carbohydrates from the Kreb's Cycle.

Chlorophyll reduction occurs in order to provide N for other plant processes (Kreuzwieser *et al*. 2002) and is likely to be triggered by upregulation of ethylene synthesis due to

flooding. A small degree of leaf chlorosis was observed in young leaves of all three species but was most pronounced in *B. littoralis*, appearing after 17 days of waterlogging and improving towards the end of the recovery period, probably in association with improved uptake due to root regeneration and/or nitrogen availability in the new potting medium.

Unusually, the nitrogen concentration of young and mature leaves of *B. baxteri* was unaltered by waterlogging. The root system was undoubtedly damaged by WL21 as indicated by the lack of regeneration. It may be that the severely arrested growth greatly depressed demand so that N content of leaves remained relatively constant or that upregulation of senescence-promoting compounds did not occur.

CONCLUSION

This study shows that species of banksia differ markedly in their response and recovery from waterlogging. For sensitive species, even a few days of complete inundation of their roots is likely to alter the physiological status of the plant and more extended waterlogging will result in severe root damage and ultimately plant death. Changes in cell metabolism, including changes in carbohydrate reserves, could alter the response of plants to infection by *P. cinnamomi*. Changes in long-distance transport in the plant, due to impaired root function affecting water uptake or to reduced transpiration, could impact on phosphite distribution and redistribution between organs such as leaves, stems and roots. This is likely to be exacerbated by impairment of photosynthetic capacity as there will be less carbohydrate and hence less amounts of minerals such as potassium ions and orthophosphate cycling from the shoot to the root. With knowledge of the magnitude and timing of the physiological responses in the three Banksia species, the next experiment can investigate the likely impact of waterlogging on the efficacy of phosphite to control *P. cinnamomi* inside stem tissue.

Waterlogging treatments of *Banksia attenuata* (Photo: D Hüberli)

Experiment 2: Effect of waterlogging on the efficacy of phosphite to reduce disease caused by *Phytophthora cinnamomi*

AIMS

The aims of this study were to determine the effect of waterlogging:

- before a phosphite treatment on phosphite redistribution in the plant and the effectiveness of phosphite in reducing disease caused by *P. cinnamomi* in *Banksia* species; and
- after a phosphite treatment on phosphite redistribution in the plant and the effectiveness of phosphite in reducing disease caused by *P. cinnamomi* in *Banksia* species.

OUTCOMES

Knowledge gained about the uptake and distribution of phosphite, and its effectiveness in controlling disease spread within the plant during waterlogging stress will provide information to managers to formulate improved operational guidelines for the use of phosphite in natural ecosystems.

METHODS

The plants

Banksia attenuata *Banksia baxteri*

Seed of *Banksia attenuata* (provenance North Dandaragan) and *B. baxteri* (provenance Mt Manypeaks) were germinated in March 2006. There were a total of 432 plants of each species. Seedlings were potted into 150 mm free-draining pots containing composted pine bark, coarse river sand and coco peat fibre (2:2:1; Richgro Garden Products, Canning Vale, WA) with added basal fertiliser (O'Gara *et al*. 1996). Each pot was top-dressed with 15 g of a 4 - 9 month slow release low phosphate fertiliser (Osmocote Plus Native Gardens, Scotts Australia, NSW). Plants were watered twice daily from overhead sprinklers.

Experimental design

In two separate experiments we examined the effect of waterlogging on the efficacy of phosphite when waterlogging occurred:

- before phosphite treatment (Experiment 2a); and
- after phosphite treatment (Experiment 2b).

There were six treatments [phosphite (two treatments) x waterlogging (three treatments)] with 12 replicate plants per treatment (a total of 216 plants of each species for each experiment). In each experiment there were three assessments made; at 1 week, 1 month and 4 months (72 plants of each species for each assessment) after the spray (Experiment 2a) or after the completion of the waterlogging treatments (Experiment 2b). The timeline of both experiments in relation to waterlogging treatments, phosphite application, physiological measurements and disease assessment is shown in Figure 23.

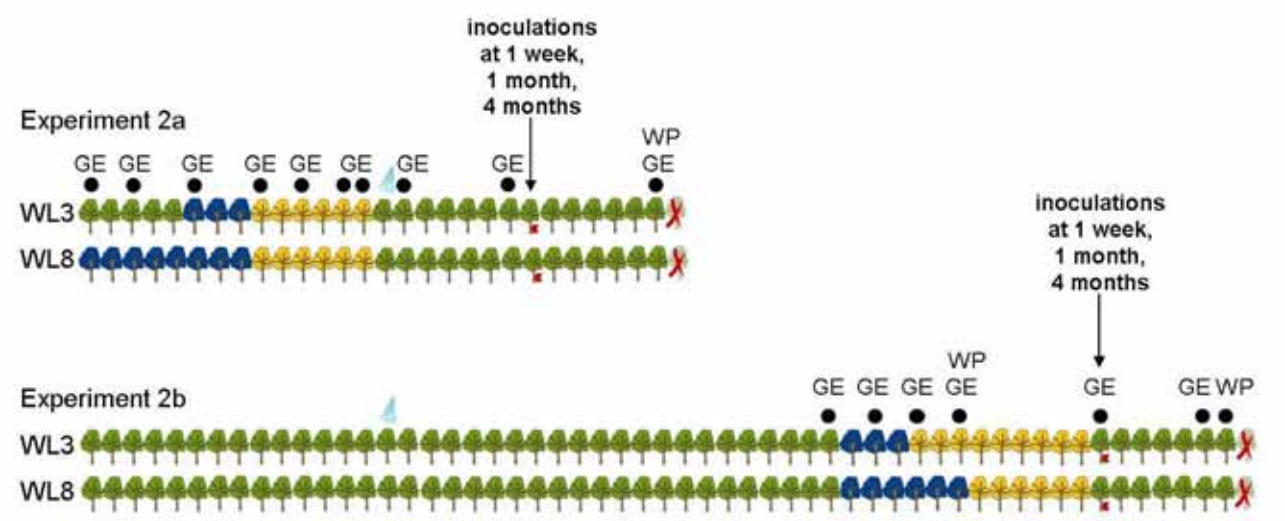


Figure 23 Daily timeline showing the phosphite treatments ( ) in relation to waterlogging ( ) and plant recovery ( ). Plants were inoculated ( ) 1 week, 1 month or 4 months after the phosphite (Experiment 2a) or after completion of waterlogging (Experiment 2b), and then harvested ( ) 1 week later. Periods of leaf gas exchange (GE) and water potential (WP) measurements are also shown.

Application of phosphite

A 40% solution of phosphite containing 0.2% (v/v) of the wetting agent BS1000® was sprayed with a Microfit low-volume fine mist applicator at 24 kg/ha to all the phosphite treated plants in both Experiments 2a and 2b. One phosphite spray ensured that the treatment received by plants in both experiments was consistent across experiments since waterlogging was relatively easy to control. In Experiment 2a, the plants were treated with phosphite 7 days after the completion of the waterlogging treatments, while in Experiment 2b, plants were treated 21 days prior to waterlogging (Figure 23).

Waterlogging
In each experiment three waterlogging treatments were applied.
Experiment 2a:

- WL0 – control plants not subjected to waterlogging;
- WL3 - plants were waterlogged for 3 days and then returned to free-draining conditions; and
- WL8 – plants were waterlogged for 8 days and then returned to free-draining conditions.

Experiment 2b:

- WL0 – control plants not subjected to waterlogging;
- WL3 - plants were waterlogged for 3 days and then returned to free-draining conditions; and
- WL6 – plants were waterlogged for 6 days and then returned to free-draining conditions.

In experiment 2b, the 8 day waterlogging treatment was reduced to 6 days, as in experiment 2a, plants failed to recover after 8 days of waterlogging.

The waterlogging treatment was achieved by lining a larger pot with a plastic bag, placing the potted plant into this and then flooding the pot. Water was maintained at 1 cm above the soil surface. All non-waterlogged plants were free draining and watered daily to container capacity, while those that were waterlogged had their water levels topped up daily to maintain water 1 cm above the soil surface.

Inoculation
Prior to the first inoculation, the *P. cinnamomi* isolate was passaged through a *B. baxteri* seedling and then reisolated onto NARPH, to maintain its pathogenicity. Plants were underbark inoculated using the method as described in Hüberli *et al*. (2001) with *P. cinnamomi* (isolate SR2). There were ten replicate inoculated plants and two control plants (inoculated with sterile Miracloth) per treatment. There were three separate inoculation events for each experiment during which the daily temperatures were recorded (Table 2).

Table 2 Mean daily average, maximum and minimum temperatures in the glasshouse recorded for 7 days following each inoculation.

		Mean daily temperature (°C)		
Experiment	**Inoculation**	**Average**	**Minimum**	**Maximum**
2a	1	20.9	14.4	32.6
	2	20.2	14.8	32.8
	3	22.6	14.2	34.1
2b	1	20.4	13.2	33.8
	2	21.7	15.8	32.7
	3	24.7	15.4	36.5

Measurements

Waterlogging conditions

During waterlogging, the temperature and dissolved oxygen in the container medium water surrounding the plant was measured (at 1, 3, 5 and 8 days for Experiment 2a, and 1, 3 and 6 days for Experiment 2b) using a portable Dissolved O_2 Meter (H1 9145 Hanna Instruments, Keysborough, Vic, Australia). Briefly, these were measured after calibration of the probe for a few minutes to the greenhouse air and then placing it into the water surrounding the pot after the plant was carefully removed. The electrode was left in the water for up to 15 - 20 min once O_2 had equilibrated. The temperature of the water for Experiment 2a ranged from 20.7 to 25.1°C and for Experiment 2b, 17.8 to 24°C (Figures 24 and 25). In Experiment 2a, the dissolved O_2 remained around 32 - 45% at day 1, 3, 5 and 8 for *B. attenuata*, while for *B. baxteri* the levels at day 1 (36 - 40%) dropped gradually to 22 - 23% by day 8 (Figure 24). In Experiment 2b, dissolved O_2 levels were about 58 - 69% for *B. attenuata,* whilst for *B. baxteri* on day 1 they were between 59 - 63%, and dropped to between 47 - 56% on day 3, and then rose back to 58 - 59% on day 6 (Figure 25).

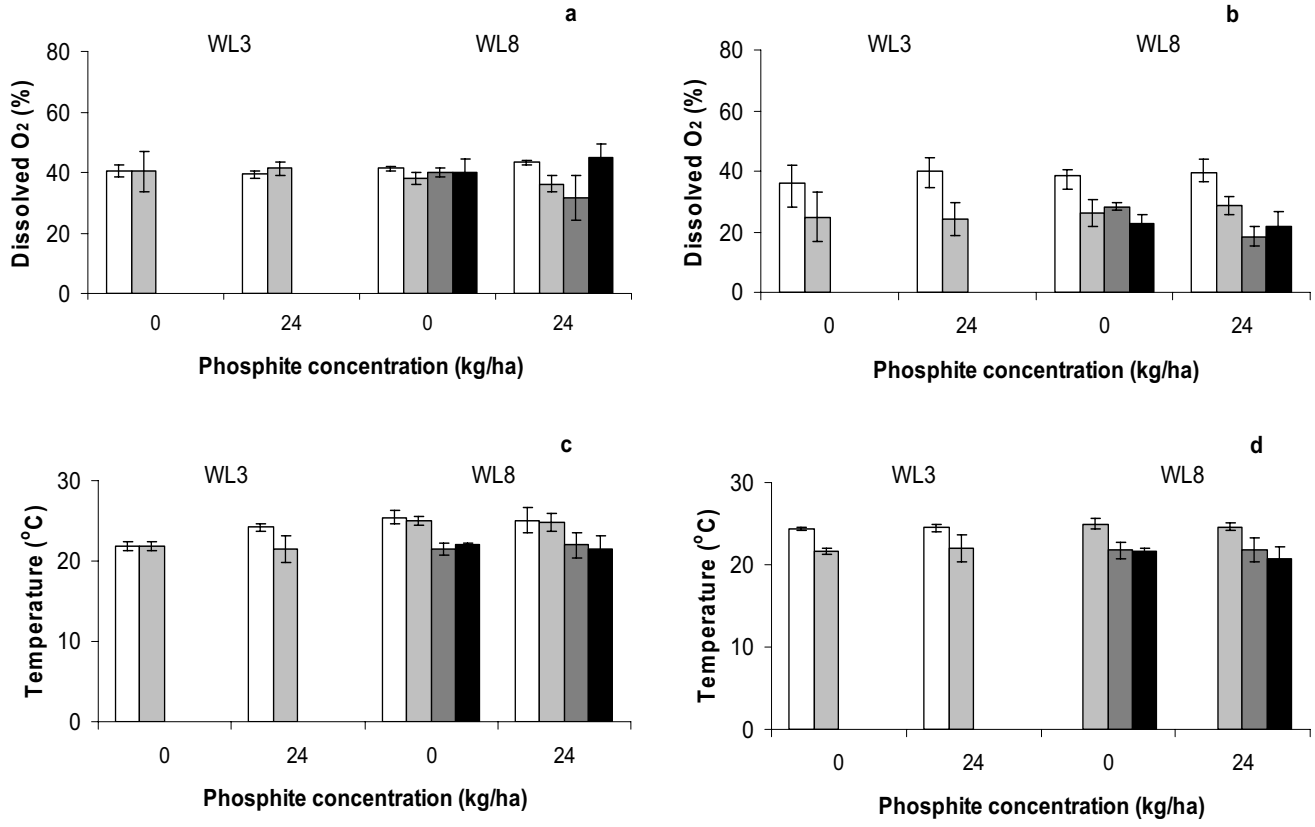


Figure 24 Mean dissolved oxygen and temperature of the water surrounding the container medium used for waterlogging the plants at 1 (□), 3 (▫) 5 (■), and 8 days (■) in Experiment 2a where phosphite was applied at 0 or 24 kg/ha to the plants 7 days after completion of waterlogging. **a** and **c**) *Banksia attenuata,* **b** and **d**) *B. baxteri. n* = 3. Plants were waterlogged for 3 (WL3) or 8 (WL8) days. Vertical bars represent two standard errors of the mean.

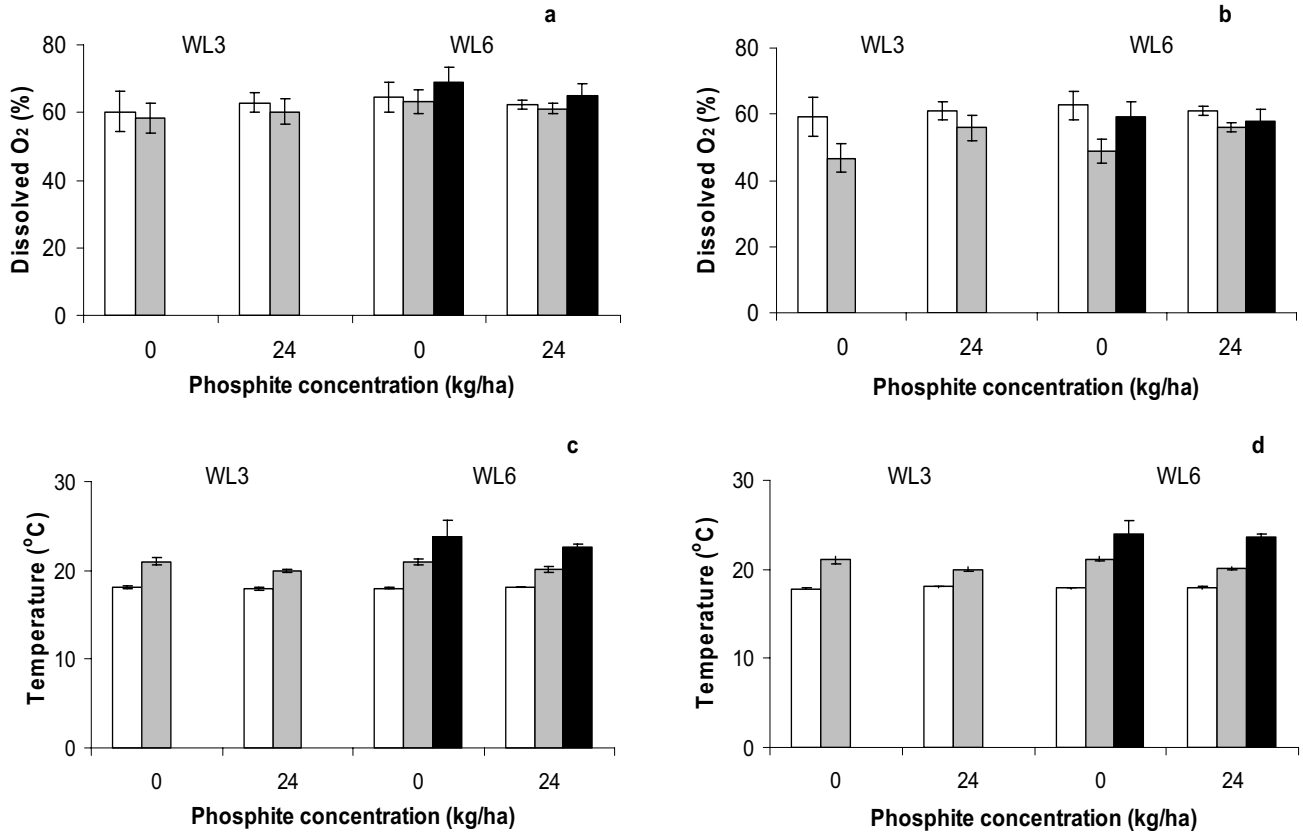


Figure 25 Mean dissolved oxygen and temperature of the water surrounding the container medium used for waterlogging the plants at 1 (□), 3 (▨) and 6 days (■) in Experiment 2b where phosphite was applied at 0 and 24 kg/ha 21 days prior to the waterlogging event. **a** and **c)** *Banksia attenuata,* **b** and **d**) *B. baxteri. n* = 3. Plants were waterlogged for 3 (WL3) or 6 (WL6) days. Vertical bars represent two standard errors of the mean.

Physiology
During waterlogging, gas exchange measurements were taken every two days. The plants were randomly selected from each treatment group. In addition, predawn water potential measurements at the end of the waterlogging treatment were taken. Gas exchange and water potential measurements at predawn were conducted 1 week, 1 month and 4 months after the spray (Experiment 2a) or after the completion of the waterlogging treatments (Experiment 2b).

Mortality
Plant mortality was recorded during both experiments.

Disease progression
At harvest, 1 week after inoculations, the inoculated main stem was removed at the soil level. The outer bark was carefully scraped back to uncover the lesion which was measured. Disease by *P. cinnamomi* may include an extension ahead of the lesion that is macroscopically asymptomatic, and in some cases may be up to 6 cm (Hüberli *et al*.

2002). To determine the extension beyond the lesion of *P. cinnamomi* in the stem, a 1 cm section of the stem from the lesion front and subsequent 1 cm section up to 5 cm beyond the lesion front were plated onto NARPH. Colonisation incorporates the sum of the lesion and extension lengths beyond the lesion.

Phosphite
Phosphite concentration of the leaf, stem and root material were determined. Tissue samples were washed in phosphate-free detergent and dried at 40^{o}C for several days. Samples were ground and sent to the WA Chemistry Centre (Perth) for phosphite analysis (see page 4).

Statistical analyses
All statistical analyses were carried out using the Statistica software V6.1 (Statsoft, Inc., Tulsa, OK, US). Following Tabachnick and Fidell (1996), data for parametric tests were screened for assumptions of homoscedasticity, presence of outliers, normality and non-correlations of means and variances. Physiological leaf measurements including photosynthetic rate, stomatal conductance, transpiration rate and predawn water potential were treated as dependent variables in separate repeated measures analysis of variance (ANOVA), with independent variables of waterlogging treatment (0, WL3, WL6/8), phosphite treatment (+/-) and species (*B. attenuata* and *B. baxteri*). Separate tests were completed on measurements taken during and post-recovery, and those taken at the beginning and end of the three inoculations.

Total lesion and colonisation lengths were correlated significantly for *B. attenuata* (r_{120} = 0.90) and *B. baxteri* (r_{171} = 0.79) in Experiment 2a and for *B. attenuata* (r_{143} = 0.87) and *B. baxteri* (r_{166} = 0.73) in Experiment 2b. Therefore, only colonisation was used as the dependent variable in analyses of variance (ANOVA). The independent variables for the separate analyses for Experiment 2a and 2b were harvest (one, two and three), phosphite treatment (+/-), waterlogging treatments (0, WL3, and WL6/8) and species (*B. attenuata* and *B. baxteri*). All significant main effects and interactions were compared using Tukey's HSD test.

Phosphite concentrations in leaf, stem, and root samples were treated as dependent variables for each of the harvests in multivariate analysis of variance (MANOVA) in each of Experiments 2a and 2b, with independent variables of waterlogging (0, WL3, WL6/8), phosphite treatment (+/-) and plant species (*B. attenuata* and *B. baxteri*). The data were analysed by two separate MANOVA tests; one tested both species at 0 and WL3 with the exclusion of WL6/8, and the second, tested all 3 waterlogging treatments for *B. baxteri* only because *B. attenuata* had not survived all WL6/8 treatments, and thus excluded the independent variable of plants species. Where appropriate, significant main effects and interactions were compared using Tukey's HSD test.

RESULTS

Physiological measurements

Leaf Water Potential

Water potentials of leaves at predawn were measured at four periods during both experiments (Figures 26 and 27). In Experiment 2a, the ANOVA test comparing water potentials immediately following free-drainage (day 9) and at the day of inoculation one (day 28), found significant main effects of Waterlogging, Species and Time, and interactions of Waterlogging x Species, Time x Waterlogging, Time x Species and Time x Waterlogging x Species (Table 3). The three-way interaction accounts for the significantly ($p < 0.001$) lower water potentials of *B. attenuata* at WL8 compared to controls and WL3 treatments (immediately after drainage), while these plants had returned to control water potential levels two weeks later (Figure 26). For *B. baxteri*, there were no differences in water potentials between waterlogging treatments at both measurement times, and these levels were not different to that of *B. attenuata* at day 28 (Figure 26). Water potentials were not affected by phosphite treatment. For Experiment 2b, there were no significant ($p > 0.05$) differences in water potentials at any of the treatments (Figures 27).

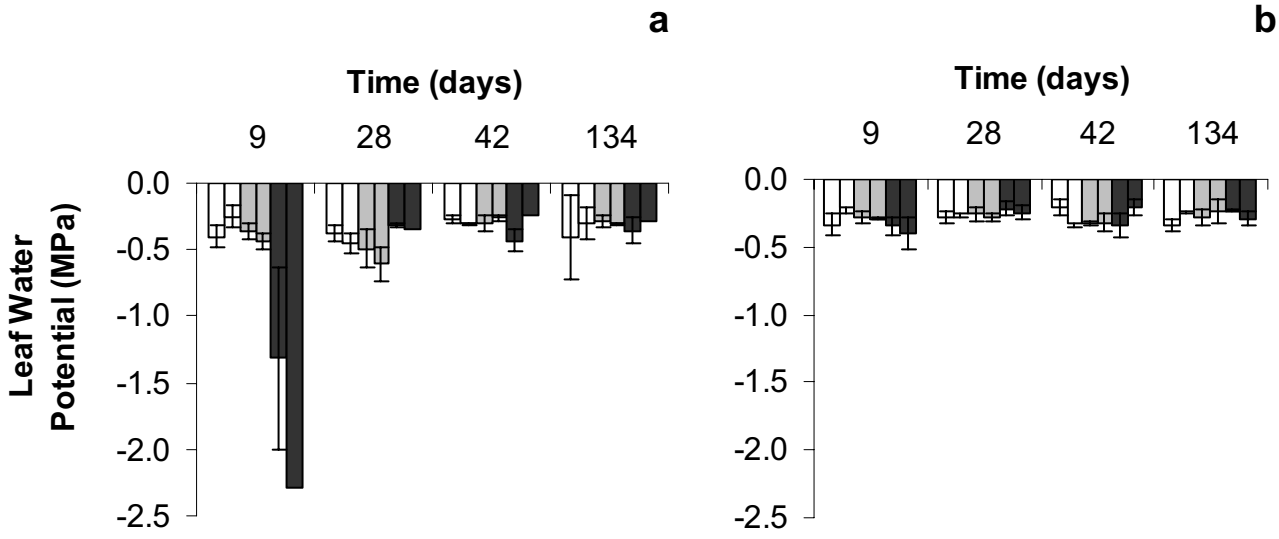


Figure 26 Mean predawn leaf water potential over a 4 month experimental period of **a**) *Banksia attenuata* and **b**) *B. baxteri* seedlings treated with 0 (left bar of each pair) or 24 kg/ha phosphite (right bar of each pair) and subjected to 0 (control □), 3 (WL3 ▒) or 8 days (WL8 ■) of waterlogging before the phosphite treatment (Experiment 2a). Waterlogging commenced at day 6 (WL3) and day 1 (WL8) with free drainage of all plants at day 9. Phosphite was applied 7 days after completion of the waterlogging events, at day 15. *n* = 3. Vertical bars represent two standard errors of the mean.

Table 3 Results of repeated measures ANOVA test for predawn leaf water potentials at free draining versus day before inoculation (day 28) of *Banksia attenuata* and *B. baxteri* subjected to 0, 3 (WL3) or 8 days (WL8) waterlogging prior to application of 24 kg/ha phosphite (Experiment 2a). Phosphite was applied 7 days after completion of waterlogging. Significant effects and interactions at $p < 0.05$ are in bold.

Effect	Df	F	p
Phosphite (+/-)	1, 24	1.4878	0.234
Waterlogging (+/-)	2, 24	**9.5016**	**<0.001**
Species	1, 24	**23.1633**	**<0.001**
Phosphite, Waterlogging	2, 24	1.7254	0.199
Phosphite, Species	1, 24	1.6294	0.214
Waterlogging, Species	2, 24	**7.9023**	**0.002**
Phosphite, Waterlogging, Species	2, 24	0.8017	0.460
Time	1, 24	**12.0309**	**0.002**
Time, Phosphite	1, 24	0.5469	0.467
Time, Waterlogging	2, 24	**18.4749**	**<0.001**
Time, Species	1, 24	**7.2780**	**0.013**
Time, Phosphite, Waterlogging	2, 24	2.1594	0.137
Time, Phosphite, Species	1, 24	0.8594	0.363
Time, Waterlogging, Species	2, 24	**13.3522**	**<0.001**
Time, Phosphite, Waterlogging, Species	2, 24	1.5852	0.226

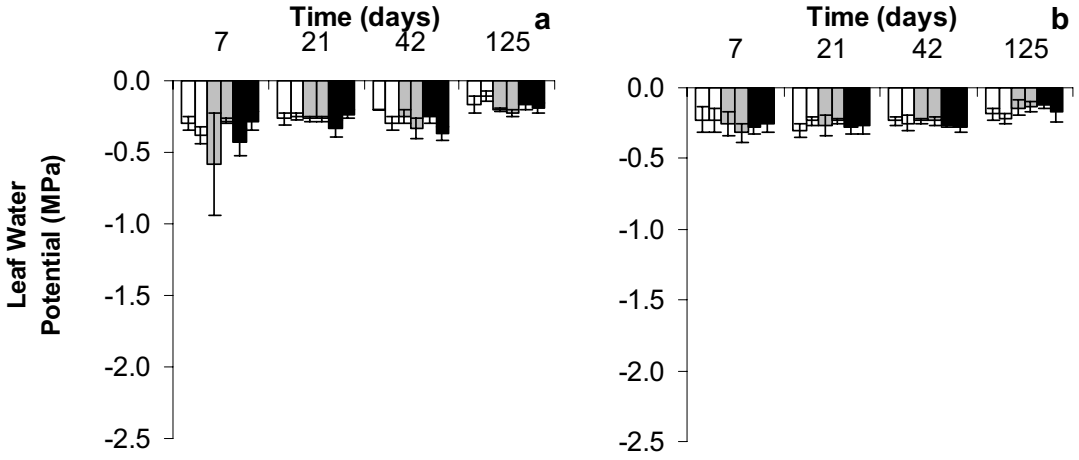


Figure 27 Mean predawn leaf water potential over a four month experimental period of **a**) *Banksia attenuata* and **b**) *B. baxteri* seedlings treated with 0 (left bar of each pair) or 24 kg/ha phosphite (right bar of each pair) and subjected to 0 (control □), 3 (WL3 ▩) or 6 days (WL6 ■) of waterlogging after the phosphite treatment (Experiment 2b). Waterlogging treatments commenced 21 days prior to phosphite application. Time scale is in relation to commencement of waterlogging treatments. *n* = 3. Vertical bars represent two standard errors of the mean.

Leaf Gas Exchange

Photosynthetic rates, stomatal conductance and transpiration responses to the waterlogging treatments for Experiments 2a and 2b are shown in Figures 28 and 29. In Experiment 2a, ANOVA tests found that all main effects and interactions were significant ($p < 0.03$) for photosynthetic and transpiration rates, except Species for transpiration rate (Table 4). For stomatal conductance, main effects of Waterlogging, Species, Time and the interaction Waterlogging x Species were significant ($p < 0.03$).

Table 4 Results of repeated measures ANOVA test for photosynthetic rates, stomatal conductance and transpiration rates over 21 days of *Banksia attenuata* and *B. baxteri* subjected to 0, 3 (WL3) or 8 days (WL8) waterlogging prior to application of 24 kg/ha phosphite (Experiment 2a). Phosphite was applied 7 days after completion of waterlogging. Significant effects and interactions at $p < 0.05$ are in bold.

		Photosynthetic rate		**Stomatal conductance**		**Transpiration rate**	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Waterlogging (0, WL3, WL8)	2, 133	**7.268**	**0.004**	**13.263**	**<0.001**	**6.851**	**0.005**
Species	1, 133	**5.449**	**0.031**	**193.201**	**<0.001**	0.867	0.362
Waterlogging, Species	2, 133	**5.994**	**0.010**	**4.445**	**0.024**	**6.308**	**0.007**
Time	7, 133	**3.265**	**0.003**	**2.251**	**0.033**	**7.216**	**<0.001**
Time, Waterlogging	14, 133	**2.719**	**0.002**	1.110	0.353	**2.898**	**<0.001**
Time, Species	7, 133	**4.085**	**<0.001**	1.079	0.380	**4.565**	**<0.001**
Time, Waterlogging, Species	14, 133	**2.071**	**0.017**	0.670	0.801	**2.373**	**0.005**

The three-way interaction for the dependent variables of photosynthetic rates and transpiration shows that *B. baxteri* was unaffected by waterlogging, while for *B. attenuata* the rates dropped after both WL3 and WL8 treatments and was more severe for WL8 than WL3 (Figure 28). Controls of *B. attenuata* were at the same level as that of *B. baxteri* treated to no waterlogging, or WL3 and WL8. *B. attenuata* at WL3 and WL8 had not recovered at the time of phosphite application (day 15) and were only just starting to recover at day 21.

Stomatal conductance also showed that the two species were different across the waterlogging treatments (Figure 28). However, the differences with time were less pronounced and thus, not significant.

In Experiment 2b, waterlogging was not a significant main effect (Table 5). No significant main effects or interactions were found for transpiration rates. Photosynthetic rates and stomatal conductance had a significant main effect of Time and the interaction Time x Species.

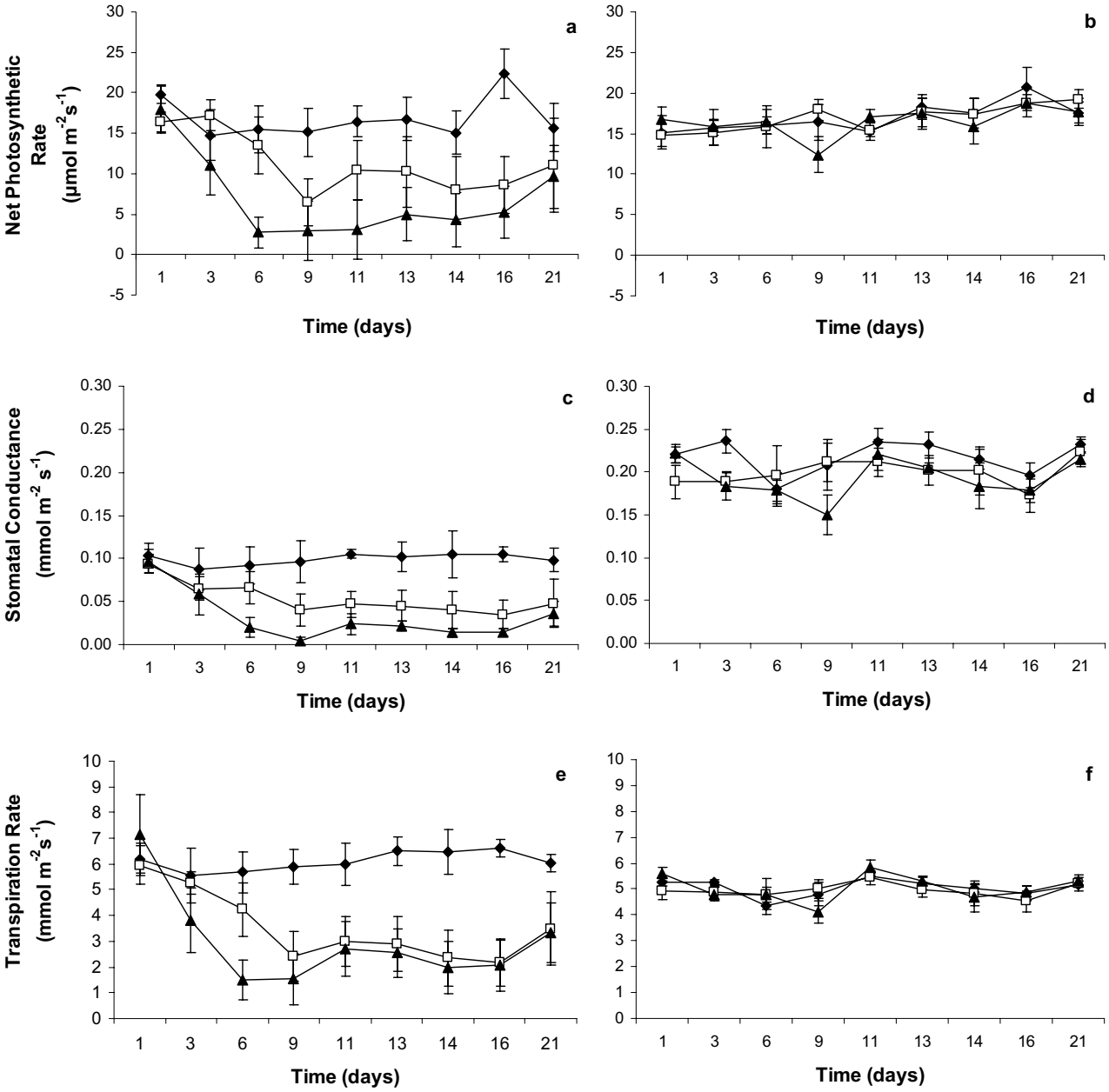


Figure 28 Mean net photosynthetic rate, stomatal conductance and transpiration rate over 21 days of **a, c** and **e**) *Banksia attenuata,* and **b, d** and **f**) *B. baxteri* seedlings subjected to 0 (cont ◆), 3 (WL3 □) or 8 days (WL8 ▲) of waterlogging before the phosphite treatment (Experiment 2a). Waterlogging treatments commenced at day 1 and ended at day 4 (WL3) and day 9 (WL8). Phosphite was applied at day 15, and since it had no significant ($p > 0.05$) effect on leaf gas exchange measurements the data were combined for waterlogging treatments. $n = 6$. Vertical bars represent two standard errors of the mean.

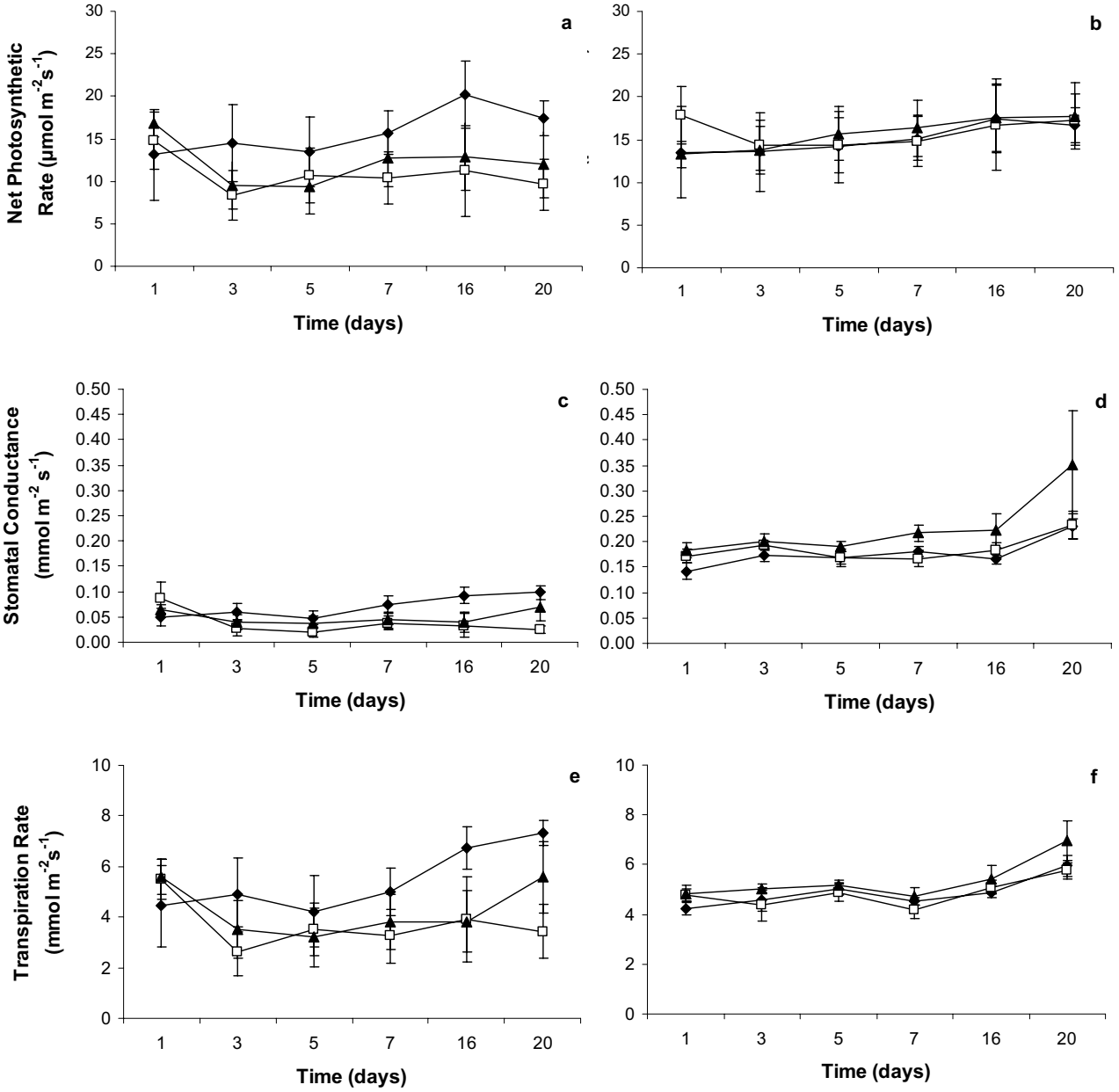


Figure 29 Mean net photosynthetic rate, stomatal conductance and transpiration rate over 20 days of **a, c** and **e**) *Banksia attenuata,* and **b, d** and **f**) *B. baxteri* seedlings subjected to 0 (cont ◆), 3 (WL3 □), or 6 days (WL6 ▲) of waterlogging after the phosphite treatment (Experiment 2b). Waterlogging treatments commenced at day 1 and ended at day 4 (WL3) and day 7 (WL6). Phosphite was applied 21 days prior to waterlogging treatments. Since phosphite treatments had no significant ($p > 0.05$) effect on leaf gas exchange measurements the data were combined for waterlogging treatments. $n = 6$. Vertical bars represent two standard errors of the mean.

Table 5 Results of repeated measures ANOVA test for photosynthetic rates, stomatal conductance and transpiration rates over 20 days of *Banksia attenuata* and *B. baxteri* subjected to 0, 3 (WL3) or 6 days (WL6) waterlogging after application of 24 kg/ha phosphite (Experiment 2b). Phosphite was applied 21 days before commencement of waterlogging. Significant effects and interactions at $p < 0.05$ are in bold.

		Photosynthetic rate		Stomatal conductance		Transpiration rate	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Waterlogging (0, WL3, WL6)	2,81	0.204	0.816	0.769	0.477	0.035	0.966
Species	1,81	1.738	0.198	**150.893**	**<0.001**	0.106	0.748
Waterlogging, Species	2,81	1.197	0.318	1.500	0.248	0.220	0.804
Time	3,81	**3.968**	**0.011**	**2.836**	**0.046**	0.534	0.660
Time, Waterlogging	6,81	1.030	0.412	1.273	0.284	1.231	0.301
Time, Species	3,81	**3.153**	**0.029**	**3.855**	**0.014**	2.594	0.060
Time, Waterlogging, Species	6,81	1.112	0.363	0.658	0.683	0.635	0.702

The initial MANOVA of physiology measurements at the 2, 5 and 27 weeks after phosphite application (Experiment 2a) found significant main effects of Harvest and Species and the interaction Harvest x Species. For all four measurements this interaction was significant ($p < 0.02$) (Table 6). The significance was attributed to *B. baxteri* having much lower levels of photosynthesis, stomatal conductance and transpiration at the final harvest, while *B. attenuata* remained relatively stable across harvests. For leaf water potentials, however, it was *B. baxteri* that had no differences across harvests, whilst *B. attenuata* had significantly ($p < 0.04$) lower water potentials at the first harvest than the other 2 harvests.

Table 6 Results of univariate ANOVA tests (following significant initial MANOVA) of photosynthetic and transpiration rates, stomatal conductance and leaf water potential across three harvests (2, 5, and 27 weeks after phosphite treatment). This shows significant main effects and interactions for *Banksia attenuata* and *B. baxteri* sprayed with phosphite after completion of waterlogging treatments of 0, 3 (WL3) or 8 days (WL8) (Experiment 2a). Significant main effects and interactions at $p < 0.05$ are in bold.

		Photosynthetic rate		Stomatal conductance		Transpiration rate		Leaf water potential	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Harvest	2,72	**12.885**	**<0.001**	**39.760**	**<0.001**	**17.122**	**<0.001**	2.487	0.090
Species	1,72	0.887	0.350	**145.812**	**<0.001**	3.173	0.079	**9.090**	**0.004**
Harvest, Species	2,72	**5.954**	**0.004**	**12.674**	**<0.001**	**4.003**	**0.023**	**4.870**	**0.010**

For Experiment 2b, the initial MANOVA only found significant main effects of Harvest and Species. Harvest was significant across all four physiological measurements, while Species was significant only for photosynthetic rates and stomatal conductance (Table 7). The last two harvests had similar and lower photosynthetic rates, stomatal conductance and transpiration rates than in the first harvest two weeks after the phosphite application.

For leaf water potentials, the first two harvests were significantly ($p < 0.001$) lower than the last harvest.

Table 7 Results of univariate ANOVA tests (following significant initial MANOVA) of photosynthetic and transpiration rates, stomatal conductance and leaf water potential across three harvests (2, 5, and 27 weeks after completion of waterlogging treatments). This shows significant main effects and interactions for *Banksia attenuata* and *B. baxteri* sprayed with phosphite prior to waterlogging treatments of 0, 3 (WL3) or 8 days (WL8) (Experiment 2b). Significant main effects and interactions at $p < 0.05$ are in bold.

		Photosynthetic rate		Stomatal conductance		Transpiration rate		Leaf water potential	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Harvest	2,70	**4.127**	**0.020**	**12.096**	**<0.001**	**6.920**	**0.002**	**19.949**	**<0.001**
Species	1,70	**4.429**	**0.039**	**91.117**	**<0.001**	0.215	0.644	0.937	0.336

Disease Progression

Colonisation

Colonisation responses of *B. attenuata* and *B. baxteri* to waterlogging treatments before (Experiment 2a) or after (Experiment 2b) the phosphite treatment are shown in Figures 30 and 31. In both experiments, the ANOVA tests found no significant ($p > 0.45$ in both cases) main effects of Waterlogging treatment (Table 8). There were, however, significant ($p < 0.05$) main effects of Harvest, Phosphite treatment and Species, and interactions of Harvest x Phosphite and Harvest x Species in Experiments 2a and 2b.

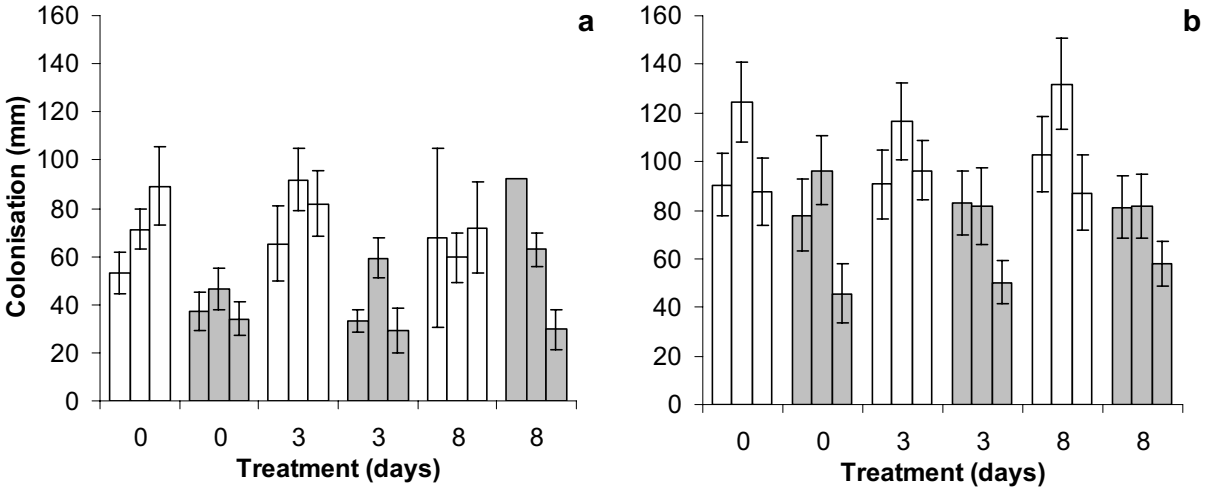


Figure 30 Mean total colonisation (mm) of *Phytophthora cinnamomi* in the stems of **a**) *Banksia attenuata,* and **b**) *B. baxteri* plants treated with 0 (□) or 24 kg/ha phosphite (▩) and subjected to 0, 3 (WL3) or 8 days (WL8) of waterlogging. The three bars of each colour set were harvested 2, 5 and 27 weeks after phosphite treatment. Phosphite was applied 7 days after completion of waterlogging events (Experiment 2a). *n* = 10 (except WL8 for *B. attenuata* where *n* = 1 - 2). Vertical bars represent two standard errors of the mean.

Table 8 Results of univariate ANOVA tests of colonisation length of *Banksia attenuata* and *B. baxteri* after underbark inoculation of the stem with *Phytophthora cinnamomi* 2, 5 and 27 weeks after phosphite treatment (Experiment 2a) or after completion of waterlogging treatments (Experiment 2b). Plants were subjected to 0, 3 (WL3), 6 (WL6, Experiment 2b) or 8 days (WL8, Experiment 2a) of waterlogging prior to (Experiment 2a) or after (Experiment 2b) the phosphite treatment. Significant effects and interactions at $p < 0.05$ are in bold.

		Experiment 2a		Experiment 2b	
Effect	**Df**	**F**	**p**	**F**	**p**
Harvest (2, 5, 27 weeks)	2,255	**4.842**	**0.009**	**7.083**	**0.001**
Phosphite (+/-)	1,255	**78.208**	**<0.001**	**40.566**	**<0.001**
Waterlogging (0, 3, 6/8 days)	2,255	0.710	0.492	0.794	0.453
Species (*B. attenuata*, *B. baxteri*)	1,255	**60.509**	**<0.001**	**55.168**	**<0.001**
Harvest, Phosphite	2,255	**5.474**	**0.005**	**4.133**	**0.017**
Harvest, Waterlogging	4,255	1.518	0.197	0.578	0.679
Phosphite, Waterlogging	2,255	0.184	0.832	0.380	0.684
Harvest, Species	2,255	**3.039**	**0.050**	**4.020**	**0.019**
Phosphite, Species	1,255	0.002	0.960	0.261	0.610
Waterlogging, Species	2,255	0.539	0.584	0.092	0.912
Harvest, Phosphite, Waterlogging	4,255	0.351	0.843	0.993	0.412
Harvest, Phosphite, Species	2,255	1.398	0.249	0.070	0.932
Harvest, Waterlogging, Species	4,255	2.378	0.052	2.179	0.072
Phosphite, Waterlogging, Species	2,255	1.550	0.214	1.359	0.259
Harvest, Phosphite, Waterlogging, Species	4,255	0.873	0.480	0.419	0.795

In Experiment 2a, colonisation was always significantly ($p = 0.005$) lower in sprayed plants across all three harvests compared to non-sprayed plants (Figure 30). There were two exceptions where sprayed *B. attenuata* subjected to WL8 had colonisation lengths that were similar to non-sprayed plants in the first two harvests. However, in these instances there were only two or fewer replicate plants per treatment, due to plant deaths, as a result of waterlogging. Despite these exceptions in the first two harvests, the final harvest of 27 weeks after spraying phosphite, found that sprayed plants had a large reduction in colonisation of about 50% compared to non-sprayed plants (Figure 30). The significant Species x Harvest interaction for Experiment 2a highlights that *B. baxteri* always had larger colonisation ($p < 0.001$) than *B. attenuata* across the first two harvests. At harvest three, however, colonisation lengths were no longer significant ($p = 15$) between the two species.

In Experiment 2b, colonisation was significantly (p = 0.02) higher in non-sprayed plants than in the sprayed in all but the final harvest (Figure 31). Unlike in Experiment 2a, *B. baxteri* had larger (p = 0.02) colonisation lengths in all but the first harvest compared to *B. attenuata*.

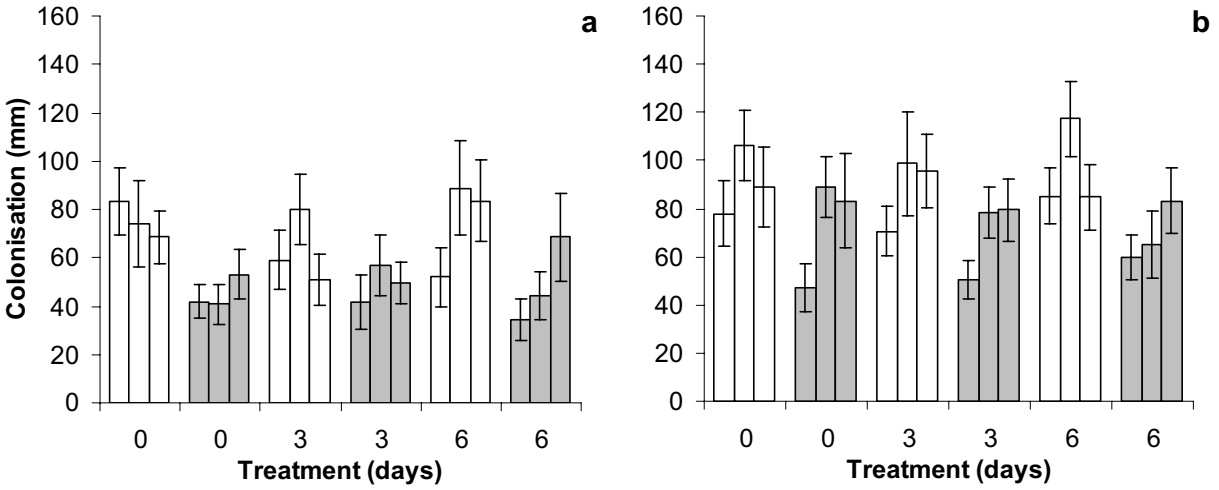


Figure 31 Mean total colonisation (mm) of *Phytophthora cinnamomi* in the stems of **a**) *Banksia attenuata,* and **b**) *B. baxteri* plants treated with 0 (□) or 24 kg/ha phosphite (▩) and subjected to 0, 3 (WL3) or 6 days (WL6) of waterlogging. The three bars of each colour set were harvested 2, 5 and 27 weeks after completion of waterlogging treatments. Phosphite was applied 21 days before commencement of waterlogging events (Experiment 2b). *n* = 10. Vertical bars represent two standard errors of the mean.

Mortality

B. attenuata was significantly (p < 0.05) more sensitive to waterlogging in both experiments than *B. baxteri* (Figure 32) with approximately 30 - 50% of plants dying after treatments 6 (Experiment 2b) or 8 days (Experiment 2a) of waterlogging. The 6 day waterlogging treatment was less severe to *B. attenuata*. In contrast, less than 10% of *B. baxteri* died after 8 days of waterlogging, whilst no plants died after 6 days waterlogging.

Phosphite analysis in stems, roots and leaves

Phosphite concentrations over time in leaf, stem and root samples of *B. attenuata* and *B. baxteri* subjected to waterlogging treatments before (Experiment 2a) and after (Experiment 2b) phosphite application are shown in Figures 33 and 34, respectively. For Experiment 2a, the initial MANOVA comparing waterlogging treatment 0 and WL3 of both species found significant main effects of Phosphite and Species, and the interaction Phosphite x Species. The waterlogging treatment was not significant (p = 0.51). Leaf phosphite concentrations were significant for all three effects, while stems and roots were only significant for Phosphite (Table 9). *B. attenuata* had significantly (p = 0.03) higher phosphite concentrations in leaves than *B. baxteri* when controls and WL3 were compared (Figure 33).

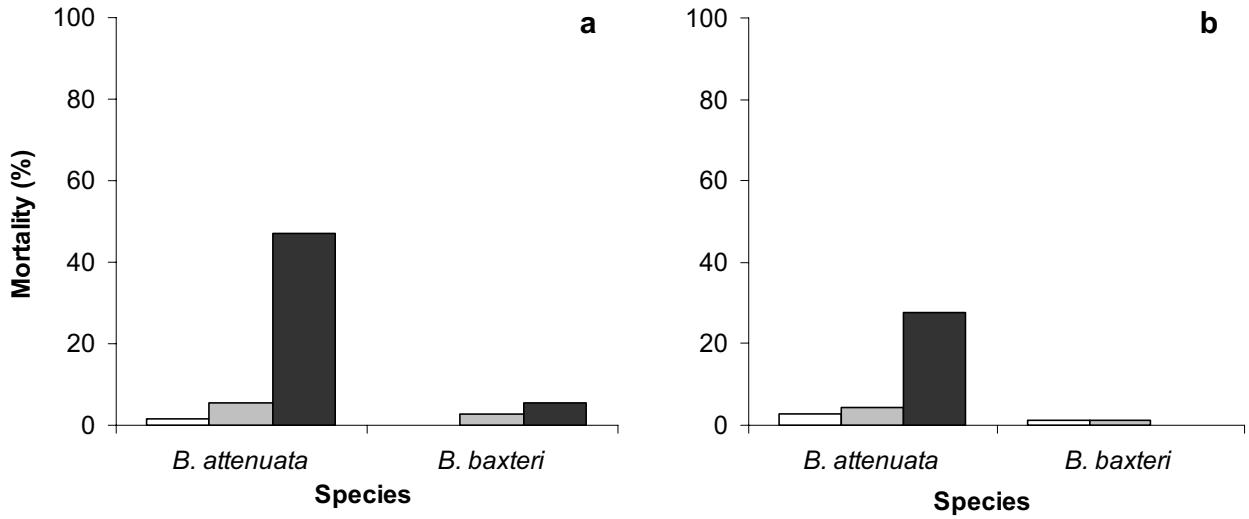


Figure 32 Percentage mortality caused by waterlogging treatments in *Banksia attenuata* and *B. baxteri* seedlings subjected to 0 (control □), 3 (WL3 ▒), 6 (WL6 ■ , Experiment 2b) or 8 days (WL8 ■ , Experiment 2a) of waterlogging. **a**) Experiment 2a - phosphite was applied 7 days after the completion of waterlogging events, while in **b**) Experiment 2b - phosphite was applied 21 days prior to waterlogging. *n* = 36.

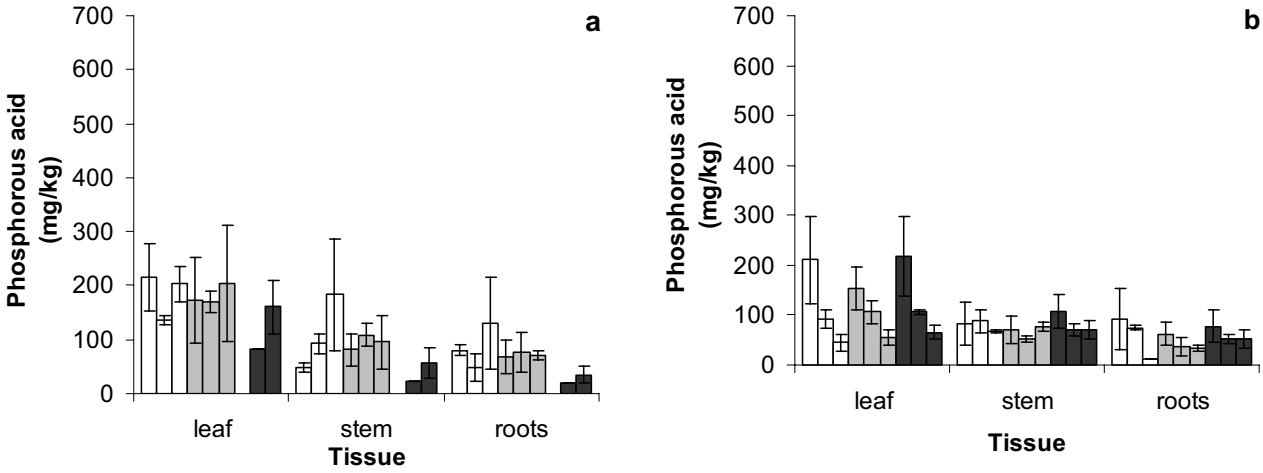


Figure 33 Mean phosphite (as phosphorous acid mg/kg) content of tissue in **a**) *Banksia attenuata* and **b**) *B. baxteri* seedlings treated with 24 kg/ha phosphite and subjected to 0 (control □), 3 (WL3 ▒) or 8 days (WL8 ■) of waterlogging. The three bars of each colour set were harvested 2, 5 and 27 weeks after phosphite application. Phosphite was applied 7 days after completion of the waterlogging treatment (Experiment 2a). *n* = 3. Vertical bars represent two standard errors of the mean.

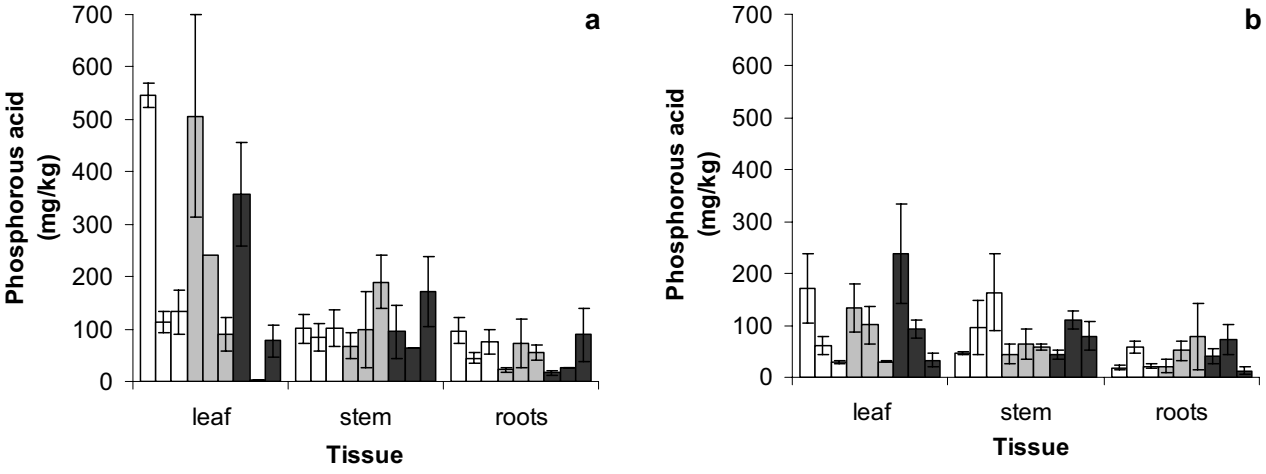


Figure 34 Mean phosphite (as phosphorous acid mg/kg) content of tissue in **a**) *Banksia attenuata* and **b**) *B. baxteri* seedlings treated with 24 kg/ha phosphite and subjected to 0 (control □), 3 (WL3 ▒) or 6 days (WL6 ■) of waterlogging. The three bars of each colour set were harvested 2, 5 and 27 weeks after the final waterlogging treatment. Phosphite was applied 21 days prior to waterlogging (Experiment 2b). *n* = 3. Vertical bars represent two standard errors of the mean.

Since *B. attenuata* had missing data for WL8, a comparison of all three waterlogging treatments can only be made for *B. baxteri*. In Experiment 2a, the initial MANOVA for this comparison found significant main effects of Harvest and Phosphite. Both main effects were significant for leaves, while as in the previous analysis, stems and roots were only significant for Phosphite (Table 10). For *B. baxteri*, leaf concentrations declined over time with the first harvest having significantly (p = 0.002) higher concentrations than the final harvest 4 months after phosphite application (Figure 33). This decline in leaf concentrations did not appear to be a trend for *B. attenuata*.

Table 9 Results of univariate ANOVA tests (following significant initial MANOVA) of plant tissue phosphite concentrations across three harvests (2, 5, and 27 weeks after phosphite treatment). This shows significant main effects and interactions for phosphite treatment after (Experiment 2a) completion of waterlogging treatments of 0 and 3 days (WL3) of *Banksia attenuata* and *B. baxteri* at the time of harvest of the inoculated stems. The 8 day waterlogging treatment (WL8) is not included in this analysis because there were no data in some treatments for *B. attenuata*. Significant main effects and interactions at p < 0.05 are in bold.

		Leaf phosphite concentration		Stem phosphite concentration		Root phosphite concentration	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Phosphite (+/-)	1, 40	**69.979**	**<0.001**	**73.068**	**<0.001**	**36.248**	**<0.001**
Species	1, 40	**5.083**	**0.030**	3.554	0.067	1.714	0.198
Phosphite, Species	1, 40	**4.961**	**0.032**	3.524	0.068	2.238	0.142

Table 10 Results of univariate ANOVA tests (following significant initial MANOVA) of plant tissue phosphite concentrations across three harvests (2, 5, and 27 weeks after phosphite treatment). This shows significant main effects and interactions for phosphite treatment after (Experiment 2a) completion of waterlogging treatments of 0, 3 (WL3) and 8 days (WL8) of *B. baxteri* at the time of harvest of the inoculated stems. Significant main effects and interactions at $p < 0.05$ are in bold.

		Leaf phosphite concentration		Stem phosphite concentration		Root phosphite concentration	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Harvest (2, 5, 27 weeks)	2,32	**6.509**	**0.004**	0.064	0.938	2.404	0.106
Phosphite (+/-)	1,32	**48.874**	**<0.001**	**68.974**	**<0.001**	**27.599**	**<0.001**

For Experiment 2b, the initial MANOVA comparing controls and WL3 treatments of both species found significant main effects of Harvest, Phosphite and Species, and the interactions Harvest x Phosphite, Harvest x Species, Phosphite x Species and Harvest x Phosphite x Species. The univariate ANOVA test attributed all significant effects to leaf phosphite concentrations, and Phosphite to stem and root phosphite concentrations (Table 11). Stems also had a significant Harvest x Phosphite interaction.

Table 11 Results of univariate ANOVA tests (following significant initial MANOVA) of plant tissue phosphite concentrations across three harvests (2, 5, and 27 weeks after completion of waterlogging treatments). This shows significant main effects and interactions for phosphite treatment before (Experiment 2b) waterlogging treatments of 0 and 3 days (WL3) of *Banksia attenuata* and *B. baxteri* at the time of harvest of the inoculated stems. Significant main effects and interactions at $p < 0.05$ are in bold. The 6 day waterlogging treatment (WL6) is not included in this analysis because there was no data in some treatments for *B. attenuata*.

		Leaf phosphite concentration		Stem phosphite concentration		Root phosphite concentration	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Harvest (2, 5, 27 weeks)	2, 41	**16.622**	**<0.001**	2.858	0.069	0.996	0.378
Phosphite (+/-)	1, 41	**70.796**	**<0.001**	**72.909**	**<0.001**	**48.095**	**<0.001**
Species	1, 41	**19.946**	**<0.001**	3.439	0.071	2.326	0.135
Harvest, Phosphite	2, 41	**15.500**	**<0.001**	**3.246**	**0.049**	0.911	0.410
Harvest, Species	2, 41	**5.710**	**0.006**	0.044	0.957	0.383	0.684
Phosphite, Species	1, 41	**19.566**	**<0.001**	3.249	0.079	2.186	0.147
Harvest, Phosphite x Species	2, 41	**5.602**	**0.007**	0.044	0.956	0.330	0.720

B. baxteri always had lower phosphite concentrations in leaves across the three harvests than *B. attenuata* and these were significant (p = 0.001) only at the 2 week harvest (Figures 34 and 35). Both species had phosphite concentrations that were higher than in non-sprayed plants, but these were only significant (p = 0.001) for *B. attenuata* at the 2 week harvest. For stems and roots, phosphite concentrations were always higher in sprayed plants (Table 11).

In the initial MANOVA tests comparing all three waterlogging treatments for *B. baxteri*, main effects of Harvest and Phosphite, and the interaction of Harvest x Phosphite were significant (p < 0.001). The univariate ANOVA tests attributed the significances to leaves for Harvest, Phosphite and Harvest x Phosphite, and Phosphite to stems and roots (Table 12). Phosphite concentrations declined with each harvest, with the 2 week harvest having significantly (p < 0.005) higher concentrations than at the 5 and 27 week harvests, while concentrations in the last two harvests were not different from each other (Figure 34).

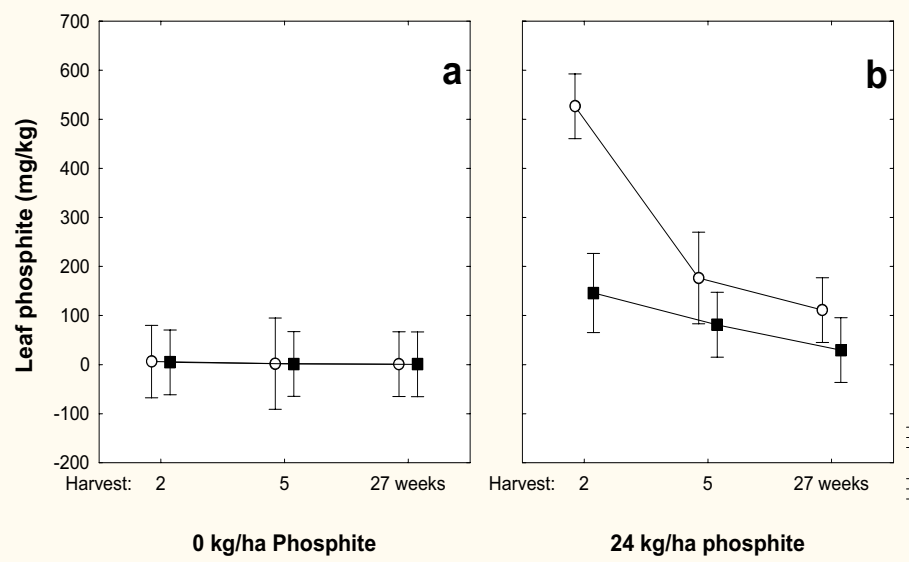


Figure 35 Three-way interaction plot of Harvest x Phosphite x Species for comparisons of mean phosphite concentrations in leaves harvested at 2, 5 and 27 weeks after the final waterlogging treatment of *Banksia attenuata* (○) and *B. baxteri* (■) treated with 0 (**a**) or 24 kg/ha (**b**) phosphite 21 days prior to waterlogging (Experiment 2b).

Table 12 Results of univariate ANOVA tests (following significant initial MANOVA) of plant tissue phosphite concentrations across three harvests (2, 5, and 27 weeks after completion of waterlogging treatments). This shows significant main effects and interactions for phosphite treatment before (Experiment 2b) waterlogging treatments of 0, 3 (WL3) and 6 days (WL6) of *B. baxteri* at the time of harvest of the inoculated stems. Significant main effects and interactions at $p < 0.05$ are in bold.

		Leaf phosphite concentration		Stem phosphite concentration		Root phosphite concentration	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Harvest (2, 5, 27 weeks)	2, 32	**11.193**	**<0.001**	1.228	0.306	1.083	0.351
Phosphite (+/-)	1, 32	**52.042**	**<0.001**	**39.494**	**<0.001**	**22.264**	**<0.001**
Harvest, Phosphite	2, 32	**10.028**	**<0.001**	1.563	0.225	1.004	0.378

DISCUSSION

The two species chosen to investigate whether waterlogging altered the efficacy of phosphite to control *P. cinnamomi* had very different responses to short-term waterlogging. *Banksia attenuata* was very sensitive to waterlogging and had markedly reduced stomatal aperture within three days which slowed photosynthesis and transpiration (Experiment 2a). By contrast, *B. baxteri* continued to maintain stomatal function and gas exchange was unimpaired. *B. baxteri* also maintained its midday leaf water potential just below -0.5 MPa whereas in *B. attenuata* leaf water potential dropped to about -2.0 MPa. Similar trends occurred in Experiment 2b, but the effects of 3 days waterlogging on *B. attenuata* were somewhat muted.

Even though waterlogging altered the physiology of the leaf in *B. attenuata*, and leaf function had not recovered to their unstressed rates by the time phosphite was applied (Experiment 2a), this did not appear to affect the uptake and distribution of phosphite in the plant. This suggests that phosphite can be applied shortly after waterlogging has occurred. Not surprisingly, there was no effect of waterlogging on uptake and distribution of phosphite in *B. baxteri*, a species less sensitive to waterlogging than *B. attenuata* in Experiment 2a. These data suggest that the main pathway for phosphite uptake by leaves is likely to be via the epidermis and uptake via the stomata is likely to be minor. Indeed, the stomata occur on the lower side of the leaf, in grooves in some species, and foliar sprays do not achieve good coverage of these areas. There is considerable literature on the uptake of other chemicals by leaves which illustrate the importance of ectotrophic channels in the cuticular layers leading into the outer wall of epidermal cells.

It might have been expected that the more stressed *B. attenuata* would have less carbohydrate, and hence less phosphite flowing to the roots as a consequence of reduced carbon assimilation and root respiration. However, this was not observed over the time course of the measurement period. Again, this indicates how *Banksia* species that are susceptible to *P. cinnamomi* can rapidly distribute phosphite to the roots once uptake occurs by the leaves.

Short-term waterlogging did not affect the concentration of phosphite in roots and stems, and nor did it reduce the efficacy of phosphite to control *P. cinnamomi*. In the absence of phosphite, waterlogging of 3 or 6 - 8 days had no effect on *P. cinnamomi* colonisation when assessed two weeks after the waterlogging event. During waterlogging, the plants are likely to be most vulnerable as has been demonstrated widely in the literature. Whether phosphite offers protection during this vulnerable period needs further investigation, especially under long-term water-stress.

Phosphite reduced *P. cinnamomi* colonisation in both *B. attenuata* and *B. baxteri* when the plants were not subjected to waterlogging treatments compared to non-phosphite treated plants. This was expected based on previous studies (Shearer and Fairman 2007). When phosphite was applied to *B. attenuata* and *B. baxteri* after they had been subjected to three days of waterlogging (Experiment 2a), phosphite continued to reduce colonisation as effectively as in non-waterlogged plants. In *B. attenuata,* colonisation assessments showed phosphite applications after 8 days of waterlogging were effective at 27 weeks post-application, but in the earlier assessments at 2 and 5 weeks after the waterlogging event, phosphite efficacy was reduced. This indicates that in at least some species, phosphite applied soon after the waterlogging event is taken up by the plants, but the plant defense systems do not become fully functional until the plants have physiologically recovered from the waterlogging event. In the case of *B. attenuata* this took longer than 5 weeks. It is interesting to note that for *B. baxteri*, a more waterlogging tolerant species, the efficacy of phosphite was not adversely affected by waterlogging. When phosphite was applied prior to waterlogging (Experiment 2b), subsequent 3 or 6 day waterlogging events had no adverse impact on the ability of either *B. attenuata* or *B. baxteri* to respond to phosphite and contain *P. cinnamomi*. These results indicate that applications of phosphite soon after prolonged waterlogging events (less than one week) should not be recommended, until further research determines the time at which plants effectively take up phosphite after waterlogging. However, if applications of phosphite occur before waterlogging there is no need for managers to be concerned and they need not spend resources and apply more phosphite.

In summary, managers do not need to be concerned about reapplying phosphite to waterlogged areas at high risk from *P. cinnamomi* if phosphite has been applied to the area either prior or subsequent to the waterlogging event. This recommendation is made for waterlogging events between 3 and 8 days. In terms of management of areas at risk from *P. cinnamomi* which have not previously been treated with phosphite, but have been subjected to waterlogging, our study shows that as soon as weather conditions are conducive to spraying (no wind and no rain forecast within one week) phosphite can be and should be applied.

REFERENCES

Beckman C, Perry RL, Flore JA (1992) Short-term flooding affects gas exchange characteristics of containerized sour cherry trees. *Hortscience* 27, 1297-1301.

Bowen BJ (1991) Fire response within the Family Proteaceae: a comparison of plants displaying the seeder and resprouter mode of recovery. PhD thesis, Perth, Western Australia: University of Western Australia.

Bradford KJ, Hsiao TC (1982) Stomatal behaviour and water relations of waterlogged tomato plants. *Plant Physiology* 70, 1508-1513.

Burgess SSO, Adams MA, Turner NC, Ong CK (1998) The redistribution of soil water by tree root systems. *Oecologia* 115, 306-311.

Burgess T, McComb J, Colquhoun I, Hardy G (1999) Increased susceptibility of *Eucalyptus marginata* to stem infection by *Phytophthora cinnamomi* resulting from root hypoxia. *Plant Pathology* 48, 797–806.

Cohen Y, Coffey MD (1986) Systemic fungicides and the control of oomycetes. *Annual Review of Phytopathology* 24, 311-338.

Crombie DS, Tippett JT, Hill TC (1988) Dawn water potential and root depth of trees and understorey species in south-western Australia. *Australian Journal of Botany* 36, 621-631.

Davies CL, Turner DW, Dracup M (2000) Yellow lupin (*Lupinus luteus*) tolerates waterlogging better than narrow-leafed lupin (*L. angustifolius*) I. Shoot and root growth in a controlled environment. *Australian Journal of Agricultural Resources* 51, 701-709.

Davison EM, Tay FCS (1986) The effect of aeration on colony diameter, sporangium production and zoospore germination of *Phytophthora cinnamomi*. *New Phytologist* 103, 735-744.

Davison EM, Tay FCS (1987) The effect of waterlogging on infection of *Eucalyptus marginata* seedlings by *Phytophthora cinnamomi. New Phytologist* 105, 585-594.

Dodd J, Bell DT (1993) Water relations of the canopy species in a *Banksia* woodland, Swan Coastal Plain, Western Australia. *Australian Journal of Ecology* 18, 281-293.

Drew MC (1983) Plant injury and adaptation to oxygen deficiency in the root environment: A review. *Plant and Soil* 75, 179-199.

Drew MC (1992) Soil aeration and plant root metabolism. *Soil Science* 154, 259-268.

Else MA, Coupland D, Dutton L, Jackson MB (2001) Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (*Ricinius communis*) despite diminished delivery of ABA from roots to shoots in xylem sap. *Physiologia Plantarum* 111, 46-54.

Florentine SK, Fox JED (2002) Morphological and physiological adaptations to waterlogging by *Eucalyptus* seedlings from the semi-arid Pilbara, Western Australia. *Journal of the Royal Society of Western Australia* 85, 61-70.

Froend RH, Drake PL (2006) Defining phreatophytic response to reduced water availability: preliminary investigations on the use of xylem cavitation vulnerability in *Banksia* woodland species. *Australian Journal of Botany* 54, 173-179.

George AS (1984) *The Banksia Book*. (Kangaroo Press in association with The Society for Growing Australian Plants NSW Ltd. Kenhurst, NSW).

Gravatt DA, Kirby CJ (1998) Patterns of photosynthesis and starch allocation in seedlings of four bottomland hardwood tree species subjected to flooding. *Tree Physiology* 18, 411-417.

Groom PK (2004) Rooting depth and plant water relations explain species distribution patterns within a sandplain landscape. *Functional Plant Biology* 31, 423-428.

Henson IE, Jensen CR, Turner NC (1989) Leaf gas exchange and water relations of lupins and wheat. I. Shoot responses to soil water deficits. *Australian Journal of Plant Physiology* 16, 401-413.

Hüberli D (1995) Analysis of Variability Among Isolates of *Phytophthora cinnamomi* Rands from *Eucalyptus marginata* Donn Ex. Sm. and *Eucalyptus calophylla* R. Br. Based on Cultural Characteristics, Sporangia and Gametangial Morphology, and Pathogenicity. Honours thesis, Murdoch, Western Australia: Murdoch University.

Hüberli D, Tommerup IC, Hardy GEStJ (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi. Australasian Plant Pathology* 29,164-169.

Hüberli D, Tommerup IC, Dobrowolski MP, Calver MC, Hardy GEStJ (2001) Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. *Mycological Research* 105,1053-1064.

Hüberli D, Tommerup IC, Colquhoun IJ, Hardy GEStJ (2002) Evaluation of clonal lines of *Eucalyptus marginata* inoculated in lateral branches and roots for resistance to *Phytophthora cinnamomi*. *Plant Pathology* 51, 435-442.

Jackson TJ, Burgess T, Colquhoun I, Hardy GEStJ (2000) Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. *Plant Pathology* 49,147–154.

Jones HG (1998) Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* 49, 387-398.

Kozlowski TT (1997) Responses of woody plants to flooding and salinity. *Tree Physiology* Monograph 1.

Kozlowski TT, Pallardy SG (1997) *Growth Control in Woody Plants*. Academic Press, San Diego.

Kreuzwieser J, Fürniss S, Rennenberg H (2002) Impact of waterlogging on the N-metabolism of flood tolerant and non-tolerant tree species. *Plant, Cell and Environment* 8, 1039-1049.

Lambers H, Chapin III FS, Pons TL (1998) *Plant Physiological Ecology*. New York, USA: Springer.

Liao CT, Lin CH (2001) Physiological adaptation of crop plants to flooding stress. *Proceedings of the National Science Council* 25, 148-157.

Marchant NG, Wheeler JR, Rye BL, Bennett EM, Lander NS, Macfarlane TD (1987) *Flora of the Perth Region*. West Australian Herbarium, Perth.

McCutchan H, Shackel KA (1992) Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. cv. French). *Journal of the American Society of Horticultural Science* 117, 607–611.

Naidoo G, Naidoo S (1992) Waterlogging responses of *Sporobolus virginicus* (L.) Kunth. *Oecologia* 90, 445–50.

O'Gara E, Hardy GEStJ, McComb J (1996) The ability of *Phytophthora cinnamomi* to infect through unwounded and wounded periderm tissue of *Eucalyptus marginata*. *Plant Pathology* 45, 955-963.

Pate JS, McComb AJ (Eds). (1981) *The Biology of Australian Plants*. University Press, Perth, Western Australia.

Pate JS, Froend RH, Bowen B, Hansen A, Kuo J (1990) Seedling growth and storage characteristics of seeder and resprouter species of Mediterranean-type ecosystems of S.W. Australia. *Annals of Botany* 65, 585-601.

Pezeshki SR, Chambers JL (1985) Response of cherrybark oak (*Quercus falcate* var. *pagodaefolia*) seedlings to short-term flooding. *Forestry Science* 31, 760-771.

Pezeshki SR, Pardue JH, DeLaune RD (1996) Leaf gas-exchange and growth of flood tolerant and flood-sensitive tree species under low soil redox conditions. *Tree Physiology* 16, 453-458.

Poot P, Lambers H (2003) Growth responses to waterlogging and drainage of woody *Hakea* (Proteaceae) seedlings, originating from contrasting habitats in south-western Australia. *Plant and Soil* 253, 57-70.

Rhodes D, Nadolska-Orczyk A (2001) *Plant Stress Physiology, Encyclopaedia of Life Sciences*. pp. 1-7. (Nature Publishing Group).

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA (1965) Sap pressure in vascular plants. *Science* 148, 339-346.

Sena Gomes AR, Kozlowski TT (1980) Growth response and adaptation of *Fraxinus pennsylvanica* seedlings to flooding. *Plant Physiology* 66, 267-271.

Shearer BL, Fairman RG (2007) Application of phosphite in a high-volume foliar spray delays and reduces the rate of mortality of four *Banksia* species infected with *Phytophthora cinnamomi*. *Australasian Plant Pathology* 36, 358-368.

Tabachnick BG, Fidell LS (1996) *Using Multivariate Statistics*, 3rd Edn. New York, USA: Harper Collins College Publishers.

Tang ZC, Kozlowski TT (1982) Physiological, morphological and growth responses of *Platanus occidentalis* seedlings to flooding. *Plant and Soil* 6, 243-255.

Tang ZC, Kozlowski TT (1984) Ethylene production and morphological adaptations of woody plants to flooding. *Canadian Journal of Botany* 62, 1659-1664.

Vartapetian BB, Jackson MB (1997) Plant adaptations to anaerobic stress. *Annals of Botany* 79, 3-20.

Veneklass EJ, Poot P (2003) Seasonal patterns in water use and leaf turnover of different plant functional types in a species-rich woodland, south-western Australia. *Plant and Soil* 257, 295-304.

Wilkinson CJ, Holmes JM, Dell B, Tynan KM, McComb JA, Shearer BL, Colquhoun IJ, Hardy GEStJ (2001) Ability of phosphite applied in a glasshouse trial to control *Phytophthora cinnamomi* in five plant species native to Western Australia. *Australasian Plant Pathology* 30, 343-351.

Yanar YP, Lipps E, Deep IW (1997) Effect of soil saturation, duration and water content on root rot of maize caused by *Pythium arrhenomanes. Plant Disease* 81, 475-480.

Zencich SJ, Froend RH, Turner JV, Gailitis V (2002) Influence of groundwater depth on the seasonal sources of water accessed by *Banksia* tree species on a shallow sandy coastal aquifer. *Oecologia* 131, 8-19.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-water deficit

Banksia woodland suffering from drought stress (Photo: G Hardy)

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-water deficit

INTRODUCTION

Water deficit due to prolonged drought, seasonal dry soil, or air saturation deficit is an annual feature of Australian vegetation in Mediterranean-type regions. These conditions normally follow or precede a wet season where soil moisture and temperature are suitable for *P. cinnamomi* to reproduce, infect plants and spread as spores in free water, by soil movement or anthropogenically. Although *Banksia* and *Eucalyptus* have evolved in a dry environment, changes in plant water status strongly influence their growth and physiology (Batchelard 1986, Vesk and Westoby 2003). Some Australian plants avoid drought by having deep penetrating root systems that access water through the unsaturated zone down to the capillary fringe of the saturated zone. Others adapt to drought using physiological traits such as osmotic adjustment, stomatal control and leaf shedding. Stomatal closure not only reduces photosynthesis but is likely to restrict phosphite uptake into the plant. Decreased stomatal conductance in drought stressed plants is associated with hydraulic signals in some plants and with chemical signals in others (Bond and Kavanagh 1999, Comstock 2002, Davies and Zhang 1991, Lovisolo *et al*. 2002).

Groom (2004) showed that, in sandplain woodlands north of Perth, shallow-rooted shrub species are the most drought-tolerant and survive low summer soil water potentials (< -7 MPa) and tissue water deficits by reducing transpirational water loss. Deep-rooted shrubs conserve leaf water loss and incur predawn water potentials between -1 and -4 MPa. Deep-rooted plants appear to decrease their stomatal conductance before the development of severe drought stress (Veneklaas and Poot 2003). Viability of near surface roots in dry soils can be patchily maintained in some species by hydraulic lift (Burgess *et al.* 1998).

Whilst there is no striking difference in disease control between plants sprayed with phosphite in spring or autumn, there is some evidence to suggest that season of application may play a role in the effectiveness of phosphite to control disease caused by *Phytophthora* (Hardy *et al*. 2001). In the southwest of Western Australia, phosphite is usually applied in autumn when most plants are not flowering and when wind conditions are minimal to avoid spray drift (Hardy *et al*. 2001). In one field study using *Xanthorrhoea preissii*, it was found that in an autumn application phosphite was not translocated to the roots, while with the winter spray; concentrations of 10 mg/g dry weight were found in the roots a month after spraying (Pilbeam *et al*. 2000). Water potential levels were within the stress zone that has been shown to restrict the growth of *P. cinnamomi* in *E. marginata* (Tippett *et al.* 1987), and this may have prevented uptake or translocation during the autumn spray.

The interaction between drought and infection by *P. cinnamomi* is complex and may result from the balance of contradictory effects on the plant and on the pathogen (Robin *et al.* 2001). Reduced stomatal conductance and transpiration (Crombie and Tippett 1990) and changes in concentrations of cytokinin and phenolics, which could indirectly influence stomatal closure, have been demonstrated in *P. cinnamomi* induced disease (Cahill *et al.* 1986, Cahill and McComb 1992) indicating that defense mechanisms may be altered during water shortage (Robin *et al*. 2001). During dry periods, *P. cinnamomi* is contained *in planta* and causes less root infections. The low water content in plants decreased the invasion of the plant by the pathogen (Tippett and Hill 1983, Tippett *et al*. 1987, Marcais *et al*. 1993), as restriction of soil water has a direct effect on the pathogen, inhibiting

inoculum build-up (Robin *et al.* 2001). If the plants are infected before they are subjected to drought conditions, then they suffer less from drought stress as they do from the disease-imposed stress. In *P. cinnamomi*-infected *Quercus ilex* subjected to drought the water content in the pots was higher than that of non-inoculated plants, due to reduced water absorption and transpiration by inoculated plants (Robin *et al.* 2001).

With the possibility of increasing restrictions in water availability due to climate change, it is becoming increasingly important to know just how resilient native species are to drought and water deficiency. The latest report on climate change predicts a mean increase in temperature of between 0.7 – 0.9°C in coastal areas and 1 - 1.2°C in inland areas of Western Australia by 2030 (CSIRO 2007). Precipitation levels in southwest Western Australia are predicted to decrease by 5 – 7.5% annually, and by as much as 10% during spring by 2030 (CSIRO 2007). Simulations also predict greater drought severity and frequency in the southwest by 2070 (CSIRO 2007, McDowell *et al.* 2008). This may affect the survival and distribution of plant species in Western Australia. Furthermore, McDowell *et al*. (2008) advises that population demographics such as growth rates and reproduction of biotic mortality agents will be exacerbated by climate change largely as a result of increased temperatures.

The Swan Coastal Plain is a natural topographical unit in southwest Western Australia separated from adjacent regions by its soils, geological history and vegetation. Banksia woodlands, dominated by *Banksia attenuata, B. menziesii* and *B. ilicifolia,* are the most common vegetation type and occur on soils which have an extremely low water holding capacity and are thus highly susceptible to a high degree of water stress (Dodd and Bell 1993). These factors mean that the potential impacts of changes in the climate of the Swan Coastal Plain could be disastrous for this vegetation group.

To investigate the efficacy of phosphite to control *P. cinnamomi* in plants treated pre- and post-drought, it is critical to understand the physiological responses used by the plants to prevent evapotranspiration from exceeding critical rates that could result in xylem water potentials associated with hydraulic and symplastic failure (McDowell *et al*. 2008). In the following experiments we examine the physiological responses of *Banksia* species to drought. To determine how water-stress at the time of phosphite application influences the efficacy of phosphite to control the pathogen, a suitable site for a field experiment was investigated and two glasshouse experiments were undertaken:

Experiment 1 The efficacy of phosphite treatments in water-stressed *Banksia attenuata* in the field. The aim of this study was to determine the effect of water deficit on phosphite redistribution in *Banksia* species following phosphite application, and the subsequent effectiveness of this phosphite in reducing disease caused by *P. cinnamomi*.

Experiment 2 The effect of water deficit on the physiological response of *Banksia attenuata* in the glasshouse. The aim of this experiment was to determine the watering regimes that could be used in Experiment 3.

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-water deficit

Experiment 3 The efficacy of phosphite treatments in water-stressed *Banksia attenuata* and *B. baxteri* in the glasshouse. Under controlled glasshouse conditions this study examined the effect of water deficit:

- before a phosphite treatment on phosphite redistribution in the plant and the ability to reduce disease caused by *P. cinnamomi* in *Banksia* species; and
- after a phosphite treatment on phosphite redistribution in the plant and the ability to reduce disease caused by *P. cinnamomi* in *Banksia* species.

Death of trees and understorey caused by *Phytophthora cinnamomi* (Photos: K Howard)

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-water deficit

Experiment 1 The efficacy of phosphite treatments in water-stressed *Banksia attenuata* in the field

AIMS

The aim of this study was to determine the effect of water deficit prior to phosphite treatment, on phosphite redistribution in the plant and the efficacy of this phosphite treatment in reducing disease caused by *P. cinnamomi* in *Banksia* species in the field. It is important that the project areas had not been sprayed previously with phosphite.

OUTCOMES

By measuring the phosphite redistribution in the leaves, stems and roots 6 months after application, and the progression of *P. cinnamomi* lesions in the stems we examined the impact of water deficit on the effectiveness of phosphite in reducing the severity of disease caused by *P. cinnamomi* in *Banksia* species. The results were later confirmed in the glasshouse experiment (Experiment 3), and determined whether phosphite applied to plants suffering water deficit stress was effective in preventing disease caused by *P. cinnamomi*, or if further treatments were required when plants were fully hydrated.

METHODS

Experimental plan

Approximately 40 saplings of two to three local species were divided into two groups. Twenty plants of each species were irrigated, while no water was applied to the remaining plants. Half of the irrigated and non-watered plants were sprayed with phosphite. Plants were then periodically assessed using a CIRAS-2 and pressure bomb chamber to determine the physiological status of the different plant species. Pressure bomb measurements were taken at predawn and midday. Stems were inoculated with *P. cinnamomi*, and then excised so that all infected tissue was removed from the field site and taken back to the laboratory for measurement of phosphite and lesion development to determine the effectiveness of the phosphite treatment. Either whole plants (if permitted) or a selection of roots, stems and leaves were collected for phosphite analysis during the project.

Control plants were watered (10 L/plant) on a weekly cycle during summer, after an application of a wetting agent (Aqua Wett; David Gray and Co P/L, O'Connor WA) to enhance the water penetration and retention. The drought stressed plants did not receive any water.

Site selection

Three potential sites were examined for their suitability for this experiment: Cape Riche on the south coast of Western Australia, Jandakot Airport close to Murdoch University, and Whiteman Park northeast of Perth. We report on the potential experimental sites that were visited, site characteristics and the preliminary works undertaken.

Cape Riche

The proposed experimental site was situated near Cape Riche on the south coast, east of Albany, Western Australia (118.72ºE, 34.57ºS) (Figure 36). The soil is fine textured, white siliceous sand with a poorly developed A horizon with no impediments to internal drainage. A more detailed site description is provided by Dunstan *et al*. (2008).

Figure 36 The *Banksia baxteri* and associated heath species of the proposed Cape Riche study site. (Photo: D Hüberli)

In 2006, a total of 100 *B. attenuata* and 100 *B. baxteri* trees were tagged and mapped. The heights of each tree were recorded. Plots were to be subdivided for treatments with or without phosphite. However, soil cores were taken and the soil found to be moist and the plants were not photosynthetically challenged nor water stressed as tested over two days in summer 2006 using the pressure bomb and CIRAS-2 machine (data not included).

Unfortunately, during the potential experimental period, the summer rainfall in this area reached approximately half that of winter rainfall. There was no guarantee of being able to drought stress these plants over the summer months. This site was consequently deemed unsuitable for the planned experiments. The rainfall for the period of the project (Figure 37) shows that this was a sound decision.

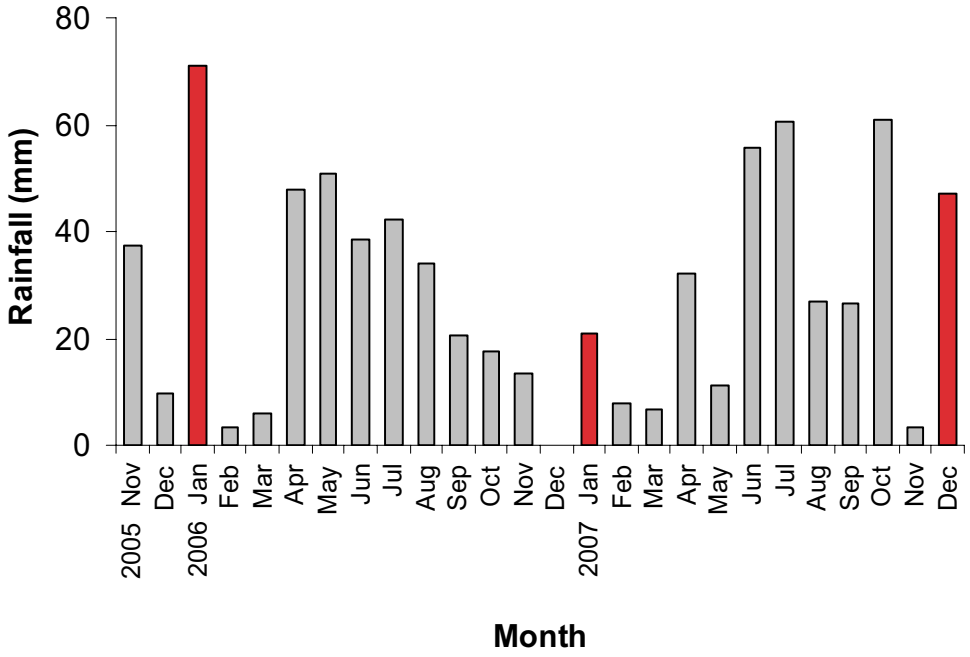


Figure 37

Rainfall at Cape Riche, Western Australia during the period of this project. Note the significant summer rainfall marked in red

Whiteman Park

Whiteman Park is approximately 22 km northeast of Perth, Western Australia. This park covers an area of more than 4200 ha with nearly half this classified as high value conservation bushland or wetland. The Park's conservation areas include typical coastal plain sand dune formations that support marri (*Corymbia calophylla*), jarrah (*Eucalyptus marginata*) and *Banksia* woodlands, extensive heathlands and *Melaleuca* wetlands. *Banksia attenuata* is common in the park (http://www.whitemanpark.com.au/park/get.asp).

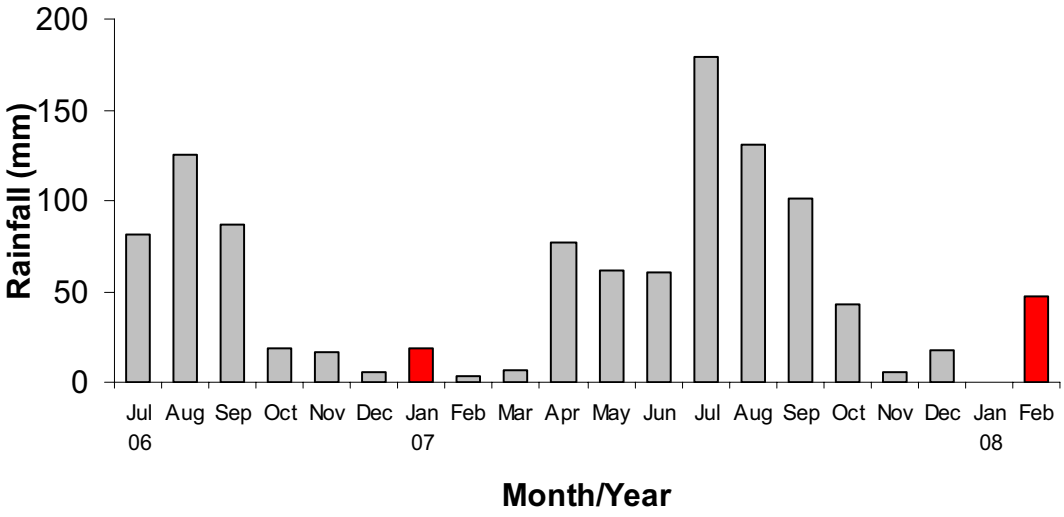
The *Banksia* spp. at Whiteman Park did not show any drought stress in the summer of 2006/2007 summer (Ray Froend, ECU *pers. comm*.). Froend and colleagues found that there were no significant differences in the leaf water potentials of the *B. attenuata* seedlings in the water-redraw sites vs. the non-redraw sites over summer and there was no indication that plants were water deficit stressed at any of the sites (unpublished data). Rainfall for the February 2008 period (Figure 38) indicates why the water stress never reached critical levels during the summer water potential measurements.

Jandakot Airport

Jandakot Airport is situated approximately 12 km south of Perth, Western Australia (32.10°S, 115.88°E). The Banksia woodland at Jandakot Airport is one of the largest and best remaining remnants of this vegetation type on the Swan Coastal Plain. Approximately 400 ha are listed on the Register of the National Estate. Some areas of this remnant of Banksia woodland contain extensive dieback caused by *P. cinnamomi*, but vegetation was shown to be of primarily good to excellent condition in the Jandakot Airport Bushland Condition Mapping of September 2006 (DEWHA 2007).

Figure 38

Rainfall at Whiteman Park, Western Australia. Note the summer rainfall events marked in red



We identified Jandakot Airport as a potential site, as it was close to Murdoch University with easy access, and had small *Banksia attenuata* trees appropriate for a small trial in the field. This site was intended to be cleared in the near future for expansion of the airport.

We required 100 *B. attenuata* saplings no taller than 1.7 m. As half of the saplings would be hand watered they needed to be within an area of no more than 10 - 12 ha. The plants were tagged and mapped. However, it was discovered that many areas were affected by *P. cinnamomi*, making it difficult to select 40 suitable plants.

Unfortunately, approval for site access by Jandakot Airport Holdings did not come through until May 2007, leaving a small window of opportunity to run the experiment. It was estimated that a period of eight to ten weeks of dry weather was required, optimally towards the end of summer. However, due to impending expansion of the airport, the site was not available for the period required to complete this experiment. Again, unseasonal summer rainfall occurred in February of 2008 (Figure 39) which further compromised the site and the experiment.

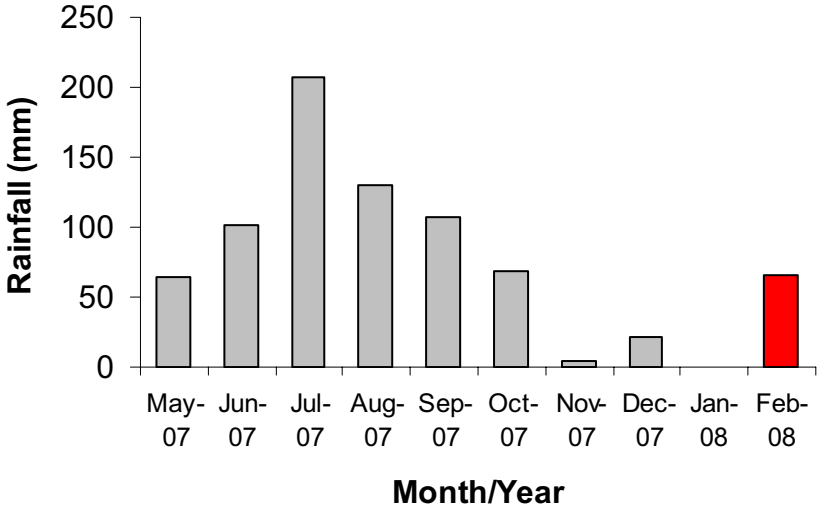


Figure 39

Rainfall at Jandakot Airport, Western Australia during the period site access was granted. Note the significant summer rainfall marked in red

CONCLUSION

Despite spending considerable time and resources to establish a field trial (as described above), we decided not to continue with the field experiments which relied on drought to occur naturally, especially because of the unseasonal rainfall events over the summer period.

It does appear that there has been an increase in summer rainfall in the southwest of WA, and the increased prevalence of extreme rainfall events in Australia generally has serious implications for the proliferation and spread of *Phytophthora* spp. which thrive in warm, moist conditions (McDowell *et al.* 2008). This is particularly pertinent in the southwest of Western Australia where approximately 41% of some 6000 described species are susceptible to *P. cinnamomi* (Shearer *et al*. 2004).

In order to have reliably undertaken the planned trial, a suitable site needed to meet several specific criteria (Table 13).

Table 13 Experimental site selection criteria for investigation of phosphite uptake of water deficit stressed plants under natural field conditions.

Criterion	Potential Field Site		
	Cape Riche	Whiteman Park	Jandakot
Plants present in sufficient numbers to conduct the experiment. Three species native to the region which are sensitive to infection from *P. cinnamomi* and respond to phosphite application. Individuals of species occurring at high densities, of similar physiological age and size, enabling phosphite spray treatment with a fine-misting wand, and leaf structure suitable for CIRAS-2 and pressure potentials measurements.	✓	✗	✗
Inoculation of plants in the experimental area and removal of all infected material from the site requires either the site to be zoned for future clearing or previous infestation of the site with *P. cinnamomi*.	✓	✓	✓
A degree of isolation and/or security to ensure minimisation of potential interference from the general public. Easy off-road access for undertaking predawn pressure potentials and hand watering of plants.	✓	✓	✓
Lengthy periods without rainfall to allow the plants to become sufficiently drought stressed to ensure experiment provides reliable and robust results.	✗	✗	✗

Since none of the three sites met the selection criteria (Table 13), the decision was made to allocate resources and time into glasshouse trials. Glasshouse trials are not subject to the vagaries of weather conditions or constraints from land managers as occur in the field. Glasshouse trials use plants of the same age and condition, and can be monitored and manipulated at will.

Banksia woodland suffering from drought stress (Photo: G Hardy)

Experiment 2 Growth and physiological measurements of *Banksia attenuata* under water deficit stress in the glasshouse

AIMS
The aim of this preliminary study was to determine the amount of water required to keep the plant alive, but at water stress levels where gas exchange parameters were impaired.

OUTCOMES
This preliminary experiment provided information on plant stress during a water deficit event prior to the main experiment being established (Experiment 3).

METHODS
Experimental design
After a water deficit event, *Banksia attenuata* seedlings were subjected to 80, 60, 40, 20 or 0% water deficit. Once wilting point was reached, the rate of photosynthesis and transpiration, and stomatal conductance was measured weekly, for 21 days at each deficit level. Growth was demonstrated by measuring plant heights over the trial. There were ten replicate plants per treatment.

Plant material and growth conditions
Eight-month old *B. attenuata* seedlings were obtained from the Australian Native Nursery (Oakford, Western Australia). The seedlings were then potted into sterilised 120 mm free-draining pots containing sterilised grey sand from the *Banksia* woodland at Murdoch University, Perth. The seedlings were staked and 15 g of Native Osmocote fertilizer (Scotts Australia P/L, NSW, Australia) was added to the soil.

The seedlings were kept in a heated glasshouse throughout the trial which was conducted during September and October 2007 (spring). The temperature during which physiological measurements were taken ranged between 27 and 32.4°C.

Water deficit treatments
Seedlings were watered until the soil was at container capacity. Simulated drought conditions were imposed on the seedlings by withholding water and allowing the substrate to dry until wilting point was reached (Figure 40). Once wilting point was reached by more than 20 seedlings, the plants were subjected to different levels of water deficit by replenishing between 20 and 80% of the water loss from container capacity (100%). The drainage holes in the base of the containers of these plants were sealed to retain the added water. The seedlings were weighed every two days and these water levels maintained for 21 days, by applying the appropriate amount of water to maintain the desired water deficit conditions. Control seedlings were maintained at container capacity, by watering for five minutes twice daily.

To determine the water needed to attain individual water deficits, each plant was weighed at container capacity and at wilting point. Then, the level of water at which each plant was maintained was calculated by: (the weight at container capacity – weight at wilting point) × water level.

Figure 40

A *Banksia attenuata* seedling at wilting point. Note the shoot apical leaves are beginning to lose turgor (arrow)

(Photo: B Palmer)

Measurements

At wilting point, the rate of photosynthesis and transpiration, and stomatal conductance of five plants from each treatment including the control plants (25 plants in total) were measured using the CIRAS-2. For this trial, the references were set: CO_2 level at 380 ppm, the chamber airflow rate at 200 mL min^{-1}, the reference humidity at 100%, the light setting was 1000 µmol $m^{-2}s^{-1}$ using a LED light, while the area of the window was 2.5 cm. Measurements were taken in exactly the same place on the third fully expanded leaf of the same plant for each of the four measurement times; at wilting point and one, two and three weeks after wilting point. The leaves did not completely fill the curvette window so the gas exchange measurements were adjusted proportionally. The height of all the plants was measured at the start and the end of the experiment.

Statistical analyses

Data were analysed using Excel for Windows (Microsoft Corporation, USA). To determine whether differences between means were significant, ANOVA tests and two sample t-tests (assuming unequal variances) were performed. Two-tailed p-values were performed and were considered as significant if they were less than 0.05.

RESULTS

Control plants at 100% container capacity never wilted and always had high gas exchange levels throughout the experiment (Figure 41) Plants subjected to water deficit conditions had very low photosynthetic and transpiration rates, and stomatal conductance at wilting point (day 0) compared to the controls (Figure 41). There was no difference in photosynthesis, transpiration or stomatal conductance between water deficit groups (20, 40, 60 and 80%) at wilting point, but by seven days post-wilting there was a significant ($p < 0.001$) difference between the different water deficit treatments. The physiological measurements of the plants watered to 60 and 80% of container capacity recovered to the rate of the control plants at seven days, but then decreased as water deficit conditions continued. At 20 and 40% of container capacity, the photosynthetic and transpiration rate, and stomatal conductance did not recover and were still falling by week three (Figure 41).

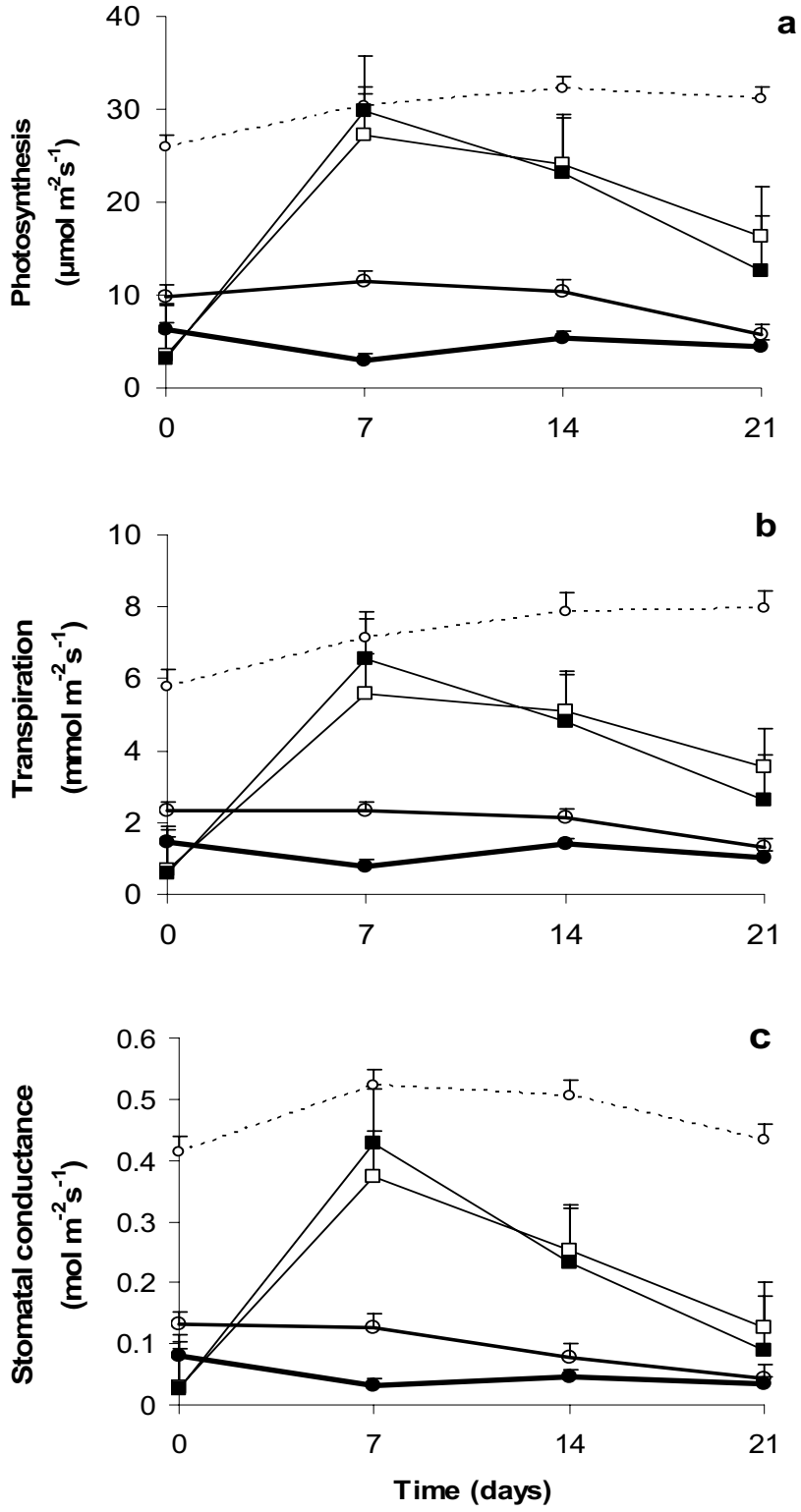


Figure 41 The mean physiological measurements; **a**) photosynthetic rate, **b**) transpiration rate, and **c**) stomatal conductance for *Banksia attenuata* seedlings subjected to water deficit levels of; 100 (control --○--), 80 (—□—), 60 (—■—), 40 (—○—), and 20% container capacity (—●—). Day 0 corresponds with wilting point. *n* = 5. Vertical bars represent one standard error of the mean.

Plant growth

There was no significant ($p = 0.82$) difference between the mean heights of each group at the commencement of the trial. However, during the 21 days of the trial there was a significant ($p < 0.001$) effect of water deficit on plant growth with a trend of decreasing plant heights with increasing water deficits (Figures 42 and 43). Plants watered to 20 and 60% container capacity had significantly ($p < 0.006$) lower growth rates compared to control plants.

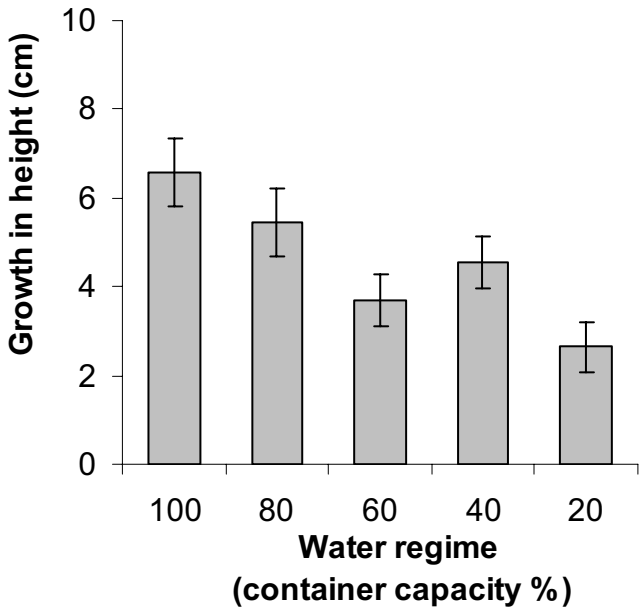


Figure 42

The mean growth in height of *Banksia attenuata* subjected to different water deficit treatments for 21 days. *n* = 10. Vertical bars represent two standard errors of the mean.

Figure 43

Banksia attenuata subjected to water deficit. From L to R: watered at 100, 80, 60, 40 and 20% container capacity

(Photo: B Palmer)

Rewatering after water deficit treatments
This experiment did not measure the consequences of rewatering the plants after the water deficit treatments. However, a large proportion of the plants (> 50%) that had been maintained at the 20 and 40% water container capacity died within one to two weeks after daily watering to container capacity had resumed.

DISCUSSION

B. attenuata was able to survive at all water deficit treatments over the three-week period. This was not surprising as *B. attenuata* occurs in areas which are subject to seasonal drying of the soil and drought (Veneklaas and Poot 2003). There was, however, considerable evidence of stress in all the plants subjected to these simulated water deficit conditions.

The photosynthesis rates, transpiration rates and stomatal conductance showed very similar response curves. Photosynthesis and transpiration rates and stomatal conductance are closely connected by a series of complex feedback loops. The exact mechanisms of these feedback loops are still largely unknown; however, it is certain that if stomatal conductance decreases so will photosynthesis and transpiration (Jones 1998).

Dodd and Bell (1993) studied the water relations of *B. attenuata* in the field. Their results for transpiration and stomatal conductance in early summer are similar to those obtained for the controls in this study. This indicates that in the field *B. attenuata* were not experiencing water stress even though water levels in the soil were well below container capacity. The reason for this is that *B. attenuata* have deep taproots which they use to access groundwater at depths of up to 8 - 9 m (Dodd and Bell 1993, Groom 2004). During the wet winter months *B. attenuata* is able to utilise the relatively high levels of water in soil. However, during the summer drought months, when soil water becomes scarce, *B. attenuata* becomes increasingly reliant on water accessed from the watertable (Groom 2004). Extended drought periods across several years can result in the lowering of water tables and drought death has been observed in the field.

In this experiment we have established:

- The growth of the *B. attenuata* plants decreased with increasing water deficits;
- At water levels below 40% of container capacity the plants were unable to carry out normal photosynthetic activity, even for short periods of time, and this negatively impacted on the plant's growth. The photosynthetic activity did not change from wilting point and some of these plants remained wilted; and
- After being at wilting point, plants watered to 60 and 80% of water container capacity were able to rapidly restore photosynthesis, transpiration and stomatal conductance. However, this rise was brief and these plants decreased their photosynthesis, transpiration and stomatal conductance to rates approximately half or less than the rates of the control plants. Perhaps when soil water content did not continue to increase beyond 60 or 80% the plants reverted back to 'survival mode', in order to decrease their water use to more sustainable levels.

While the results of this study are of limited relevance to adult plants in the field, they highlight the importance of juvenile plants developing taproots in the field before the summer drought periods begin (Groom 2004). Without access to groundwater supplies, *B. attenuata* may be exposed to severe restrictions in water availability during the summer months, and might therefore, experience a high degree of water stress. Hydraulic failure occurs in small plants, as a result of excessive cavitation and drying experiments with potted plants often result in rapid mortality. The limited rooting volume explored by plants exposes them to more negative soil water potentials than plants with larger root systems (McDowell *et al*. 2008).

This work has allowed us to understand some of the physiological responses to drought stress in a controlled situation. In a recent review on the mechanisms of plant survival and mortality during drought, McDowell *et al.* (2008) points out a paucity of knowledge on the physiological mechanisms underlying drought survival and mortality.

For Experiment 3, careful plant size selection will be critical to ensure all plants are exposed to similar water deficit impact. In the current experiment, we found wilting occurred within two to three days when watering was stopped, but was mainly influenced by plant size. Due to high mortality occurring after the resumption of watering in plants maintained for three weeks at 20 to 40% container capacity, a change to the watering regime was necessary for Experiment 3. Additionally, plants that were maintained with a continued water deficit after wilting point did not return to normal photosynthetic activity which would impact on phosphite uptake. Maintaining plants for each watering regime is labour intensive if they have to be individually watered to weight on a daily basis. Hence, in Experiment 3 it is proposed that plants will be brought to wilting point, and maintained at just above wilting point until after the phosphite application, at which point full watering to container capacity will be resumed, thus mimicking a short period of drought.

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-water deficit

Experiment 3 The efficacy of phosphite treatments in water-stressed *Banksia attenuata* and *B. baxteri* in the glasshouse

AIMS

In this study, under glasshouse conditions we examined the effect of water deficit in *Banksia* species:

- on leaf gas exchange and leaf water potentials during six days of withholding watering, and the recovery of plants after this treatment (preliminary study);
- on leaf gas exchange and leaf water potentials during and into recovery from the water stress;
- before a phosphite treatment on the redistribution of phosphite in the plant and its ability to reduce disease caused by *P. cinnamomi*;
- during a phosphite treatment on the redistribution of phosphite in the plant and its ability to reduce disease caused by *P. cinnamomi*; and
- after a phosphite treatment on the redistribution of phosphite in the plant and its ability to reduce disease caused by *P. cinnamomi*.

OUTCOMES

To provide information on the effectiveness of phosphite treatments before/during and after a water deficit stress event in preventing disease caused by *P. cinnamomi*, or if further phosphite treatments are required.

METHODS

Plants

Banksia attenuata *Banksia baxteri*

Six month-old *Banksia attenuata* and *B. baxteri* were grown from seed (Nindethana Seed, Albany) and potted into 150 mm free-draining plastic pots containing coarse river sand. Each pot was top-dressed with 15 g of a slow-release low phosphate fertiliser on 31 January 2008. On two separate occasions (December 2007 and January 2008), the lowest recommended (30 mL/10 L water) of Kelpak (Agrichem, Loganholme, Qld) was applied. Plants were watered by trickle irrigation for 30 min twice daily.

Preliminary study

Ten seedlings of *Banksia attenuata* and *B. baxteri* were subjected to six days of increasing water deficit (no watering), while watering of the four control plants was continued at container capacity. All water deficit treatment plants were weighed daily. The plant's leaf gas exchange was assessed daily using an LCpro+ portable system. Leaf water potentials were measured at predawn on day 4 of the water deficit and 15 days after the water deficit treatment using a pressure bomb chamber. The midday leaf water potential was measured 6 days after the water deficit treatment.

Photosynthetic rates declined rapidly for both species during the water deficit treatment (Figure 44). After watering resumed at day 7, *B. baxteri* began to recover and within a day was photosynthesising at almost half of the rate prior to the water deficit treatment. *B. attenuata* had a much slower recovery rate and achieved only 10% of the normal photosynthetic rate after watering had resumed.

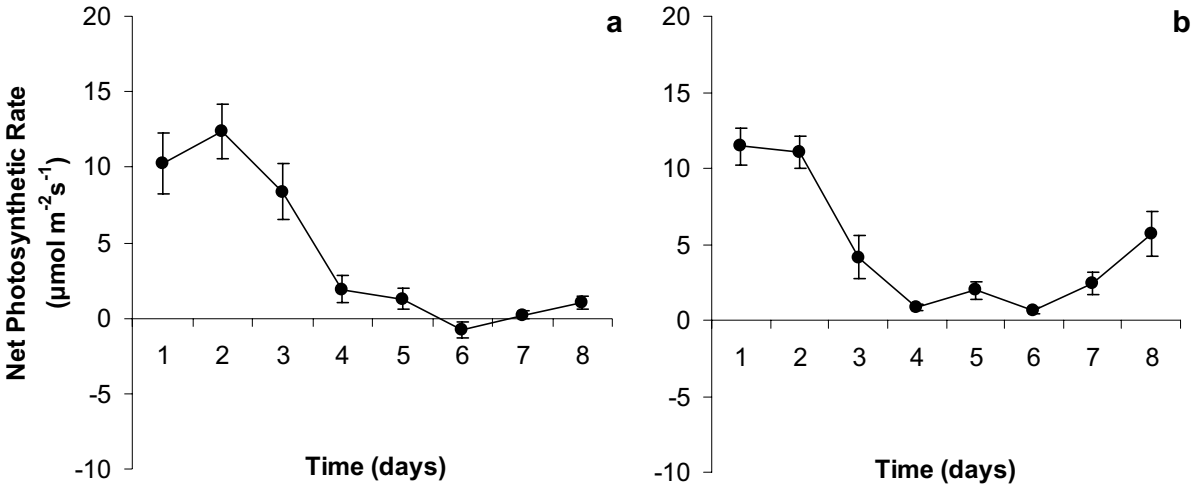


Figure 44 Mean photosynthetic rate of **a**) *Banksia attenuata* and **b**) *B. baxteri* seedlings subjected to 6 days of water deficit (days 1 - 6). On day 7 watering to container capacity was returned to twice daily. *n* = 10. Vertical bars represent two standard errors of the mean.

Both *Banksia* species had significantly ($p < 0.001$) lower leaf water potentials at predawn after 4 days water deficit stress compared to the controls (Figure 45). Water deficit stressed plants had not fully recovered after 6 days of watering to container capacity compared to controls. Control plants' water potentials at midday were significantly lower ($p < 0.001$) than water deficit stressed plants because they were photosynthetically active, while water deficit stressed plants were not functioning photosynthetically at full capacity and thus were retaining their water. After 12 days of recovery from water deficit stress, no differences in predawn leaf water potentials were observed in the controls and water deficit treated plants.

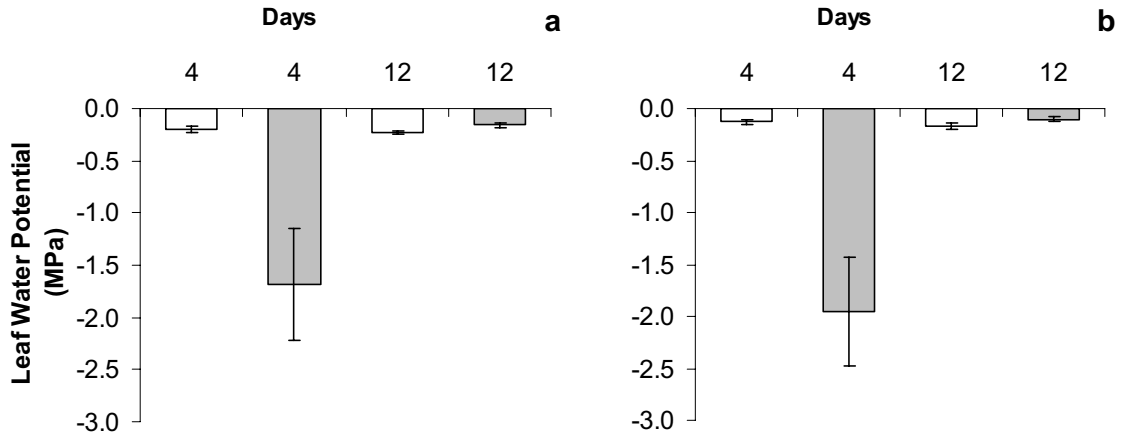


Figure 45 Mean predawn leaf water potential after 4 days of water deficit and 12 days after recovery from the water deficit treatment of **a**) *Banksia attenuata* and **b**) *B. baxteri* seedlings subjected to no water stress (□) or water deficit stress for 6 days (■). *n* = 10. Vertical bars represent two standard errors of the mean.

Three and four plants of *B. attenuata* and *B. baxteri*, respectively, died at the conclusion of the preliminary experiment. The water deficit period of 6 days was therefore deemed to be too severe. Also, *B. attenuata* (Figure 44) was not observed to recover. Thus, a shorter water deficit period with some minimal watering is required to maintain the plants at just above wilting point.

Main study

Experimental plan

One-hundred and twenty seedlings of *B. attenuata* and *B. baxteri* were divided into three groups (phosphite treatments before (48 plants), during (24 plants) and after water stress (48 plants)). Plants were subjected to one single water stress event with three different phosphite application times to ensure that water deficit stress was consistent across all plants (Figure 45). Half of the plants in each group were not sprayed with phosphite. The control plants were irrigated, while no water was applied to the remaining 72 plants. The experiment was an unbalanced design as only plants of very similar size were selected to reduce any experimental error. Half of the irrigated and non-watered plants were sprayed with phosphite. Plants were assessed using an LCpor+ portable system and pressure bomb chamber to determine the plant's physiological status. Pressure bomb measurements were taken at predawn and midday. Stems were inoculated with *P. cinnamomi* to determine the effectiveness of the phosphite treatment. A selection of leaves, stems and roots were collected for phosphite analysis at the conclusion of the experiment.

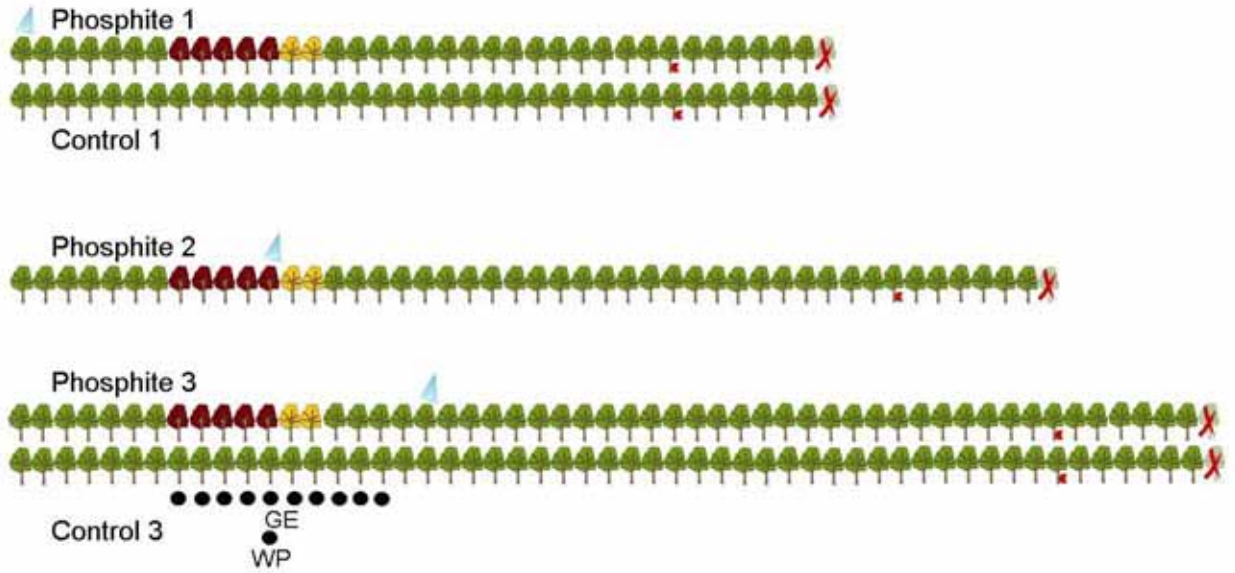


Figure 45 Daily time line showing the three phosphite treatments ( ) in relation to water deficit stress ( ) and plant recovery ( ). Plants were inoculated ( ) 4 weeks after the phosphite treatment and then harvested ( ) 1 week later. Periods of leaf gas exchange (GE) and water potential (WP) measurements are also shown.

Water deficit treatment

During the water deficit treatment the irrigation was turned off for 5 days for the water deficit treated plants, while watering was uninterrupted for the control plants. To ease plants into the water deficit treatment, 30 mL of water was added to each of the water deficit treated plants on the first afternoon the irrigation was switched off. At wilting point, another 15 mL of water was applied. After the phosphite application, water deficit treated plants were returned to trickle irrigation watering to container capacity. The daily average, maximum and minimum temperatures during the water deficit treatment were 31.0, 45.4 and 21.0°C, respectively.

Application and analysis of phosphite

Phosphite was applied on three separate occasions; 1 week prior to water deficit stress, during water deficit stress (once all plants had reached wilting point), or 1 week after water deficit stress (Figure 45). A 40% solution of phosphite containing 0.2% (v/v) of the wetting agent BS1000® was applied as a spray with a Microfit low-volume fine mist applicator at 24 kg/ha (see page 4).

All leaves, stems and roots were collected from three replicate plants eight days after each of the three inoculations. Samples were then prepared for phosphite analysis and sent to the WA Chemistry Centre (Perth) for phosphite analysis (see page 4).

Inoculation
Prior to the inoculations, the *P. cinnamomi* isolate was passaged through a *B. baxteri* seedling and then reisolated onto NARPH, to maintain its pathogenicity. Plants were underbark inoculated with *P. cinnamomi* (isolate SR2) four weeks after each of the phosphite treatments using the method as described in Hüberli *et al*. (2001). There were nine replicate inoculated plants and three control plants (inoculated with sterile Miracloth) per treatment. There were three separate inoculation events during which the daily temperatures were recorded (Table 14).

Table 14 Mean daily average, maximum and minimum temperatures in the glasshouse recorded for 7 days following each inoculation.

	Mean daily temperature (°C)		
Inoculation	**Average**	**Minimum**	**Maximum**
prior to water deficit stress	27.2	18.5	39.7
during water deficit stress	23.2	15.0	34.1
after water deficit stress	23.5	16.1	35.0

Physiological measurements
Leaf gas exchange measurements were taken daily on three replicate plants per treatment during the water deficit treatment and up to 1 week after the water deficit treatment (Figure 45). The temperature of the leaf chamber was set at 29°C and ranged from 32 to 34°C during measurement days. A predawn and midday leaf water potential measurement was made immediately following the wilting (4 days of water deficit stress).

Measurement of infection
At harvest, 1 week after inoculations, the inoculated main stem was removed at the soil level. The outer bark was carefully scraped back to uncover the lesion which was measured. Disease by *P. cinnamomi* may include an extension ahead of the lesion that is macroscopically asymptomatic, and in some cases may be up to 6 cm (Hüberli *et al*. 2002). To determine the extension beyond the lesion of *P. cinnamomi* in the stem, a 1 cm section of the stem from the lesion front and subsequent 1 cm section up to 5 cm beyond the lesion front were plated onto NARPH. Colonisation incorporates the sum of the lengths of the lesion and extension beyond the lesion.

Statistical analyses
All statistical analyses were done using the Statistica software V6.1 (Statsoft, Inc., Tulsa, OK, US). Following Tabachnick and Fidell (1996), data for parametric tests were screened for assumptions of homoscedasticity, presence of outliers, normality and non-correlations of means and variances. Physiological leaf measurements including photosynthetic rate, stomatal conductance, transpiration rate and predawn water potential were treated as dependent variables in separate repeated measures analysis of variance (ANOVA), with independent variables of water deficit treatment (+/-), phosphite treatment (+/-) and species (*B. attenuata* and *B. baxteri*).

Total lesion and colonisation lengths were correlated significantly for *B. attenuata* (r_{90} = 0.91) and *B. baxteri* (r_{90} = 0.74). Therefore, only colonisation was used as the dependent variable (after $\log_{10}$ transformation to correct for correlation between means and variances across the cells of the design) in analyses of variance (ANOVAs). Data for pre- and post-water deficit phosphite applications and data for water deficit affected plants across pre-, during- and post- water deficit phosphite applications were tested separately because the phosphite treatment during-water deficit did not include non-water stress treatment. The independent variables in the first analysis were phosphite application time (pre- and post-water deficit), phosphite treatment (+/-), water deficit treatment (+/-) and species (*B. attenuata* and *B. baxteri*), while in the second analysis, they included all variables as well as the during-water deficit phosphite application time, but did not include the water deficit treatment variable. Phosphite data were analysed in the same manner as colonisation data. All significant main effects and interactions were compared using Tukey's HSD test.

RESULTS

All water deficit treated plants wilted within 6 h of each other after four days of withholding water. Controls remained fully turgid throughout the experiment. After the wilting point was reached and phosphite was applied to some of the plants, watering was resumed for all plants. No plants died throughout the entire experiment.

Leaf gas exchange and water potentials

The repeated measures ANOVA for leaf water potentials found that for both predawn and midday potentials only the plants that were subjected to four days of water stress had a significant ($p < 0.003$) main effect of water deficit stress only. For predawn water potentials, control plants were higher ($p < 0.001$) than the water stressed plants (Figure 46). The reverse was true for midday water potentials with control plants having lower water potentials ($p = 0.003$) than water deficit stressed plants.

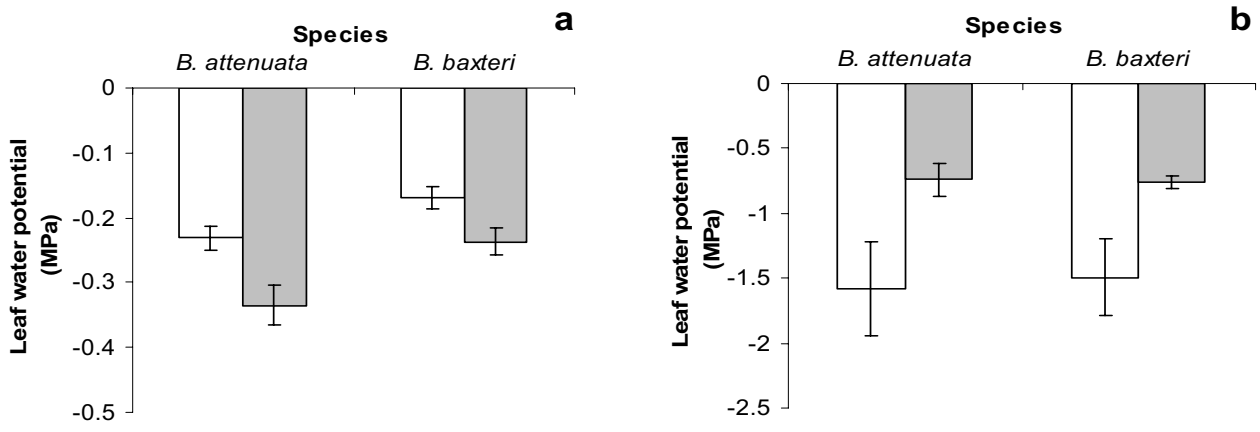


Figure 46 Mean predawn **a**) and midday **b**) water potential of *Banksia attenuata* and *B. baxteri* seedlings of control watered plants (□) or water deficit stressed plants after 4 days (■). *n* = 12. Vertical bars represent two standard errors of the mean.

The ANOVA test found significant ($p < 0.02$ in all cases) main effects of Water deficit, Species, and Time and interaction effects of Water deficit x Time and Species x Time for photosynthetic rates (except species), stomatal conductance and transpiration rates (Table 15). Water deficit stress resulted in a decline in each of the leaf gas exchange measurements during the water deficit stress and recovery once watering was resumed (Figure 47). *B. baxteri* always had the highest stomatal conductance and transpiration rates compared to *B. attenuata*, but the reverse was true for photosynthetic rates. Whilst there is an indication of a delayed response to the water deficit by *B. baxteri* compared to *B. attenuata*, which responded immediately after watering stopped, only for stomatal conductance was this three-way interaction of Water deficit x Species x Time found to be significant (Table 15).

Table 15 Results of repeated measures ANOVA test for photosynthetic and transpiration rates, and stomatal conductance of *Banksia attenuata* and *B. baxteri* over 10 days. Plants were either watered to container capacity (controls) or subjected to 4 days of water deficit. Half the plants within each treatment were sprayed with 24 kg/ha phosphite. Significant effects and interactions at $p < 0.05$ are in bold.

		Photosynthetic rate		Stomatal conductance		Transpiration rates	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Phosphite (+/-)	1,24	1.707	0.204	0.208	0.653	0.160	0.692
Water deficit (+/-)	1,24	**5.761**	**0.024**	**13.642**	**0.001**	**9.994**	**0.004**
Species	1,24	1.568	0.222	**84.370**	**<0.001**	**8.129**	**0.009**
Time (days 1 - 10)	9,216	**14.523**	**<0.001**	**12.075**	**<0.001**	**23.240**	**<0.001**
Phosphite, Water deficit	1,24	0.140	0.711	1.346	0.259	0.594	0.448
Phosphite, Species	1,24	0.016	0.900	0.407	0.530	<0.001	0.988
Water deficit, Species	1,24	1.753	0.198	3.277	0.085	0.373	0.547
Phosphite, Time	9,216	0.855	0.567	0.551	0.836	0.981	0.456
Water deficit, Time	9,216	**6.177**	**<0.001**	**2.342**	**0.016**	**4.161**	**<0.001**
Species, Time	9,216	**2.535**	**0.009**	**2.245**	**0.021**	**3.083**	**0.002**
Phosphite, Water deficit, Species	1,24	0.038	0.846	0.050	0.824	0.324	0.574
Phosphite, Water deficit, Time	9,216	0.303	0.973	0.303	0.973	0.790	0.626
Phosphite, Species, Time	9,216	1.292	0.243	1.066	0.390	1.464	0.163
Water deficit, Species, Time	9,216	1.599	0.117	**2.017**	**0.039**	1.080	0.378
Phosphite, Water deficit, Species, Time	9,216	0.667	0.738	0.571	0.820	0.972	0.464

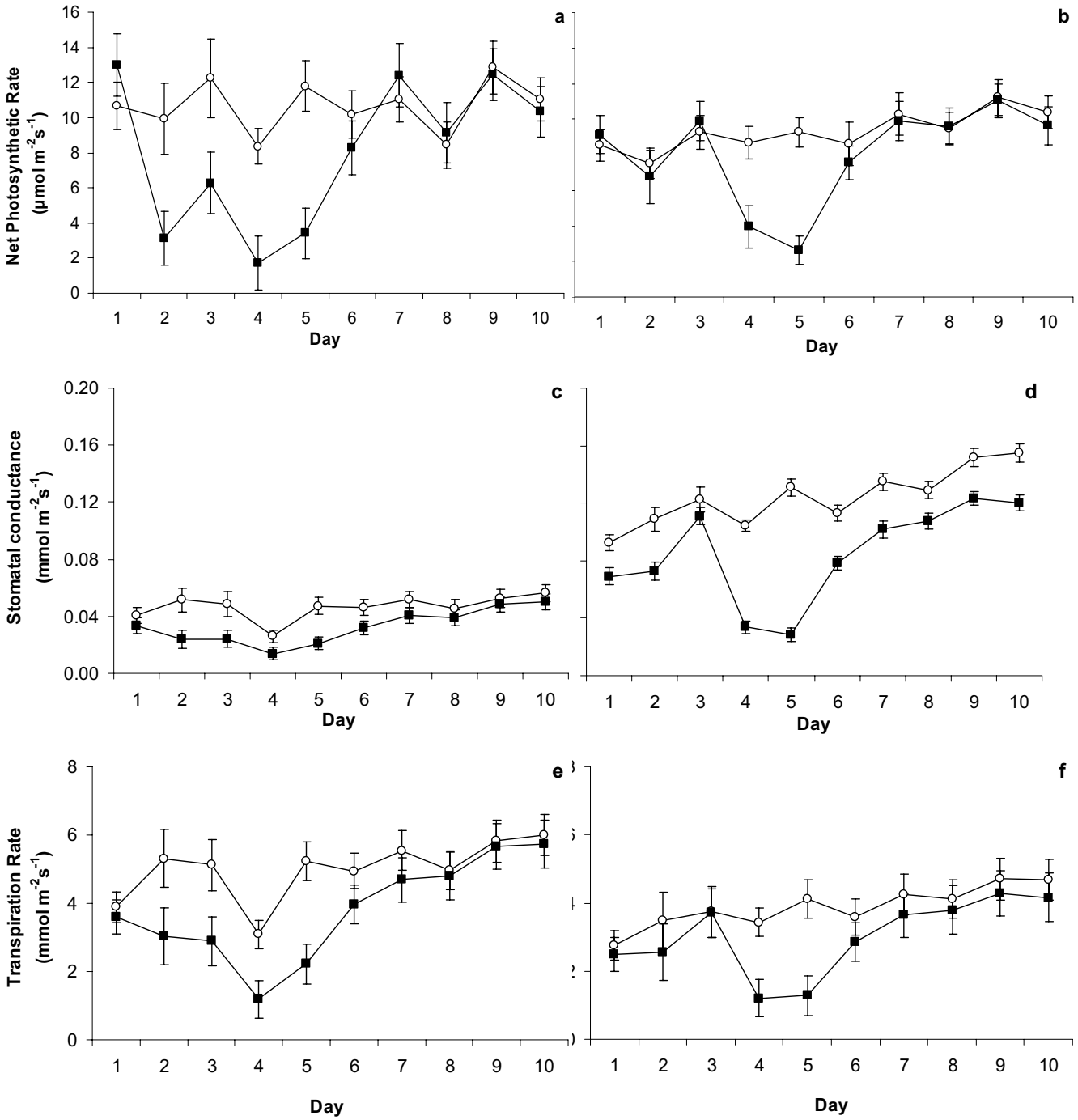


Figure 47 Mean photosynthetic rate, transpiration rate, and stomatal conductance for seedlings of *Banksia attenuata* (**a, c** and **e**) and *B. baxteri* (**b, d** and **f**) subjected to no water deficit (control; —○—) or water deficit (—■—) for days 1 to 5. Phosphite treatments had no significant ($p > 0.20$ in all cases) effect on leaf gas exchange measurements, so the data were combined for water deficit treatments. $n = 6$. Vertical bars represent two standard errors of the mean.

Colonisation

The ANOVA comparing the pre- and post- phosphite applications times showed that water deficit and its interaction with other factors was never significant ($p > 0.10$ in all cases) (Table 16). Main effects of Phosphite application time, Phosphite treatment and Species, and the interaction of Phosphite treatment x Species were significant ($p < 0.04$ in all cases). With regard to phosphite application time, colonisation was always longer in all plants (0 and 24 kg/ha phosphite treated) sprayed after the water deficit stress than those sprayed before the water deficit stress (Figure 48). Phosphite treatment, irrespective of water stress, always reduced colonisation significantly. Overall, *B. baxteri* had longer colonisation lengths than *B. attenuata* and this was significant ($p < 0.001$) for phosphite treated plants only. Phosphite treatment of *B. attenuata* reduced colonisation more than treatment of *B. baxteri* (Figure 48).

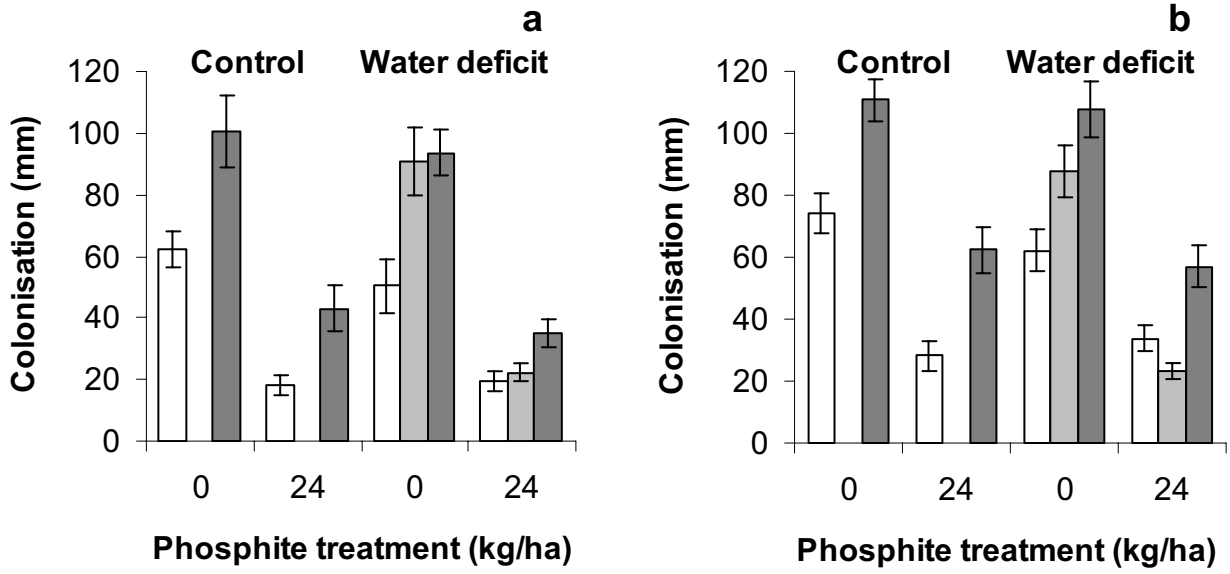


Figure 48 Mean colonisation (mm) of stems (including lesions and extension beyond the lesion) underbark inoculated with *Phytophthora cinnamomi* in **a)** *Banksia attenuata* and **b)** *B. baxteri* plants under well watered (control) conditions or subjected to water deficit stress for 5 days prior to the inoculations. Phosphite was applied 1 week before water deficit (□), when plants reached wilting point (▨), or 1 week after watering was returned (■), and plants in each of these three categories were inoculated 4 weeks later. There were no control plants for phosphite applied at wilting point. *n* = 9. Vertical bars represent two standard errors of the mean.

Table 16 Results of univariate ANOVA test of colonisation length of *Banksia attenuata* and *B. baxteri* after underbark inoculation of the stem with *Phytophthora cinnamomi* 4 weeks after plants were sprayed with 24 kg/ha phosphite before or after water deficit stress. Significant effects and interactions at $p < 0.05$ are in bold.

Effect	Df	F	p
Application time (before or after water deficit stress)	1, 128	**75.93**	**<0.001**
Phosphite (+/-)	1, 128	**152.73**	**<0.001**
Water deficit (+/-)	1, 128	0.81	0.37
Species	1, 128	**22.22**	**<0.001**
Application time, Phosphite	1, 128	1.38	0.24
Application time, Water deficit	1, 128	0.01	0.91
Phosphite, Water deficit	1, 128	1.54	0.22
Application Time, Species	1, 128	0.27	0.61
Phosphite, Species	1, 128	**4.32**	**0.04**
Water deficit, Species	1, 128	0.34	0.56
Application time, Phosphite, Water deficit	1, 128	2.82	0.10
Application time, Phosphite, Species	1, 128	0.06	0.80
Application time, Water deficit, Species	1, 128	0.27	0.60
Phosphite, Water deficit, Species	1, 128	0.01	0.94
Application time, Phosphite, Water deficit, Species	1, 128	0.00	0.97

The ANOVA test comparing only water deficit treated plants across all three phosphite application times found a significant ($p < 0.04$ in all cases) difference for the main effects of Phosphite application time, Phosphite treatment and Species, and the interaction of Phosphite treatment x Species (Table 17). Colonisation was significantly ($p < 0.001$) more extensive in plants treated (0 and 24 kg/ha phosphite) after the water stress than at the other two application times (Figure 48). Phosphite reduced colonisation in treated plants and colonisation was more extensive in *B. baxteri* than *B. attenuata*. Colonisation in non-treated plants was significantly ($p < 0.006$) reduced in the pre-water deficit application time than at the other two application times. Pre- and during-water deficit applications of phosphite were more effective ($p < 0.001$) in reducing colonisation compared to the post-water deficit phosphite application. Overall, phosphite treatments, regardless of when the application occurred with respect to the water deficit treatment, were always effective in reducing colonisation significantly ($p < 0.001$).

Table 17 Results of univariate ANOVA test of colonisation length of *Banksia attenuata* and *B. baxteri* stems after underbark inoculation of the stem with *Phytophthora cinnamomi* 4 weeks after plants were sprayed with 24 kg/ha phosphite before, during or after water deficit stress. Analysis includes only water stressed plants as there were no non-stressed controls for the phosphite application during water deficit stress. Significant effects and interactions at $p < 0.05$ are in bold.

Effect	Df	F	p
Application time (before, during or after water deficit stress)	2, 96	**20.126**	**<0.001**
Phosphite (+/-)	1, 96	**148.633**	**<0.001**
Species	1, 96	**10.575**	**0.002**
Application time, Phosphite	2, 96	**5.418**	**0.006**
Application time, Species	2, 96	2.398	0.10
Phosphite, Species	1, 96	2.036	0.16
Application time, Phosphite, Species	2, 96	0.261	0.77

Phosphite concentrations in plant tissue

Phosphite concentrations in leaf, stem and root samples of *B. attenuata* and *B. baxteri* at 5 weeks after spraying with phosphite 1 week before water deficit, at wilting point, or 1 weeks after watering was returned, are presented in Figure 49. Since there were no non-stressed controls for the application at wilting point, two separate MANOVA tests were conducted; one comparing phosphite applications before and after water deficit stress, and a second, comparing all the three phosphite application times across water deficit stressed plants only.

The first initial MANOVA found significant ($p < 0.001$ in all cases) main effects of Application time and Phosphite and an interaction between Application time x Phosphite. Water deficit stress was not a significant main effect and nor its interactions with the other independent variables ($p > 0.08$ in all cases). The univariate ANOVA showed that the significant main effects and the interactions were significant for phosphite concentrations in leaf, root and stem samples (Table 18). Phosphite concentrations in all three tissue were higher ($p = 0.001$) in plants sprayed with phosphite before the water deficit stress than those sprayed after the deficit stress (Figure 49). Only for leaves were phosphite concentrations in plants sprayed after water deficit significantly ($p = 0.001$) higher than in the non-sprayed plants, while all sprayed tissue were higher ($p < 0.001$) than controls in the spray treatment applied before water deficit was imposed.

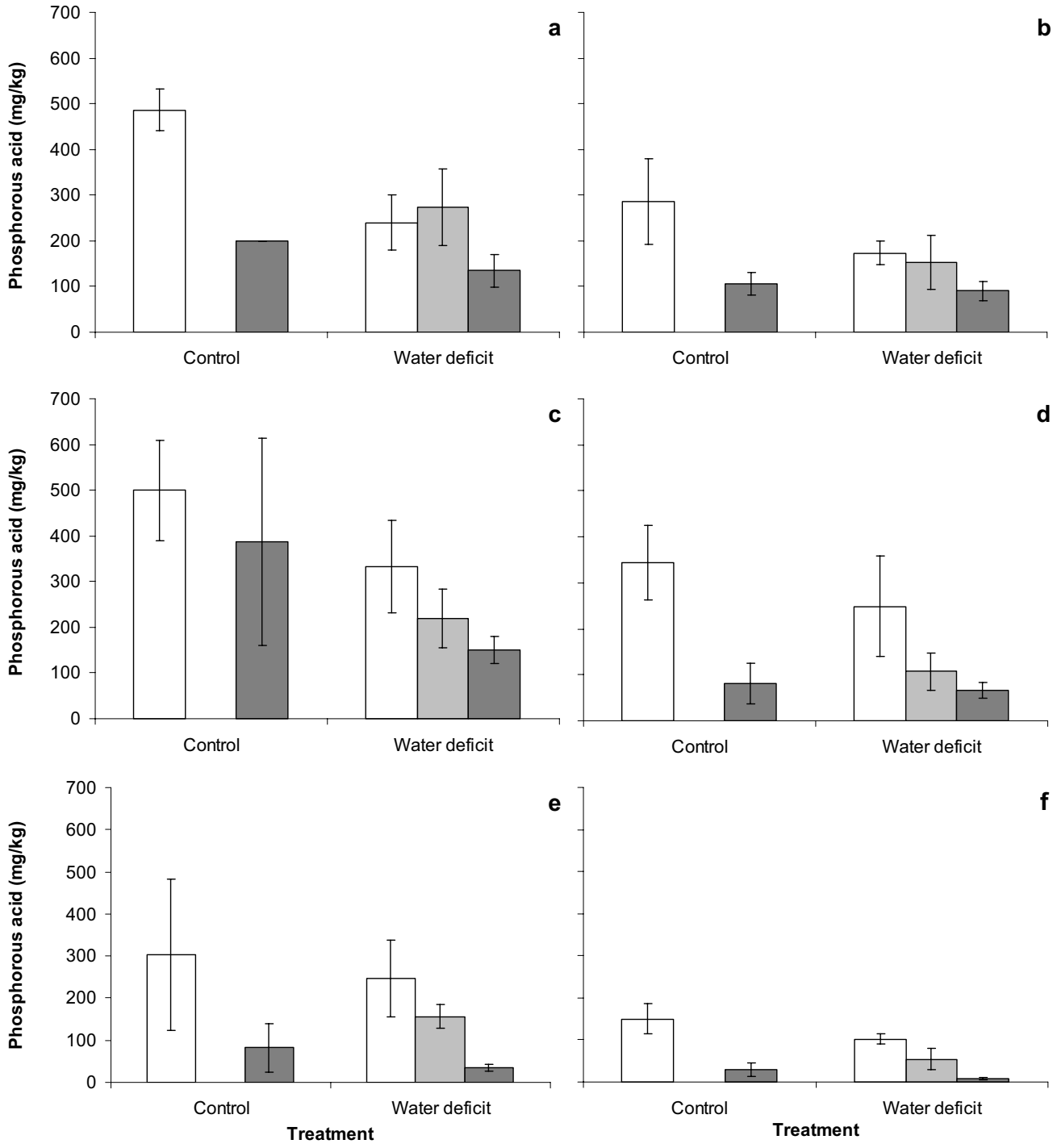


Figure 49 Mean phosphite concentration (phosphorous acid mg/kg) of **a** and **b)** leaf, **c** and **d)** stem and **e** and **f)** root samples of *Banksia attenuata* (**a, c, e**) and *B. baxteri* (**b, d, f)** plants under well watered (control) conditions or subjected to water deficit stress for 5 days prior to harvests. Plants were sprayed with 24 kg/ha phosphite 1 week before water deficit (□), when plants reached wilting point (▣), or 1 week after watering was returned (■), and plants in each of these three categories were harvested 5 weeks later. There were no control plants for phosphite applied at wilting point. *n* = 3. Vertical bars represent two standard errors of the mean.

Table 18 Results of univariate ANOVA tests (following significant initial MANOVA) of phosphite concentration in leaf, stem and root samples of *Banksia attenuata* and *B. baxteri* 5 weeks after plants were sprayed with 24 kg/ha phosphite before or after water deficit stress. Significant effects and interactions at $p < 0.05$ are in bold.

		Leaf phosphite concentration		Stem phosphite concentration		Root phosphite concentration	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Application Time (before or after water deficit stress)	1, 28	**17.773**	**<0.001**	**15.462**	**<0.001**	**8.210**	**0.008**
Phosphite (+/-)	1, 28	**115.700**	**<0.001**	**54.590**	**<0.001**	**13.033**	**0.002**
Application Time, Phosphite	1, 28	**17.012**	**<0.001**	**14.954**	**<0.001**	**8.122**	**0.008**

In the second initial MANOVA test, comparing all three phosphite application times in water deficit stressed plants only, significant ($p < 0.001$) main effects of Application time, Phosphite, Species, and interactions between Harvest x Phosphite and Phosphite x Species were found. Univariate tests within the significant effect of Phosphite attributed significance to all three-plant tissues (Table 19). The remaining significant effects and interactions were attributed only to roots. Phosphite concentrations resulting from application of phosphite before water deficit stress were significantly ($p < 0.001$) higher than from applications after water deficit stress, while the application at the wilting point was not different ($p > 0.10$) to either of the other application times (Figure 49). While the Phosphite x Species interaction attributed the significance to sprayed *B. attenuata* having higher concentrations of phosphite in roots than controls, and for sprayed *B. baxteri* these concentrations were not significant, overall *B. attenuata* always had higher phosphite concentrations than *B. baxteri* (Figure 49).

Table 19 Results of univariate ANOVA tests (following significant initial MANOVA) of phosphite concentration in leaf, stem and root samples of *Banksia attenuata* and *B. baxteri* 5 weeks after plants were sprayed with 24 kg/ha phosphite before, during or after water deficit stress. Analysis includes only water stressed plants as there were no non-stressed controls for the phosphite application during water deficit stress. Significant effects and interactions at $p < 0.05$ are in bold.

		Leaf phosphite concentration		Stem phosphite concentration		Root phosphite concentration	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Application time (before, during or after water deficit stress)	2, 22	1.604	0.224	2.925	0.074	**5.239**	**0.0138**
Phosphite (+/-)	1, 22	**51.690**	**<0.001**	**33.375**	**<0.001**	**27.808**	**<0.001**
Species	1, 22	2.630	0.119	2.243	0.148	**5.878**	**0.024**
Application time, Phosphite	2, 22	1.558	0.233	2.836	0.080	**5.085**	**0.015**
Phosphite, Species	1, 22	2.430	0.133	2.060	0.165	**5.815**	**0.025**

DISCUSSION

This study demonstrated that phosphite treatment of *Banksia* seedlings, regardless of the timing of the application with respect to a short-term water deficit event, were effective in reducing stem colonisation by *P. cinnamomi* 4 weeks after the phosphite treatment. However, the best reduction in colonisation was obtained for applications of phosphite either before or during water deficit stress. This is the first report to demonstrate that a short-term water deficit event does not have an impact on phosphite uptake and translocation. However, spraying plants immediately after drought stress is not recommended in the field as prolonged stress may have other physiological consequences for uptake and long-distance transport of phosphite.

The level of water stress reached in our main study was low compared to the preliminary work with *B. attenuata*. Whether long-term water deficit stress, such as that observed in the preliminary work with *B. attenuata* in the current study where photosynthesis was maintained for three weeks at a third of the control levels and growth was significantly reduced compared to the controls, can alter the plant response to *P. cinnamomi*, is unknown.

Pilbeam *et al*. (2000) showed that no phosphite was detected in the roots one month after an autumn application of phosphite onto water deficit stressed *Xanthorrhoea preissii*. No other studies of this type, as far as we are aware, have been conducted on Australian natives. Other work which showed that translocation is reduced by water stress is limited to studies of herbicide applications in annual plants (Reynolds *et al.* 1988, Peregoy *et al.* 1990, Morrison *et al.* 1995).

Further work needs to determine how long-term water stress impacts on phosphite uptake and translocation. If long-term water stress results in similar physiological responses to those observed in the current study, then the limitation to phosphite uptake may be small.

Stem inoculations with *P. cinnamomi* have been shown as a useful and practical means of assessing susceptibility of several keystone Australian native species (e.g. Tippett *et al.* 1985, Shearer *et al.* 1988, Pilbeam *et al*. 2000, Hüberli *et al*. 2002). Susceptibility of stems has also been shown to reflect susceptibility in roots for *Eucalyptus marginata* (Hüberli *et al*. 2002). In the current study, stem inoculations were good predictors of the ability of phosphite to control colonisation of *P. cinnamomi* of *B. attenuata* and *B. baxteri*. The use of stem inoculations is a conservative estimate of the effectiveness of phosphite, since root phosphite levels were more concentrated than in the stem. This concurs with other studies using *Banksia telmetia, Lambertia multiflora* (Komorek and Shearer 1997) and *Corymbia calophylla* (Fairbanks 2001, S Barrett unpublished results).

Colonisation was smaller in the first inoculation than at the two later inoculations. During the later two inoculations, glasshouse temperatures were more favourable for the pathogen with daily averages of average 23.2 - 23.5°C compared to an average of 27.2°C. Interestingly, temperature did not appear to affect colonisation of the phosphite treated plants of both species because colonisation resulting from the first two inoculations was significantly smaller than in the final inoculation. In this instance, it appears that either less phosphite was taken up than in the other two application times or plants received less

phosphite although every effort was made to ensure levels applied were the same over the different phosphite applications.

B. baxteri was less responsive to phosphite than *B. attenuata*, since only in phosphite treated plants was there a significant difference between the species while in controls there was no difference between species. Overall, *B. baxteri* was also less responsive to the water deficit stress with no observed changes in photosynthesis, transpiration and stomatal conductance until after 2 days, whilst the response of *B. attenuata* was immediate. This trend, however, was only significant for stomatal conductance; three-way interaction of Water deficit x Species x Time. The reduced response to phosphite is therefore not related to the species' differences in physiological response to water stress.

The application of phosphite to short-term water deficit stressed plants did not result in significantly less phosphite uptake than non-stressed plants. Sufficient phosphite is taken up to induce host-defense responses in the plants to effectively contain *P. cinnamomi.* We do not know whether severe and prolonged water deficit stress impacts on phosphite uptake and translocation.

Plating plant tissue to detect *Phytophthora cinnamomi* (Photo: D Hüberli)

REFERENCES

Batchelard EP (1986) Effects of soil moisture stress on the growth of seedlings of three eucalypt species. III. Tissue-water relations. *Australian Forestry Research* 16, 155-163.

Bond BJ, Kavanagh KL (1999) Stomatal behaviour of four woody species in relation to leaf-specific hydraulic conductance and threshold water potential. *Tree Physiology* 19, 503-510.

Burgess T, McComb J, Hardy G, Colquhoun I (1998) Influence of low oxygen levels in aeroponics chambers on eucalypt roots infected with *Phytophthora cinnamomi. Plant Disease* 82, 368-373.

Cahill DM, Weste GM, Grant BR (1986) Changes in cytokinin concentrations in xylem extrudate following infection of *Eucalyptus marginata* Donn. ex Sm. with *Phytophthora cinnamomi* Rands. *Plant Physiology* 81, 1103-1109.

Cahill DM, McComb JA (1992) A comparison of changes in phenylalanine ammonia-lyase activity, lignin and phenolic synthesis in the roots of *Eucalyptus calophylla* (field resistant) and *E. marginata* (susceptible) when infected with *Phytophthora cinnamomi. Physiological and Molecular Plant Pathology* 40, 315-332.

Comstock JP (2002) Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. *Journal of Experimental Botany* 53, 195-200.

Crombie DS, Tippet JT (1990) A comparison of water relations, visual symptoms, and changes in stem girth for evaluating impact of *Phytophthora cinnamomi* dieback on *Eucalyptus marginata*. *Canadian Journal of Forest Research* 20, 233-240.

CSIRO (2007) *Climate Change in Australia* http://www.climatechangeinaustralia.gov.au/resources.php (accessed October 19, 2007).

Davies WJ, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology* 42, 55-76.

DEWHA (2007) Recommendation Report on the Commercial Development on Jandakot Airport (EPBC 2007/3599). The Australian Government Department of the Environment, Water, Heritage and the Arts. http://www.environment.gov.au/epbc/notices/pubs/recommendation-report-07-3599.pdf

Dodd J, Bell DT (1993) Water relations of the canopy species in a Banksia woodland, Swan Coastal Plain, Western Australia. *Australian Journal of Ecology* 18, 281-293.

Dunstan WA, Rudman T, Shearer BL, Moore NA, Paap T, Calver MC, Armistead R, Dobrowolski MP, Morrison B, Howard K, O'Gara E, Crane C, Dell B, O¹Brien P, McComb JA, Hardy GEStJ (2008) Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia. Eradication of *Phytophthora cinnamomi* from spot infections in native plant communities in Western Australia and Tasmania. Prepared by the Centre for Phytophthora Science and Management for the Australian Government Department of the Environment, Water, Heritage and the Arts.

Fairbanks M (2001) The effect of the fungicide phosphite on some aspects of plant reproduction. PhD Thesis, Murdoch University, Perth, Australia.

Groom PK (2004) Rooting depth and plant water relations explain species distribution patterns within a sandplain landscape. *Functional Plant Biology* 31, 423-428.

Hardy GEStJ, Barrett S, Shearer BL (2001) The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology* 30, 133-139.

Hüberli D, Tommerup IC, Dobrowolski MP, Calver MC, Hardy GEStJ (2001) Phenotypic variation in a clonal lineage of two *Phytophthora cinnamomi* populations from Western Australia. *Mycological Research* 105, 1053-1064.

Hüberli D, Tommerup IC, Colquhoun IJ, Hardy GEStJ (2002) Evaluation of clonal lines of *Eucalyptus marginata* inoculated in lateral branches and roots for resistance to *Phytophthora cinnamomi*. *Plant Pathology* 51, 435-442.

Jones HG (1998) Stomatal control of photosynthesis and transpiration. *Journal of Experimental Botany* 49, 387-398.

Komorek BM, Shearer BL (1997) The control of *Phytophthora* in native plant communities. In *Control of* Phytophthora *and Diplodia canker in Western Australia.* (Ed D Murray), pp. *1-59.* Department of Conservation and Land Management, Perth.

Lovisolo C, Hartung W, Schubert A (2002) Whole-plant hydraulic conductance and root to-shoot flow of abscisic acid are independently affected by water stress in grapevines. *Functional Plant Biology* 29, 1349-1356.

Marcais B, Dupuis F, Desprez-Loustau ML (1993) Influence of water stress on susceptibility of red oak (*Quercus rubra*) to *Phytophthora cinnamomi*. *European Journal of Forest Pathology* 23, 295-305.

McDowell N, Pockman WT, Allen CD, Breshears DD, Cobb N, Kolb T, Plaut J, Sperry J, West A, Williams DG, Yepez1 EA (2008) *Tansley review:* Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist* 178, 719-739.

Morrison RG, Lownds NK, Sterling TM (1995) Picloram uptake, translocation, and efficacy in relation to water status of Russian knapweed *(Acroptilon repens). Weed Science* 43, 34-39.

Peregoy RS, Kitchen LM, Jordan PW, Griffin JL (1990) Moisture stress effects on the absorption, translocation and metabolism of haloxyfop in johnsongrass *(Sorghum halepense)* and large crabgrass *(Digitaria sanguinalis). Weed Science* 38, 331-337.

Pilbeam RA, Colquhoun IJ, Shearer B, Hardy GEStJ (2000) Phosphite concentration: its effect on phytotoxicity symptoms and colonisation by *Phytophthora cinnamomi* in three understorey species of *Eucalyptus marginata* forest. *Australasian Plant Pathology* 29, 86-95.

Reynolds DB, Wheless TG, Basler E, Murray DS (1988) Moisture stress effects on absorption and translocation of four foliar-applied herbicides. *Weed Technology* 2, 437-441.

Robin C, Capron G, Desprez-Loustan ML (2001) Root infection by *Phytophthora cinnamomi* in seedlings of three oak species. *Plant Pathology* 50, 708-716.

Shearer BL, Michaelsen BJ, Somerford PJ (1988) Effects of isolate and time of inoculation on invasion of secondary phloem of *Eucalyptus* spp. and *Banksia grandis* by *Phytophthora. Plant Disease* 72**,** 121-126.

Shearer BL, Crane CE, Cochrane A (2004) Quantification of the susceptibility of the native flora of the South-West Botanical Province, Western Australia to *Phytophthora cinnamomi. Australian Journal of Botany* 52, 435-443.

Tabachnick BG, Fidell LS (1996) *Using Multivariate Statistics*, 3rd Edn. New York, USA: Harper Collins College Publishers.

Tippett JT, Hill TC (1983) The relationship between bark moisture and invasion of *Eucalyptus marginata* by *Phytophthora cinnamomi. Australasian Plant Pathology* 12, 40-41.

Tippett JT, Hill TC, Shearer BL (1985) Resistance of *Eucalyptus* spp. to invasion by *Phytophthora cinnamomi. Australian Journal of Botany* 33, 409-418.

Tippett JT, Crombie DS, Hill TC (1987) Effect of phloem water relations on the growth of *Phytophthora cinnamomi* in *Eucalyptus marginata. Phytopathology* 77, 246-250.

Veneklass EJ, Poot P (2003) Seasonal patterns in water use and leaf turnover of different plant functional types in a species-rich woodland, south-western Australia. *Plant and Soil* 257, 295-304.

Vesk PA, Westoby M (2003) Drought damage and recovery – a conceptual model. *New Phytologist* 160, 7-14.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-fire

A controlled burn in the native forest of southwestern Australia (Photo: G Freebury)

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-fire

INTRODUCTION

Fire is a frequent event in the Australian landscape (Dixon and Barrett 2003) and influences the biological productivity and biotic composition of many ecosystems (Williams *et al.* 1994). Its effects are complex, ranging from the reduction or elimination of above-ground biomass to impacts on below-ground physical, chemical and microbial processes (Neary *et al.* 1999).

In fire-prone environments, prescribed burning is commonly used to reduce fuel loads to protect life and property (Conroy 1996), and to maintain biodiversity (Cowling *et al.* 1990, Keith 1996). Each year, about 1M ha of native vegetation throughout Australia is burnt with low-intensity, prescribed fires in order to reduce the likelihood of wildfires or to minimise their impact when they occur. Prescribed burning has been a forest management strategy in most States for approximately 30 years (Hall 1994). Nationally, over 54M ha were affected by bushfires in the 2002-2003 dry season (Ellis *et al*. 2004). In the southwest of Western Australia (WA), between 50 and 250K ha are subject to prescribed burns annually (CALM 2006). The total area of wildfires in WA for 2005 - 2006 was 2.7M ha (CALM 2006). The effect of more frequent fires has been studied in some vegetation and soil types (e.g. McKenzie *et al*. 2004, Clarke *et al*. 2005) but importantly their effect on biological interactions, such as symbiotic, parasitic and pathogenic, are yet to be examined in detail in many fire-prone Australian ecosystems.

Climate change is likely to increase fire frequency and severity in already fire-prone regions of the world (e.g. Brown *et al*. 2004, Pearce *et al*. 2005). In eastern Australia, the combined frequencies of days when the Forest Fire Danger Index is extreme have been projected to increase 4 - 25% by 2020 and 15 - 70% by 2050 (Hennessy *et al*. 2005). A similar pattern is expected for the southwest of WA where it is predicted that the climate is likely to get drier, with lower winter rainfall and increased average temperatures resulting in a longer 'fire season' and a greater proportion of the landscape that is dry enough to burn (Burrows and Wardell-Johnson 2003, DEC 2007). Furthermore, the expected increase in summer rainfall events in the southwest of WA is likely to exacerbate the impact and severity of *P. cinnamomi* which thrives under warm wet conditions.

Although fire is a frequent event in the Australian landscape and is expected to increase with climate change, we know very little about the relative uptake of phosphite by vegetation pre- and post-fire, or how fire may alter the long-distance transport and longevity of phosphite within woody plants. Only one study showed that phosphite was able to reduce disease extension of *P. cinnamomi* at a landscape level in *Banksia* woodland after an unplanned fire (Shearer *et al*. 2004). In this study it was assumed, but not assessed, that phosphite remained in woody roots of injected plants and remained effective thus slowing the disease front.

The effect of fire on native species

Life-history strategies that plants have evolved to cope with fire include: resprouting from deep-seated buds in stem bark and/or lignotubers; and repopulating from seed banks in fire-sensitive species. The time taken for resprouting individuals to flower is referred to as the secondary juvenile period. Resprouting species generally take longer to flower from seed than obligate seeding species (Abbott 1985, Bell 2001, Knox and Clarke 2004).

Knox and Clarke (2004) found basal resprouting to be the most common regeneration mode of the resprouters.

The proportion of resprouters in southwestern Australian plant communities ranges from 66 to 80% (Bell 2001). About half of the *Adenanthos*, *Banksia* and *Hakea* species are resprouters. Bell (2001) considers that the relatively high percentage of resprouters probably indicates that the WA vegetation experiences a harsher fire stress regime than do other Mediterranean type climate areas. Following a fire, the rate of seedling establishment in the first year was about 10 times higher, and seedling survivorship over the first 5 years was seven times higher, for the non-sprouters (Enright and Goldblum 1999).

Reseeders tend to have shallow, fibrous root systems whereas resprouters have massive, deep, woody root systems. The latter store more starch than the former. Reseeders tend to conserve nutrients to a greater extent than resprouters through leaf retention (Bell 2001). Some *Banksia* species that are killed by fire, e.g. *B. nutans*, require at least 7-year intervals between fires to enable seed set to occur (Wooller *et al*. 2002).

In some plant communities, both *P. cinnamomi* and changing fire regimes are threatening processes and can place threatened and endangered species and communities at risk. However, there is little substantial knowledge on the interaction of fire with *P. cinnamomi* and the disease process in susceptible plant species and communities. Twenty five threatened plant species are endemic to the Stirling Range NP and their survival is critically endangered by *P. cinnamomi* and inappropriate fire regimes (Brown *et al.* 1998). A vegetation survey of mountains in WA identified high levels of disease impact within frequently burnt sites in the eastern Stirling Range NP communities (Barrett 1996). The increased susceptibility of sites may be attributed to changes in soil microclimate or hydrology, which are further intensified by the slower regeneration times at higher elevations (Barrett 1996, Barrett and Gillen 1997). A 12 month preliminary study found that fire in *P. cinnamomi* infested communities of the SRNP has the potential to increase both the severity and extent of disease in communities, and to reduce the regeneration capabilities of susceptible species. The study identified changes in soil chemistry, reduced microbial activity and enhanced production of viable sporangia in soil solutions collected from recently burnt plots (0 - 1 year) confirming the increase in disease incidence observed in the wild. For example, viable sporangia production was significantly higher for two sites burnt in 2004 and 2005, while the corresponding site burnt more than 30 year ago produced significantly less and higher numbers of viable and nonviable sporangia, respectively (Moore 2005).

Uptake of phosphite

The season of burn can affect shoot recovery and post-fire reproduction (Bowen and Pate 2004). For example, after fire, photosynthates produced initially by new shoots of *Stirlingia latifolia* are diverted preferentially into further shoot growth and inflorescence production, and no priority is given at this time to replenishment of root reserves (Bowen and Pate 1993). This could temporarily restrict long-distance transport of phosphite to the roots. It is not known whether other resprouters in the Proteaceae have similar physiology to *S. latifolia*.

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-fire

AIMS

In this study we examine the effect of fire on the ability of phosphite to reduce the severity of disease caused by *P. cinnamomi* in three members of the Proteaceae, one reseeder and two resprouters, treated pre- and post-fire. Two separate experiments were conducted to determine the following specific aims: if the application of phosphite pre-fire (Experiment 1) or post-fire (Experiment 2) will adversely affect the efficacy and persistence of the phosphite's ability to contain *P. cinnamomi.*

OUTCOMES

We will provide land managers with information on whether a phosphite treatment before/after a fire is still effective in preventing disease caused by *P. cinnamomi*, or whether further treatment is required.

METHODS

Site selection

In 2006, two potential sites were assessed on the south coast of WA: Cape Riche and South Stirling in the Stirling Ranges. Both sites were deemed suitable for a prescribed burn during the autumn of 2006. The site in South Stirling contained *Banksia* species, but not *B. attenuata* or *B. baxteri.* Some parts of this site had been devastated by *Phytophthora*, but most of it was in good condition. However, after detailed examination this site was abandoned due to the low diversity of suitable plant candidates and insufficient numbers for experimental purposes. At Cape Riche, access to the site was considered to be a major problem and so this site was not pursued. After further reconnaissance, a site in the Stirling Range NP was selected. The experiment commenced at this site in August 2006 and a prescribed burn occurred in November 2006.

The study site was located on the west of Chester Pass Road in the Stirling Range NP (Figures 50 and 51a). The site was several km along the southern boundary of the park (34.651667° S, 118.070000° E) and was chosen to meet the following important criteria:

1. The area had not been burned for 10 years, had a sufficient fuel load to carry a fire, and contained *P. cinnamomi* susceptible plants capable of surviving and regenerating after a fire.
2. Permission from government agency to undertake a burn was available.
3. The area was large enough to incorporate a site suitable for burning and buffers to protect the control sites (no fire).
4. There were plant species present in sufficient numbers necessary to conduct the experiment. We required 3 species native to the region that were sensitive to infection from *P. cinnamomi* and responded to phosphite application. These species needed to occur at high densities, have similar physiological age and size, that allowed phosphite spray treatment with a fine-misting wand, and leaf structure suitable for CIRAS and pressure potential measurements.
5. The area was already infested with *P. cinnamomi* so that onsite inoculations would be permitted.
6. The area had easy off-road access to the sites for undertaking predawn pressure potentials, while sufficiently removed from potential interference from the general public.

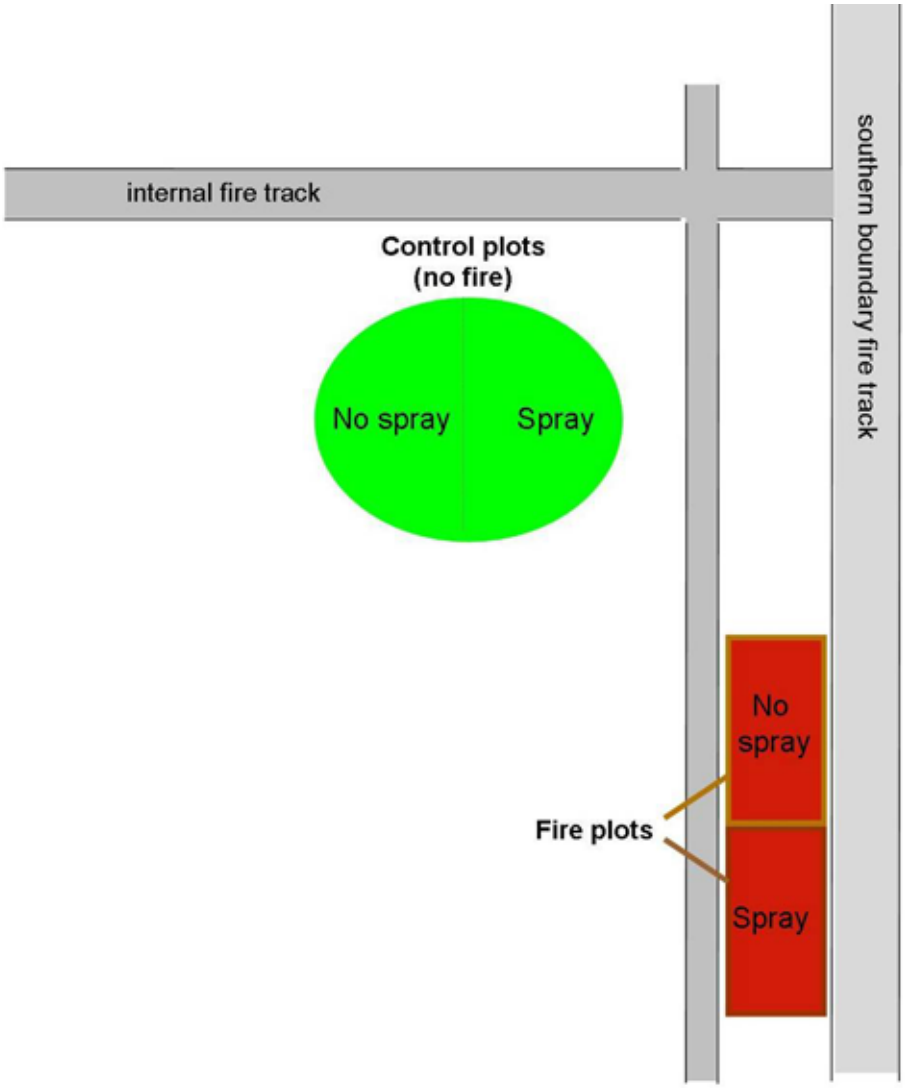


Figure 50 Experimental study site on the western side of Chester Pass Road, Stirling Range National Park in the south coast region of Western Australia. The site is several kilometres along the southern boundary of the park (34.651667°S, 118.070000°E). Map not to scale.

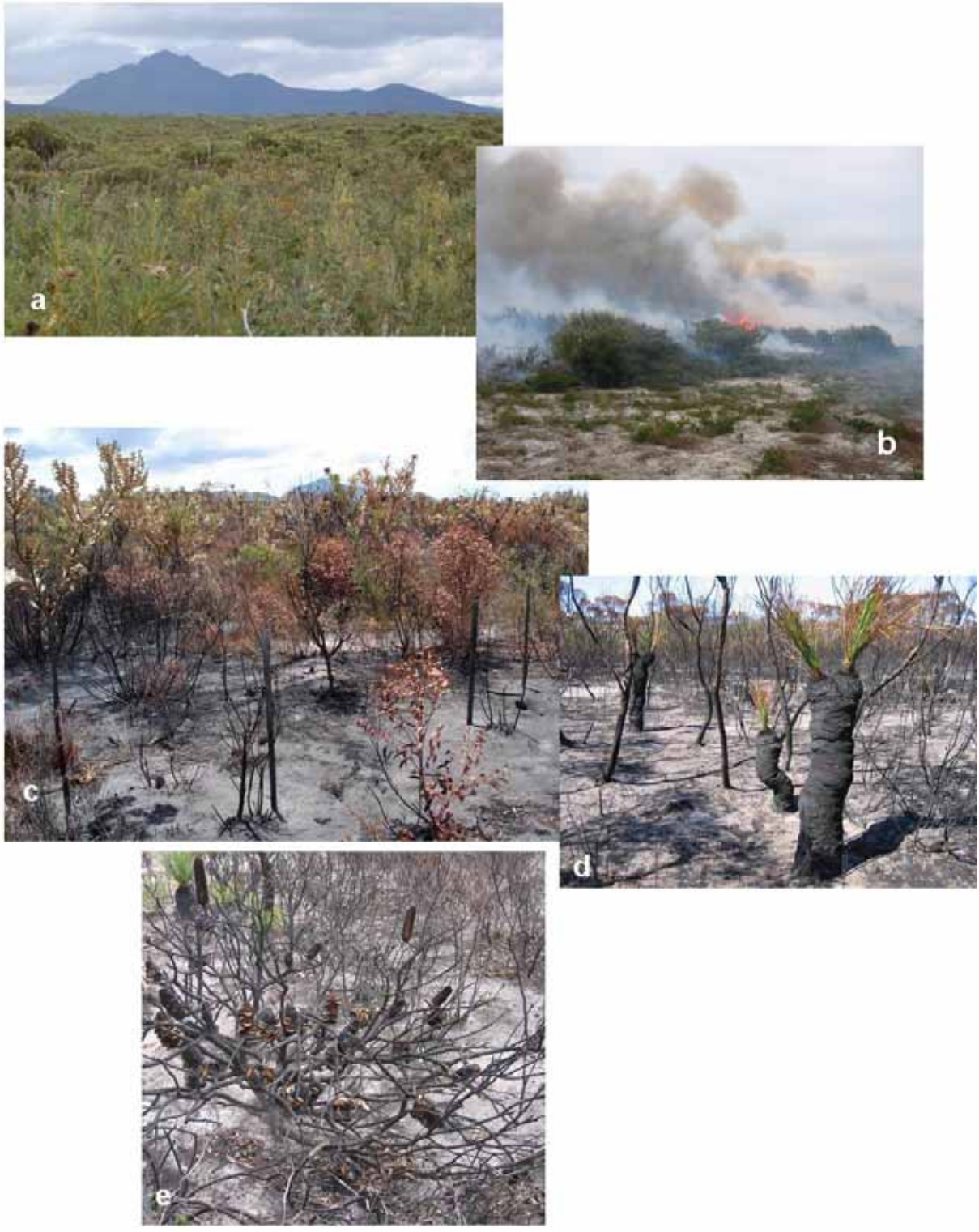


Figure 51 The study site **a)** before the burn, **b)** the burn took place on 14th November 2006, **c** and **d)** the site immediately after the fire, and **e)** *Banksia baueri* after fire, a reseeder species which is killed by fire.

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-fire

Experimental design

The study site was divided into four treatment plots. The treatments were fire and phosphite spray, fire without phosphite spray, control (no fire) with phosphite spray, and control without phosphite spray. The treatment plots were each divided into two subplots. The two subplots represented phosphite treatment before burning (Experiment 1) or after burning (Experiment 2). The treatment plots were approximately 1.6 ha (fire with phosphite), 1.2 ha (fire without phosphite), 1 ha (no fire with phosphite) and 0.7 ha (no fire without phosphite).

Experiment 1 plots were sprayed pre-fire on 7th September 2006, whilst Experiment 2 were sprayed post-fire on 3rd October 2007 when plants had re-sprouted with sufficient foliage. Plant physiological and colonisation measurements and phosphite tissue analysis taken during Experiments 1 and 2 are shown in Figure 52. The sites were burnt on the 14th November 2007 (Figure 51b - e).

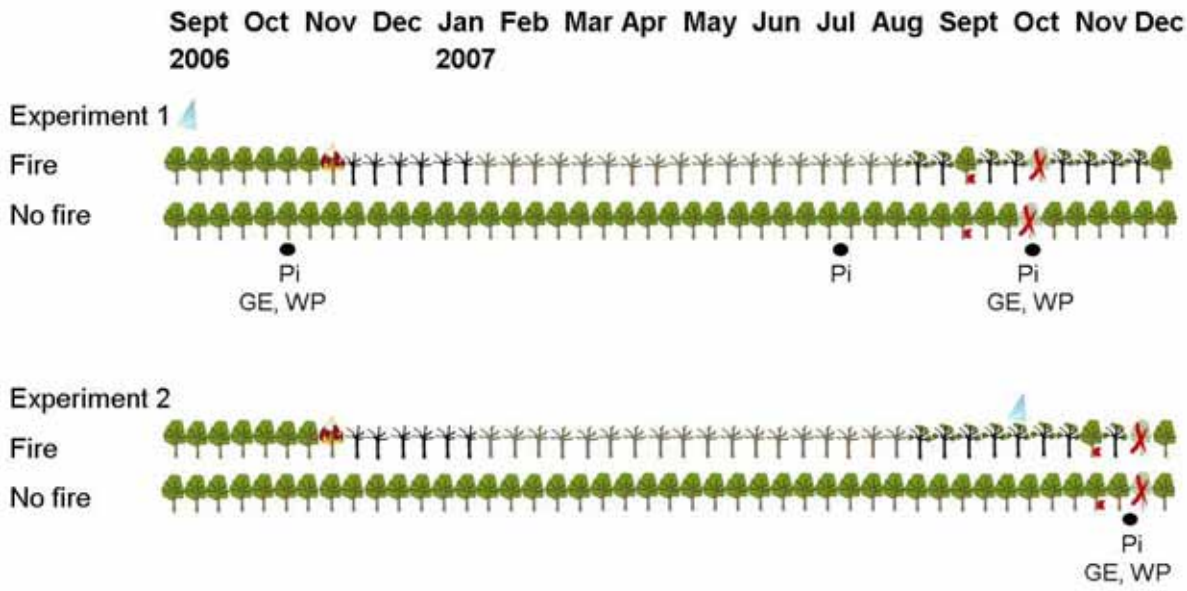


Figure 52 Timeline showing the two experiments with phosphite treatments ( ) in relation to fire ( ) and plant recovery (,). Plants were inoculated ( ) 12 months (Experiment 1) and 5 weeks (Experiment 2) after the phosphite treatment and then harvested ( ) 2.5 weeks later. Periods of leaf gas exchange (GE), water potential (WP) and phosphite concentration (Pi) measurements are also shown.

Soil analysis

Soil was collected to a depth of 5 cm prior to the fire, and to 2.5 cm after the fire (sampling the ash bed). Six soils samples were collected from each treatment plot bulked and mixed well before being analysed by CSBP Limited (Bibra Lake, WA, Australia).

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-fire

The plants

Banksia attenuata *Banksia baxteri* *Adenanthos cuneatus*

Three species were selected: *Banksia attenuata* (resprouter), *B. baueri* (reseeder) and *Adenanthos cuneatus* (resprouter). These species were chosen as they are representative species of the south coast region and are susceptible to *P. cinnamomi*. Little is known of the response of *B. baueri* to phosphite application adding to the importance of including this species.

Climate

The mean monthly minimum and maximum air temperatures, and mean monthly rainfall for January 2006 to April 2008 at Moingup Springs Ranger Station (34.651667°S, 118.070000°E), the nearest weather station, are shown in Figure 53. In January and December 2006 there were higher than average rainfall events. The 30 year (1971 - 2000) average rainfall for the nearby Mt Barker station (34.63°S, 117.64°E) was 29.2 and 23.5 mm for January and December, respectively. In 2006, Moingup Springs recorded 80.4 and 43.2 mm for January and December, respectively.

Fire treatment

The two fire plots were burnt on 14th November 2006 under high fire danger conditions. Wind speed was ESE 35 - 40 km/h at 22°C with 42% RH. The fuel moisture content in Mallee Heath was not measured because heathlands do not accumulate much leaf litter. Flame heights varied from 1 to 2 m where there was limited vegetation, and up to 6 m in dense vegetation. The forward rate of fire spread was about 4000 m/h and the fire intensity was moderate to strong. Burning commenced at midday and lasted for about 4.5 h.

Phosphite application

Phosphite was sprayed as a 40% solution of phosphite (Agrifos 600; Agrichem, Loganholme, Qld, Australia) using a Microfit low-volume fine mist applicator (Micron Sprayers Ltd., Herefordshire, UK) at 24 kg/ha. An adjuvant [0.2 % (v/v) of BS1000® (100% alcohol alkoxylate; Crop Care Australasia, Murarrie, QLD)] was added and agitated prior to spraying. The Microfit applicator simulates as closely as possible the aerial application of phosphite to natural ecosystems (Barrett *et al.* 2002). Phosphite was sprayed pre-fire on 7th September 2006 about 9 weeks before the fire in Experiment 1, whilst Experiment 2 was sprayed post-fire on 3rd October 2007, approximately 11 months after the fire (Figure 52).

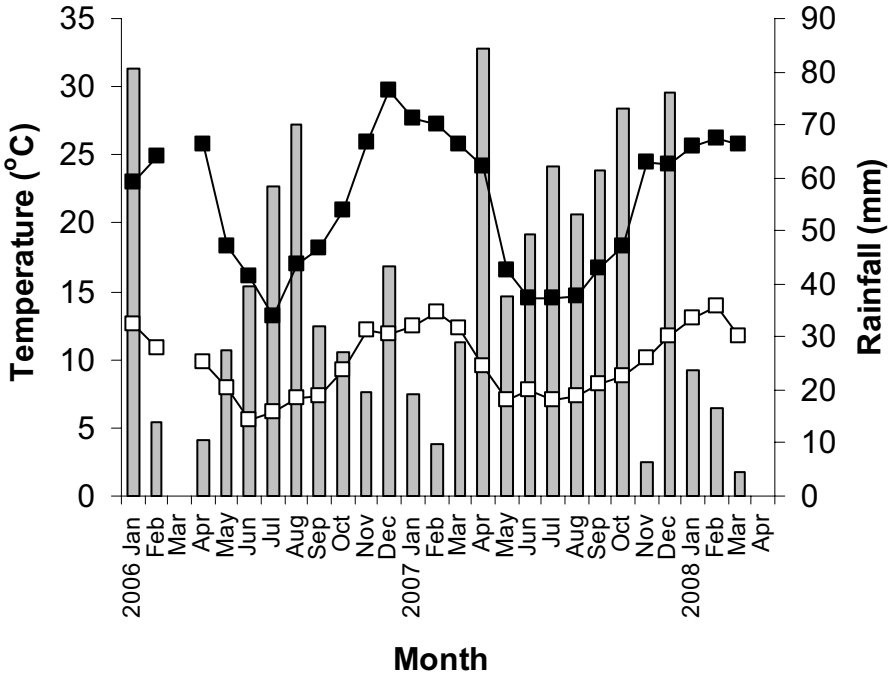


Figure 53

Mean monthly minimum (□) and maximum air temperatures (■), and total monthly rainfall (▒) for Moingup Springs (34.651667°S, 118.070000°E) for January 2006 to April 2008.
No data for March 2006 available.

Measurements

Physical

An effort was made to select plants of similar sizes across the species, fire and phosphite treatments to reduce the potential for variability between replicates (Table 20). All plants were under 150 cm in height to facilitate phosphite application by the Microfit applicator. The average height of *B. attenuata*, *B. baueri* and *Adenanthos cuneatus* across the treatments were 134.2, 98.4 and 106.1 cm, respectively.

The effect of phosphite treatment on the ability of *B. baueri* to successfully survive fire was determined in Spring 2007 when the seedlings in each site were counted. Preliminary (before fire) data showed that the numbers of potential seed present in follicles for *B. baueri* across the burn treatments were similar at each site (Table 20).

Physiological data

Before the fire, within each of the subplots, three replicate plants per species of equal size and in close proximity to one another were selected for each of the physiological measurements. The leaf water potential and gas exchange rates were measured (Figure 54) pre-fire in October 2006 and at harvests (October and November 2007) (Figure 52). Plant water potentials were measured at predawn (3.30 – 5.30 am) and midday (11.00 – 3.00 pm) using a pressure chamber (Model 1000; PMS Instrument Company, Albany, Oregon, USA). Photosynthetic and transpiration rates and stomatal conductance were measured in the morning (9.00 am to 1.00 pm) using a portable gas exchange system (CIRAS-2; PP Systems, Amesbury, Massachusetts, USA; or LCpro+; ADC Bioscientific, Herts, UK).

Calibration of the LCpro+ portable system ensured uniformity between measurement periods. For example, the mean photosynthetically active radiation (PAR) for Experiment 1 measurements (taken on 3 Oct 2007) were 1444 ± 47 µmol $m^{-2}s^{-1}$, while the curvette temperature ranged from 30.5 to 33.5°C with a mean of 32.3°C. For Experiment 2 (29 November 2007) the mean PAR values were 1648 ± 28 µmol $m^{-2}s^{-1}$, while the temperature ranged from 33.1 to 35.0°C with a mean of 34.2 °C.

Table 20 Plant height and mean canopy width of *Banksia attenuata, B. baueri* and *Adenanthos cuneatus,* and cones on *B. baueri* in control sites and sites sprayed with 24 kg/ha phosphite pre- and post- fire at commencement of experiments. *n* = 15 - 20.

Treatment	Species	Height in cm (±SE)	Mean canopy width in cm (±SE)	Mean number of cones with follicles (±SE)
With phosphite/ pre-fire	*B. attenuata*	132.3 (5.7)	150.6 (7.3)	
	B. baueri	98.3 (12.3)	113.3 (7.7)	3.6 (0.6)
	A. cuneatus	85.0 (10.0)	133.3 (5.0)	
With phosphite/post-fire	*B. attenuata*	135.8 (6.1)	154.4 (5.0)	
	B. baueri	-	-	4.5 (0.5)
	A. cuneatus	127.2 (6.1)	178.0 (11.0)	
With phosphite/ no fire (control)	*B. attenuata*	134.7 (8.2)	144.2 (9.7)	
	B. baueri	98.6 (3.7)	107.9 (5.2)	4.7 (1.9)
	A. cuneatus	106.1 (5.8)	147.3 (6.6)	

Figure 54 Using the LCpro+ to measure leaf gas exchange in the field. (Photo: T Paap)

Inoculation and measurement of infection

Randomly selected plants from each subplot were inoculated with *P. cinnamomi.* Plants were inoculated in October 2007 and November 2007 for Experiments 1 and 2, respectively (Figure 52). In each treatment plot and subplot, five replicate plants of each species were under-bark inoculated using 5 mm diameter Miracloth (Calobiochem, USA) disks colonised with *P. cinnamomi* (isolate SR2). Inoculations were made in the centre of lateral branches, such that the distance from the inoculation area to the main stem was at least 30 cm (Figure 55). Three lateral branches were inoculated per plant. All lateral branches were inoculated at least 30 cm above ground level to minimise the risk of introducing *P. cinnamomi* infection into the soil at the study sites. Inoculation wounds were wrapped in Parafilm and then silver ducting tape. A fourth lateral branch (control) per replicate plant was inoculated similarly but with a sterile Miracloth disk. After 2½ weeks, inoculated branches were excised from the plants and transported back to the laboratory in cooler-boxes. The branch diameter at the region of inoculation was measured, and after bark removal, the length of any lesions was recorded. If a lesion was present, five 1 cm sections above and below the lesion were plated onto *Phytophthora* selective media NARPH (Hüberli *et al*. 2000) to determine the extension beyond the lesion of *P. cinnamomi*. Colonisation incorporates the sum of the lengths of the lesion and extension beyond the lesion. If no lesion was present, a segment from the region of inoculation was used to re-isolate *Phytophthora,* and five sections of 1 cm from above and below the inoculation area were plated sequentially to determine colonisation. After 2 to 5 days at 24^{o}C in the dark, plates were examined for the presence of *P. cinnamomi*. Since each of

the 3 inoculated branches on each plant was a pseudo-replicate, lesion and colonisation lengths of the 3 branches were averaged for the plant and presented and analysed as a plant average.

Figure 55 Three inoculated lateral branches of *Banksia attenuata* with *Phytophthora cinnamomi* (red flagging tape) and control inoculation (white flagging tape).

Phosphite concentration analysis

Plant material was removed for phosphite analysis six weeks and 10 months after the pre-fire (Experiment 1) phosphite application, and again at each harvest of inoculated branches (13 months after pre-fire (Experiment 1) phosphite application and 8 weeks after post-fire (Experiment 2) phosphite application). A total of three of the five plants per species that were inoculated for each of the four plots were excavated and transported back to the laboratory. Samples of roots, lignotubers (where present), stems and leaves were washed, then dried at 40°C for 1 to 2 weeks, ground to a powder and sent to the WA Chemistry Centre (Perth) for phosphite analysis (see page 4). All work conducted on the South Stirling Ranges fire site was included under the Department of Environment and Conservation Permit Numbers CE001428, CE001795, SW010976 and SW011677.

Statistical analysis

All statistical analyses were done using the Statistica software V6.1 (Statsoft, Inc., Tulsa, OK, US). Following Tabachnick and Fidell (1996), data for parametric tests were screened for assumptions of homoscedasticity, presence of outliers, normality and non-correlations of means and variances. Physiological leaf measurements including photosynthetic rate, stomatal conductance, transpiration rate, and predawn and midday water potentials were treated as dependent variables in multivariate analysis of variance (MANOVA) in each of Experiments 1 and 2, with independent variables of fire (+/-), phosphite treatment (+/-) and species (*B. attenuata* and *A. cuneatus*). Since *B. baueri* was only treated with phosphite in the unburnt plots, this species was excluded from the analysis.

Total lesion and colonisation lengths were correlated significantly for *B. attenuata* (r_{20} = 0.58) and *A. cuneatus* (r_{18}= 0.68) in Experiment 1, and *B. attenuata* (r_{20} = 1.0) and *A. cuneatus* (r_{20}= 0.98) in Experiment 2. For *B. baueri*, lesions were often not visible so it was decided that colonisation was the best measurement. Therefore, only colonisation was used as the dependent variable (after $\log_{10}$ transformation in Experiment 2 only to correct for correlation between means and variances across the cells of the design) in analyses of variance (ANOVAs). Data comparing the effects of fire with *B. baueri* were excluded and data comparing all three species in the unburnt plots were tested separately because *B. baueri* was a reseeder and present only in the unburnt plots at the time of phosphite treatments. The independent variables in the first analysis were phosphite treatment (+/-), fire treatment (+/-) and species (*B. attenuata* and *A. cuneatus*), while in the second analysis, they included phosphite treatment (+/-) and species (*B. attenuata*, *B. baueri* and *A. cuneatus*). All significant main effects and interactions were compared using Tukey's HSD test where appropriate.

Phosphite concentrations in leaf, stem, lignotuber and root samples were treated as dependent variables for each of the harvests in multivariate analysis of variance (MANOVA) in each of Experiments 1 and 2, with independent variables of fire (+/-), phosphite treatment (+/-) and species (*B. attenuata* and *A. cuneatus*). For Experiment 1, the data were analysed by two separate MANOVA tests; one tested the initial harvest (October 2006) 6 weeks after the phosphite spray prior to the prescribed burn and thus, excluded the independent variable of fire (+/-), and the second, tested the last 2 post-fire harvests (July and October 2007) and included a fourth independent variable of time. Since, as a re-seeder *B. baueri* was not present on the burned plots, it was tested against the other two species on unburnt plots in 3 separate MANOVA analyses for harvests in October 2006 (Experiment 1), July and October 2007 (Experiment 1), and November 2007 (Experiment 2). In these 3 MANOVAs specific to unburnt plots, the dependent variables were phosphite concentrations in leaf, stem, and root samples; lignotuber were excluded in this analysis because *B. baueri* does not have lignotubers. The independent variables were phosphite treatment (+/-) and species (*B. attenuata, B. baueri* and *A. cuneatus*), and time only for the Experiment 1 (July and October 2007) MANOVA. Where appropriate, significant main effects and interactions were compared using Tukey's HSD test.

RESULTS

Soil chemical characteristics before and after fire

Significant increases were found in phosphorus, potassium, sulphur, conductivity and pH post-fire (Table 21).

Physiological Measurements

Physiological measurements on the 17th October 2006 (prior to the prescribed burn) showed that *A. cuneatus* had midday water potentials higher than -2 MPa, which were higher than those of the other two species. There were no differences amongst species at predawn water potentials. Photosynthetic rates, transpiration rates and stomatal conductance were similar to the measurements in the unburnt plots the following year.

Table 21 Soil chemical properties (nutrients, electrical conductivity and pH) pre- and post fire for the study site in the Stirling Range National Park in the south coast region of Western Australia. Data are means of six soil samples with SE in brackets.

Parameter	Unit	PRE-FIRE	POST-FIRE
Colour	-	Grey to dark grey	Grey to grey-brown and grey-black
Nitrate	mg/kg	1.7 (0.7)	1.0 (0)
Ammonium	mg/kg	9.0 (3.5)	8.8 (2.8)
Colwell P	mg/kg	2.7 (0.7)	12.0 (3.7)
Potassium	mg/kg	57.0 (10.5)	159.3 (43.1)
Sulphur	mg/kg	4.6 (1.4)	31.2 (9.2)
Org. Carbon	%	1.2 (0.2)	1.4 (0.3)
Reactive Fe	mg/kg	74.5 (14.3)	94.2 (22.9)
Conductivity	dS/m	0.04 (0)	0.21 (0.04)
pH_{CaCl2}	-	4.9 (0.1)	6.5 (0.3)
pH_{H2O}	-	6.1 (0.1)	7.0 (0.3)

In Experiment 1 (phosphite applied pre-fire), the initial MANOVA of all physiological measurements for *B. attenuata* and *A. cuneatus* on burnt and unburnt plots, and for all three species on unburnt plots showed that there was a significant ($p < 0.001$) main effect of Species (Figures 56 and 57). The univariate ANOVA on *B. attenuata* and *A. cuneatus* showed that the only significant main effect for all physiological variables was Species, and Fire or Phosphite or any interactions between Fire, Phosphite and Species were not significant (Table 22). Of the three species, *A. cuneatus* had the highest ($p < 0.001$) midday water potentials (Figure 56c). *B. attenuata* had the highest photosynthetic and transpiration rates (Figure 57a and b), and *A. cuneatus* had the highest stomatal conductance (Figures 57c).

Table 22 Results of univariate ANOVA tests (following significant initial MANOVA) of plant physiological measurements in October 2007 for pre-fire phosphite applications (Experiment 1) of *Banksia attenuata* and *Adenanthos cuneatus*, harvested 11 months post-fire. Significant main effects at $p < 0.05$ are in bold. Predawn water potentials not included because there were no significant main effects of plant species.

		Midday water potentials		Photosynthetic rate		Transpiration rate		Stomatal conductance	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Species	1, 16	**212.63**	**0.001**	**56.88**	**0.001**	**10.85**	**0.005**	**6.80**	**0.019**

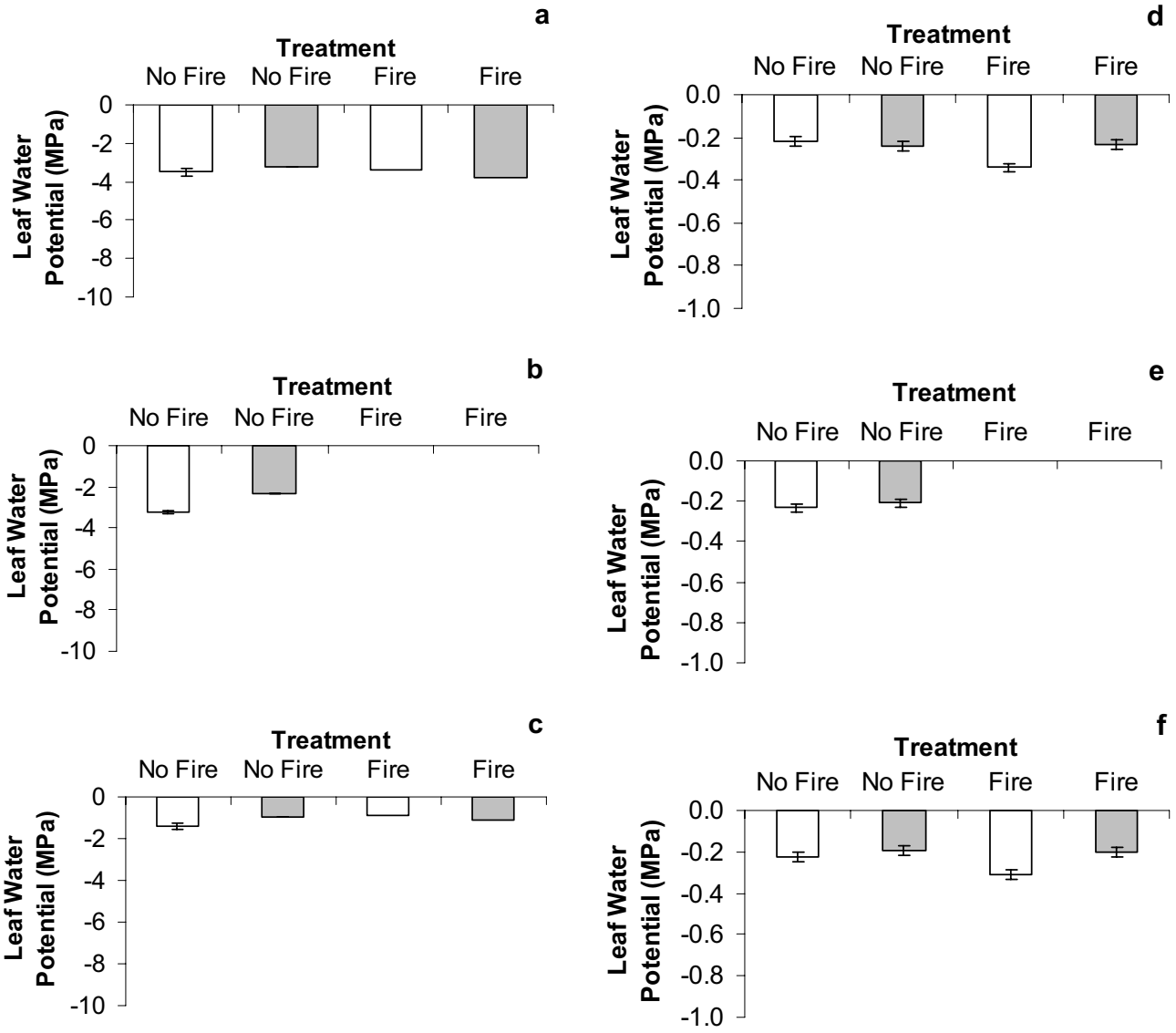


Figure 56 Midday (**a - c**) and pre-dawn (**d - f**) leaf water potentials on 3rd October 2007 (11 months post-fire). **a** and **d)** *Banksia attenuata,* **b** and **e)** *B. baueri* and **c** and **f)** *Adenanthos cuneatus* at Stirling Range National Park, Western Australia. Phosphite was applied at 0 (control □) and 24 kg/ha (■) pre-fire (Experiment 1). Vertical bars represent two standard errors of the mean; *n*= 3. No data provided for the re-seeder species, *B. baueri*, post-fire.

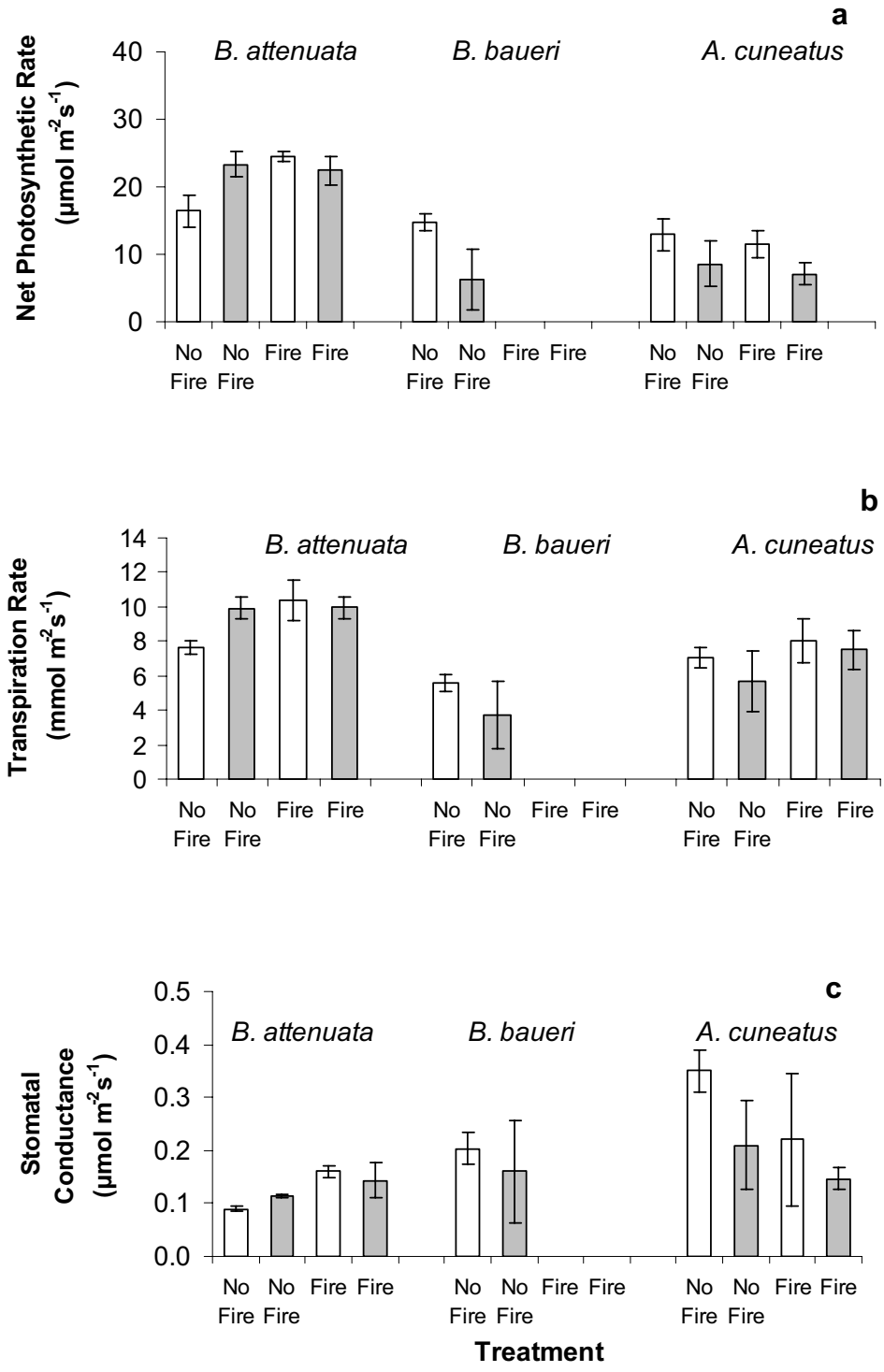


Figure 57 Physiological measurements on 3rd October 2007 (11 months post-fire). **a)** *Banksia attenuata,* **b)** *Adenanthos cuneatus* and **c)** *B. baueri* at Stirling Range National Park, Western Australia. Phosphite was applied at 0 (control □) and 24 kg/ha (■) pre-fire (Experiment 1). Vertical bars represent two standard errors of the mean; *n*= 3. No data for the re-seeder species, *B. baueri*, post-fire.

In Experiment 2 (phosphite applied post-fire), the univariate ANOVA of all physiological measurements for *B. attenuata* and *A. cuneatus* on burnt and unburnt plots showed there were significant main effects and interactions for all the independent variables tested except for the Phosphite x Species interaction (Table 23). As for Experiment 1, all but the predawn water potentials, had significant main effect of Species. A main effect of Fire was significant only for photosynthetic rate. There was also a significant interaction of Fire x Species for all three gas exchange measurements (Table 23). Midday water potentials were again overall higher for *A. cuneatus* (Figure 57c).

Table 23 Results of univariate ANOVA tests (following significant initial MANOVA) of plant physiological measurements in November 2007 for post-fire phosphite applications (Experiment 2) of *Banksia attenuata* and *Adenanthos cuneatus*, harvested 12 months post-fire. Significant main effects and interactions at $p < 0.05$ are in bold.

		Midday water potentials		Photosynthetic rate		Transpiration rate		Stomatal conductance	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Fire	1, 16	0.07	0.792	**29.32**	**0.001**	0.01	0.987	0.08	0.785
Phosphite	1, 16	2.23	0.155	0.10	0.757	**15.77**	**0.001**	2.90	0.108
Species	1, 16	**93.25**	**0.001**	**123.4**	**0.001**	**65.03**	**0.001**	**59.69**	**0.001**
Fire, Phosphite	1, 16	0.39	0.541	0.96	0.341	**36.02**	**0.001**	**35.79**	**0.001**
Fire, Species	1, 16	0.25	0.626	**43.13**	**0.001**	**5.57**	**0.031**	**8.83**	**0.009**
Fire, Phosphite, Species	1, 16	2.79	0.114	0.57	0.460	0.09	0.767	**22.23**	**0.001**

Photosynthetic rates for Experiment 2 were significantly ($p < 0.001$) higher on burnt plots than unburnt plots for *B. attenuata*, while there was no difference ($p = 0.85$) for *A. cuneatus* (Figure 59a). Transpiration rates were significantly ($p < 0.001$) higher for unsprayed than phosphite sprayed plants on unburnt plots, while for burnt plots there was no significant ($p = 0.50$) difference between unsprayed and sprayed plants. Across the burnt and unburnt plots, *B. attenuata* had significantly ($p < 0.004$ in both cases) higher transpiration rates than *A. cuneatus* (Figure 59b). Stomatal conductance did not vary across fire and phosphite treatments for *B. attenuata*. For *A. cuneatus*, stomatal conductance was significantly ($p < 0.001$) higher in unburnt plots for unsprayed plants than for sprayed plants, while the contrary was true on burnt plots ($p < 0.009$) (Figure 59c).

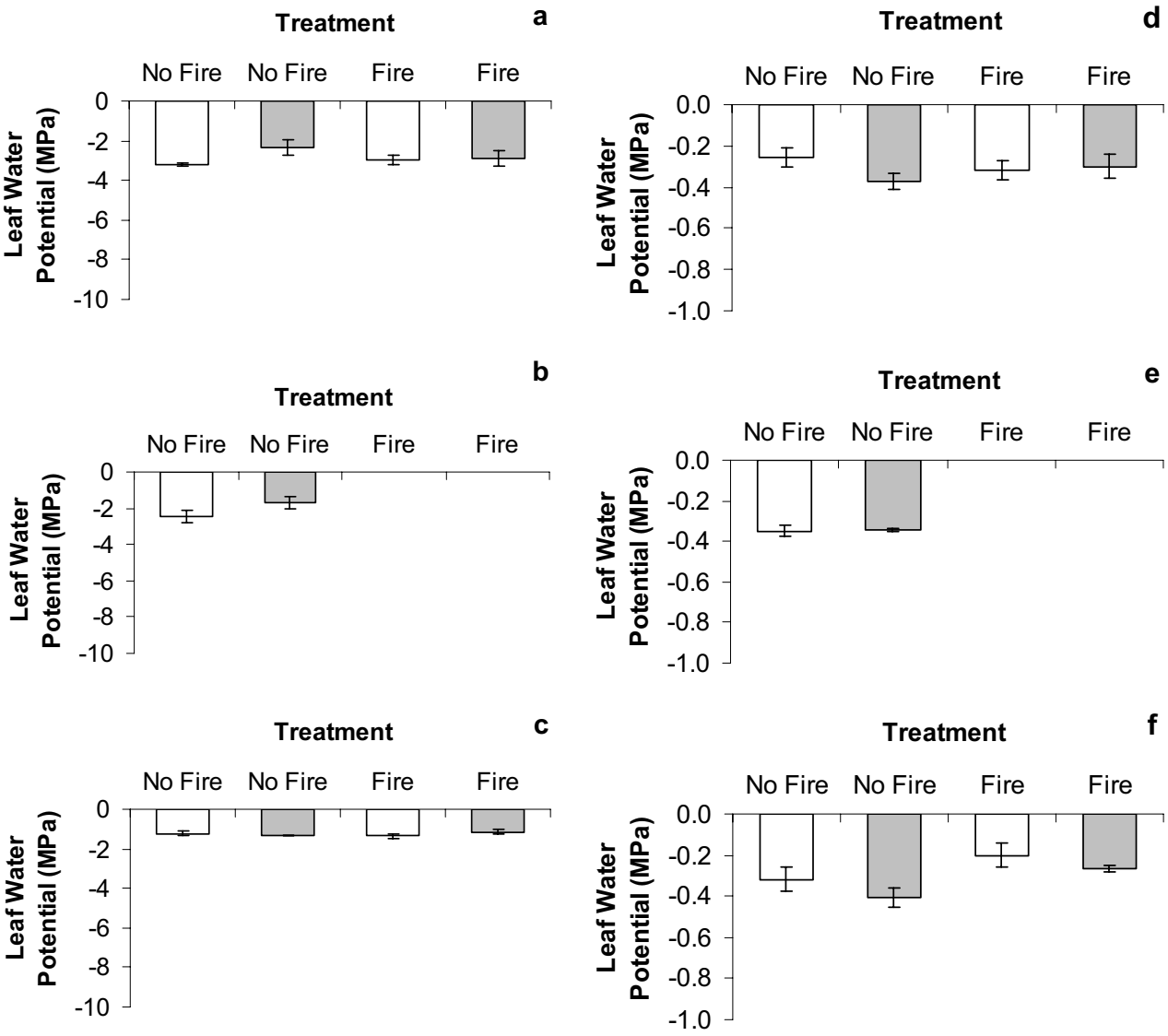


Figure 58 Midday (**a - c**) and pre-dawn (**d - f**) leaf water potential on 29th November 2007 (12 months post-fire). **a** and **d)** *Banksia attenuata,* **b** and **e)** *B. baueri* and **c** and **f)** *Adenanthos cuneatus* at Stirling Range National Park, Western Australia. Phosphite was applied at 0 (control □) and 24 kg/ha (■) post-fire (Experiment 2). Vertical bars represent two standard errors of the mean; *n*= 3. No data for the re-seeder species, *B. baueri*, post-fire.

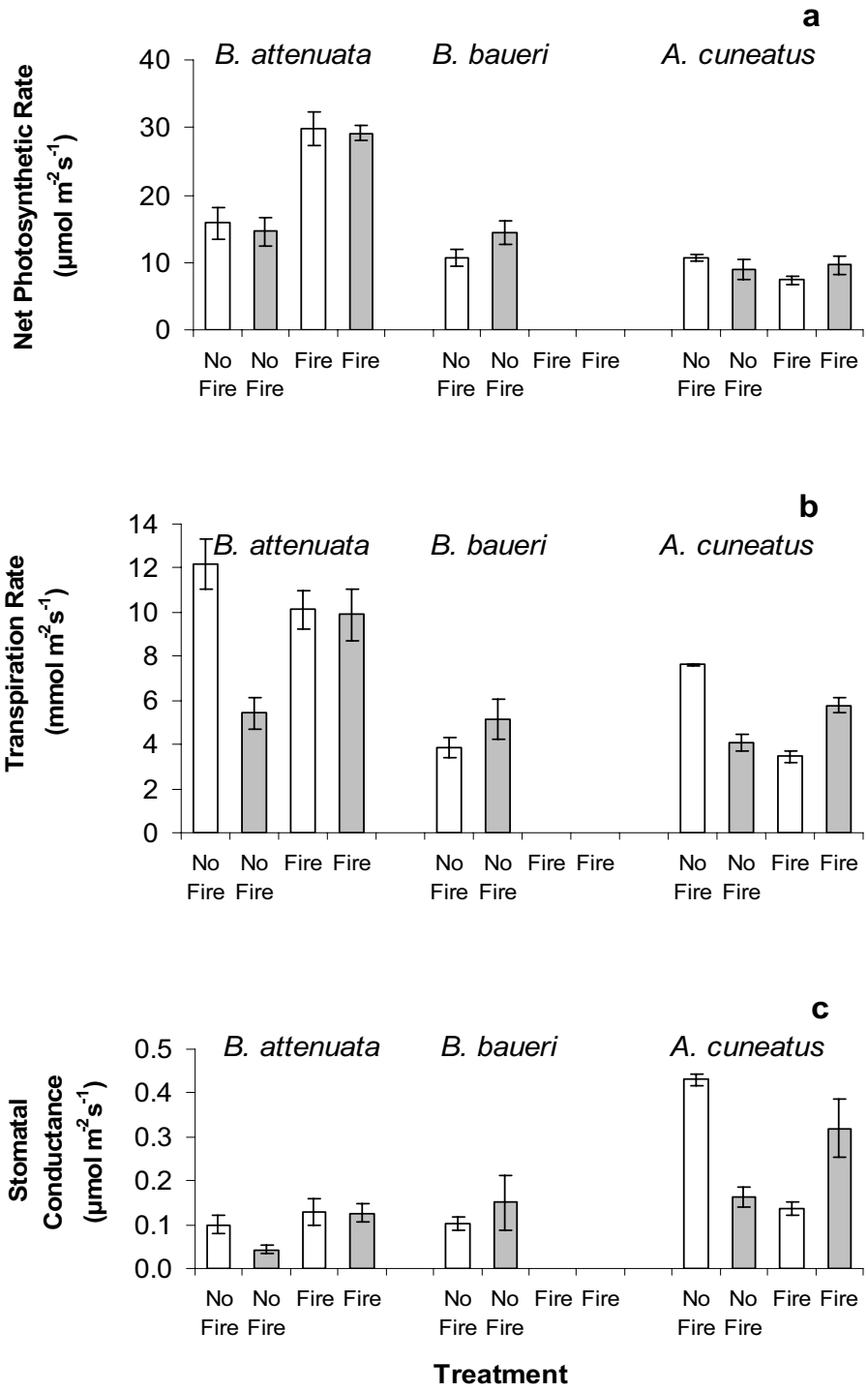


Figure 59 Physiological measurements on 29th November 2007 (12 months post-fire). **a)** *Banksia attenuata,* **b)** *Adenanthos cuneatus* and **c)** *B. baueri* at Stirling Range National Park, Western Australia. Phosphite was applied at 0 (control □) and 24 kg/ha (■) post-fire (Experiment 2). Vertical bars represent two standard errors of the mean; *n* = 3. No data for the re-seeder species, *B. baueri* post-fire.

Colonisation of stems by *P. cinnamomi*

Fire did not have a significant (p = 0.16) main effect on colonisation of *P. cinnamomi* in plants sprayed with phosphite prior to the fire (Experiment 1), but had a significant main effect (p < 0.001) on plants sprayed post-fire (Experiment 2) (Table 24; Figures 60 and 61).

In Experiment 1, significant (p < 0.05 in all cases) main effects were found for Phosphite and Species for the ANOVA comparing *B. attenuata* and *A. cuneatus* on burnt and unburnt plots and also for the unburnt plots comparing all 3 species. Overall, phosphite treatments were effective for all species except for burnt *A. cuneatus* and unburnt *B. baueri*, where phosphite treatments did not appear to reduce colonisation (Figure 60).

Table 24 Results of univariate ANOVA tests of colonisation of stems of *Banksia attenuata* and *Adenanthos cuneatus* inoculated with *Phytophthora cinnamomi* in pre- (Experiment 1) and post-fire (Experiment 2) phosphite applications. Significant main effects and interactions at p < 0.05 are in bold.

		Experiment 1		**Experiment 2**	
Effect	**Df**	**F**	**p**	**F**	**p**
Fire	1, 30	2.07	0.160	**49.13**	**0.001**
Phosphite	1, 30	**5.27**	**0.029**	1.11	0.300
Species	1, 30	**8.34**	**0.007**	**8.92**	**0.005**
Fire, Phosphite	1, 30	0.15	0.701	0.67	0.419
Fire, Species	1, 30	1.40	0.246	0.19	0.666
Phosphite, Species	1, 30	1.02	0.322	3.77	0.061
Fire, Phosphite, Species	1, 30	1.02	0.320	0.85	0.365

In addition to Fire, significant (p = 0.005) main effects for Species were found in Experiment 2 (Table 24). Although not significant, there was a general trend of small colonisation lengths in phosphite treated plants compared to untreated plants, with the exception of the burnt *A. cuneatus* when phosphite was applied post-fire (Figure 60). Colonisation was always larger (p = 0.001) in burnt plots than unburnt plots (Figure 61). Colonisation, for all three species, was larger in plants that had phosphite applied prior to the fire than those which had phosphite applied after the fire (Figure 60 and 61).

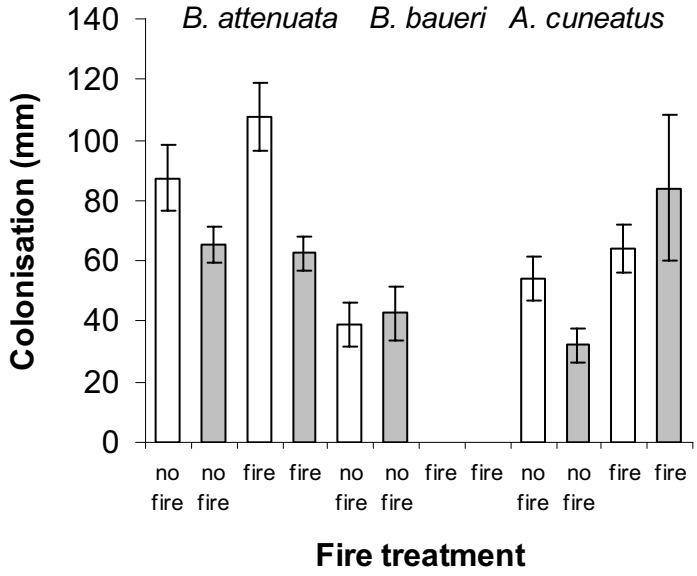


Figure 60 Mean colonisation of the stem by *Phytophthora cinnamomi* of *Banksia attenuata, Adenanthos cuneatus* and *B. baueri* at the Stirling Range National Park, Western Australia, treated with 0 (control □) and 24 kg/ha (▣) phosphite pre-fire (Experiment 1). Vertical bars represent two standard errors of the mean; *n* = 5. No data for the re-seeder species, *B. baueri*, post-fire.

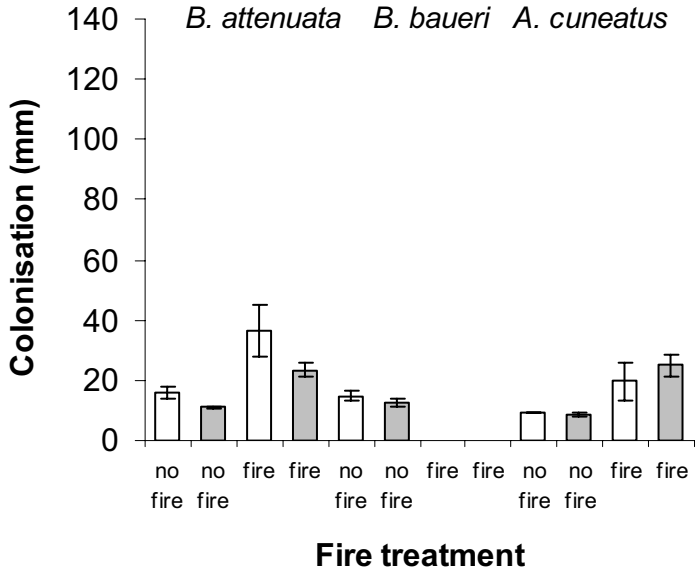


Figure 61 Mean colonisation of the stem by *Phytophthora cinnamomi* of *Banksia attenuata, Adenanthos cuneatus* and *B. baueri* at the Stirling Range National Park, Western Australia, treated with 0 (control □) and 24 kg/ha (▣) post-fire (Experiment 2). Vertical bars represent two standard errors of the mean; *n* = 5. No data for the re-seeder species, *B. baueri*, post-fire.

Phosphite concentrations in the different plant tissues

Six weeks after phosphite application in Experiment 1, Phosphite was the only significant ($p < 0.04$ in all cases) main effect for all plant tissues and species. After the burn, Phosphite was also a significant ($p < 0.03$ in all cases) main effect as well as Time and Fire, and the interactions of Time x Fire, Time x Phosphite, Fire x Phosphite and Time x Fire x Phosphite in the initial MANOVA.

Lignotubers were the only plant tissue with the main effect of Phosphite only (Table 25) in plants sprayed pre-fire. Phosphite concentrations in leaves and stems had a significant interaction of Fire x Phosphite. Except at 6 weeks after spray (prior to the fire), burnt plants had significantly ($p < 0.01$ in both cases) higher phosphite concentrations in leaves and stems than in unburnt plants (Figure 62). Phosphite concentrations in stems also had a significant Time interaction with Phosphite and Fire, and a three-way interaction with Time, Fire and Phosphite (Table 25). In July 2007, phosphite concentrations in stems were not different between burnt and unburnt plants, but in October 2007 (13 months post-spray), burnt plants had higher ($p < 0.001$) phosphite concentrations than unburnt plants (Figure 62). Root phosphite concentrations, like stems, had a significant ($p < 0.04$) Time x Phosphite interaction (Table 25).

The general trend was that in *B. attenuata* roots, phosphite concentrations in burnt plots remained higher at both harvest times, while in unburnt plots the phosphite concentrations declined with time. While for *A. cuneatus*, root phosphite concentrations remained similar in both unburnt and burnt plots.

Table 25 Results of univariate ANOVA tests (following significant initial MANOVA) of plant tissue phosphite (Pi) concentrations in July and October 2007 for pre-fire phosphite applications (Experiment 1) of *Banksia attenuata* and *Adenanthos cuneatus,* harvested 8 and 11 months post-fire. Significant main effects and interactions at $p < 0.05$ are in bold. *B. baueri* was not included in the analysis because there were no data for burnt plots and there was no lignotuber data as they do not form lignotubers.

		Leaf Pi concentration		Stem Pi concentration		Lignotuber Pi concentration		Root Pi concentration	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
Time	1, 30	0.69	0.412	**9.52**	**0.004**	0.44	0.513	**4.44**	**0.043**
Fire	1, 30	**6.82**	**0.014**	**21.57**	**0.001**	0.001	0.997	0.03	0.857
Phosphite	1, 30	**10.78**	**0.003**	**37.01**	**0.001**	**17.14**	**0.001**	**22.45**	**0.001**
Time, Fire	1, 30	0.92	0.344	**18.28**	**0.001**	2.88	0.100	0.14	0.710
Time, Phosphite	1, 30	0.74	0.396	**9.61**	**0.004**	0.62	0.437	**4.43**	**0.043**
Fire, Phosphite	1, 30	**6.85**	**0.014**	**21.70**	**0.001**	0.01	0.966	0.03	0.855
Time, Fire, Phosphite	1, 30	1.12	0.299	**18.15**	**0.001**	2.72	0.110	0.14	0.711

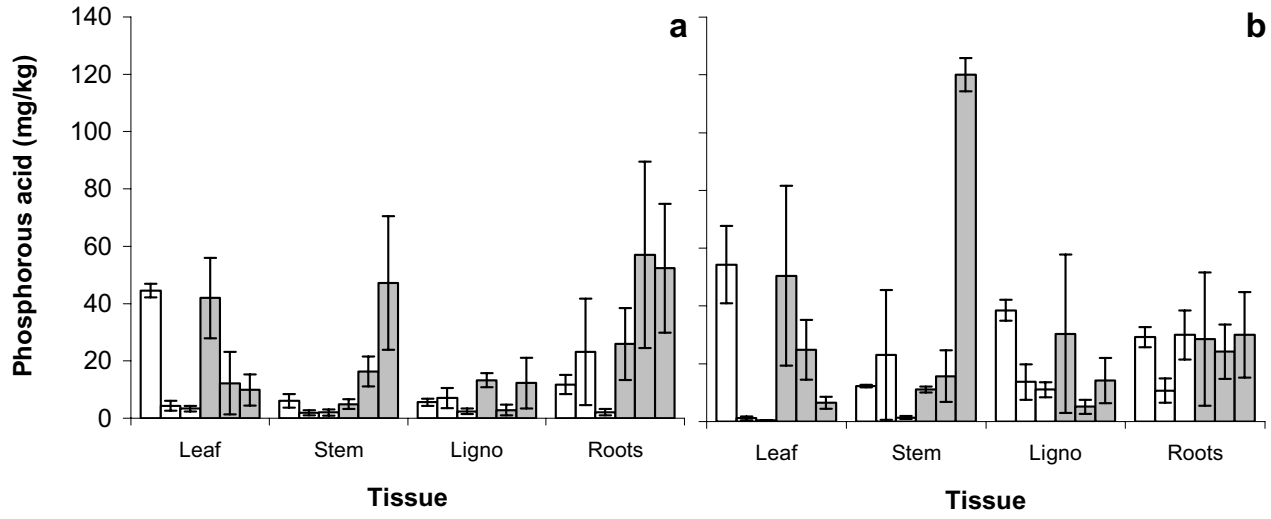


Figure 62 Mean phosphite concentration (phosphorous acid mg/kg) of tissue in **a**) *Banksia attenuata* and **b**) *Adenanthos cuneatus* subjected to no fire (control □) or fire (▩) and sprayed with 24 kg/ha phosphite pre-fire (Experiment 1). The three bars of each colour set were harvested at: 6 weeks (prior to prescribed burn), and 10 and 13 months after phosphite application. Vertical bars represent two standard errors of the mean; *n* = 3.

The initial MANOVA test comparing phosphite concentrations in leaves, stems and roots in all three species in the unburnt plots only found significant main effects for Phosphite and Species, and for the Phosphite x Species interaction. Stem phosphite concentrations did not vary significantly ($p > 0.14$ for all effects), while for leaves and roots there was a significant interaction effect of Phosphite x Species (Table 26). In *B. baueri*, phosphite concentrations in leaves and roots in the unburnt plots were always lower than in the other two species (Figures 62 and 63).

Table 26 Results of univariate ANOVA tests (following significant initial MANOVA) of plant tissue phosphite concentrations in July and October 2007 for pre-fire phosphite applications (Experiment 1) of *Banksia attenuata*, *B. baueri* and *Adenanthos cuneatus* in unburnt plots harvested 8 and 11 months post-fire. Significant main effects and interactions at $p < 0.05$ are in bold. Lignotubers are not included in the analysis because *B. baueri* does not form these.

		Leaf phosphite concentration		**Stem phosphite concentration**		**Root phosphite concentration**	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Phosphite	1, 23	3.38	0.079	1.56	0.224	**18.22**	**0.001**
Species	2, 23	1.57	0.229	0.70	0.506	**5.51**	**0.011**
Phosphite, Species	2, 23	**4.22**	**0.028**	0.73	0.494	**5.52**	**0.011**

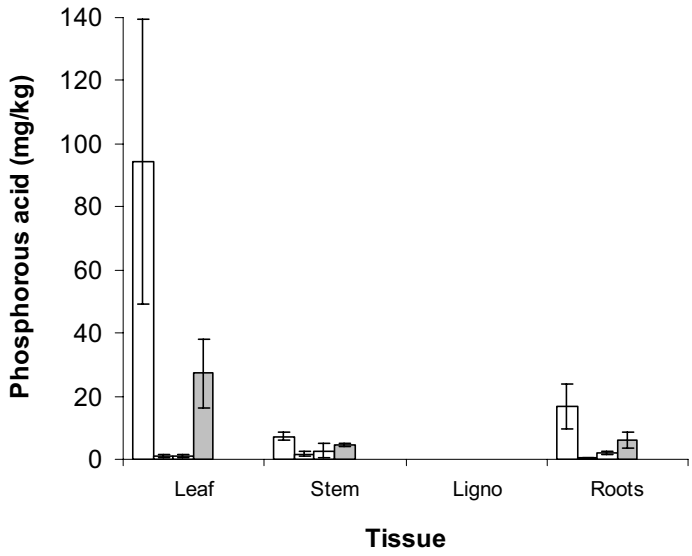


Figure 63 Mean phosphite concentration (phosphorous acid mg/kg) of tissue in *Banksia baueri* in unburnt (control □) or burnt plots (before the burn, ▣) and sprayed with 24 kg/ha phosphite pre-fire (Experiment 1). The three bars of each colour set were harvested at: 6 weeks (prior to prescribed burn), 10 and 13 months after phosphite application. Vertical bars represent two standard errors of the mean; *n* = 3. No post-fire data provided.

Table 27 Univariate ANOVA tests (following significant initial MANOVA) of plant tissue phosphite concentrations in November 2007 for post-fire phosphite applications (Experiment 2) of *Banksia attenuata* and *Adenanthos cuneatus,* harvested 12 months post-fire. Significant main effects and interactions at p < 0.05 are in bold. *B. baueri* is not included in the analysis because there were no data for burnt plots and no lignotuber data.

		Leaf phosphite concentrations		**Stem phosphite concentrations**		**Root phosphite concentrations**	
Effect	**Df**	**F**	**p**	**F**	**p**	**F**	**p**
Phosphite	1, 17	**15.70**	**0.001**	**22.86**	**0.001**	**8.32**	**0.010**
Species	1, 17	1.04	0.322	4.39	0.051	4.39	0.051
Fire, Phosphite	1, 17	0.24	0.631	0.81	0.380	**5.43**	**0.032**
Fire, Species	1, 17	2.36	0.143	1.73	0.206	3.37	0.084
Phosphite, Species	1, 17	0.99	0.333	**4.74**	**0.044**	**5.09**	**0.037**
Fire, Phosphite, Species	1, 17	2.43	0.137	1.95	0.180	2.81	0.112

For phosphite applications post-fire (Experiment 2), the initial MANOVA found significant main effects of Phosphite and interactions of Fire x Phosphite and Phosphite x Species.

All plant tissue, except lignotubers, had a significant ($P < 0.01$ in all cases) difference between sprayed and non-sprayed plants (Table 27). For all *B. attenuata* tissues, phosphite concentrations were higher, but not significantly so, in non-burnt than burnt plants (Figure 64a), but they were significant for *A. cuneatus* in roots (Figure 64b). Phosphite concentrations in all three tissue were comparable in the reseeder species, *B. baueri*, to the two resprouter species (Figure 64 and 65).

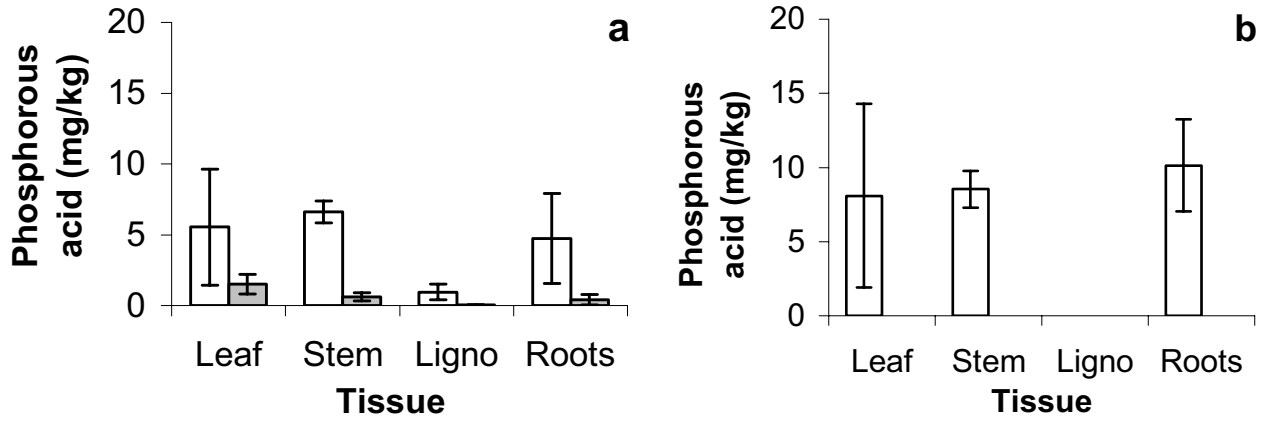


Figure 64 Mean phosphite concentration (phosphorous acid mg/kg) of tissue in **a**) *Banksia attenuata* and **b**) *Adenanthos cuneatus* subjected to no fire (control □) or fire (▩) and sprayed with 24 kg/ha phosphite post-fire (Experiment 2). Plants were harvested 11 months post-fire and 8 weeks after phosphite application. Vertical bars represent two standard errors of the mean; *n* = 3.

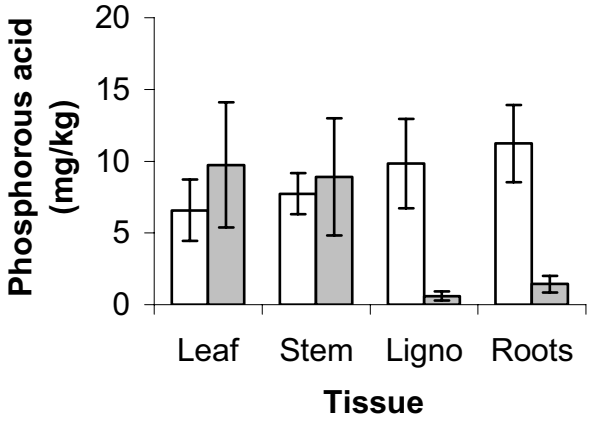


Figure 65 Mean phosphite concentration (phosphorous acid mg/kg) of *Banksia baueri*, in unburnt plots and sprayed with 24 kg/ha phosphite post-fire (Experiment 2). Plants were harvested 11 months post-fire and 8 weeks after phosphite application. Vertical bars represent two standard errors of the mean; *n* = 3.

DISCUSSION

None of the three plant species examined were under severe stress during the course of this study. For example, predawn water potentials were in the range of -0.2 to -0.4 MPa and did not reach stressed levels of -1.5 to -2.5 MPa such as achieved by *Banksia attenuata* after 8 days of waterlogging. Physiological measures of leaf gas exchange and water status were taken for comparative purposes and to allow the plant stress status to be assessed before and after treatment. This is essential as fire can affect vegetation at different times of the year when stress from non-fire factors, e.g. water stress and heat stress, may be present in vegetation components.

In general, physiological measurements over time mostly reflected species differences. However, three physiological responses due to other factors are worth noting. Firstly, only in *B. attenuata* did fire have an affect on photosynthesis. It is possible that the increase in photosynthesis was due to resprouting foliage having greater photosynthetic capacity than the foliage produced pre-fire. It would be interesting to follow leaf function over time after fire to ascertain whether there is a functional difference in leaves produced pre and post-fire and how long this functional trait takes to reach the pre-fire leaves. Secondly, the application of phosphite to *B. attenuata* depressed transpiration only on unburnt plots. That there was not a concomitant change in stomatal aperture suggests that the change may have been due to the water uptake and supply pathway being modulated by phosphite. We know nothing about how phosphite influences fine root growth and function in the field. Thirdly, in *Adenanthos cuneatus* stomatal conductance was reduced by the application of phosphite in unburnt plots, but not in burnt plots. Again, root function in this species needs study to determine the mechanism behind the observed response.

The observed differences in leaf physiology between the species are unlikely to impact severely on either the uptake of phosphite or the long-distance transport of phosphite in the plants studied. Regardless of differences in physiology all three plant species took up phosphite and distributed it throughout the plant. After fire there was a trend for phosphite to increase in stems and leaves, this did not happen in non-burnt plants. Therefore, new growth in *B. attenuata* and *A. cuneatus* acted as a sink for photosynthates and phosphite was redistributed from older tissues to young actively growing tissues. In *B. attenuata* and *A. cuneatus* treated with phosphite before fire, phosphite concentrations decreased over time in leaves, but increased in the stems and remained unchanged in the lignotubers with time after the burn. In contrast, when *B. attenuata* were treated with phosphite after fire, phosphite levels were reduced in all tissues in burnt plants compared to un-burnt plants. Whilst for *A. cuneatus* there were no differences between leaves and stems between fire treatments, but there were lower phosphite levels in lignotubers and roots of the burnt plants.

Phosphite applications prior to the fire were effective in controlling stem colonisation of *P. cinnamomi* in the resprouter species, *B. attenuata*, for almost one year after the fire. This is the first study to report the effect of fire on the uptake and translocation of phosphite in any plant species. Phosphite concentrations in stems and roots of *B. attenuata* were equivalent especially in October 2007. This is encouraging given that pathogen activity often increases after a fire (Moore, 2005). That phosphite can persist after a fire, concurs with Shearer *et al*. (2004) who showed that *Banksia* species injected with phosphite, including *B. attenuata*, were protected from an active disease front, after a wildfire event.

We would expect based on our study and that of Shearer *et al*. (2004), that when plants are treated with phosphite at least two months before a fire, roots would be similarly protected from a challenge by the pathogen, as shown in our stem inoculations. In addition, our study shows that phosphite concentrations in roots increased after fire in *B. attenuata*.

In contrast to *B. attenuata*, the other resprouter species, *A. cuneatus*, phosphite treatments were not effective in controlling colonisation in the burnt plots, irrespective of whether treatments were applied pre- or post-fire, while treatments were effective on plants that were not burnt. Despite the lack of control of stem colonisation in burnt plots, phosphite concentrations in stems were higher in burnt plants than unburnt plants, and they were also higher than those of *B. attenuata*. In this species, it appears that phosphite, whilst present in the tissue in high concentrations, is unable to induce host defense mechanisms after fire and as a result cannot control a challenge inoculation with the pathogen. Further research is required to address this question and to also determine how many other susceptible resprouter species respond in a similar fashion.

Phosphite application was not effective in controlling *P. cinnamomi* colonisation of *B. baueri*. In contrast, both *B. attenuata* and *A. cuneatus* had significantly lower colonisation in phosphite treated plants than in controls, despite having similarly low concentrations of phosphite in their stems as in *B. baueri*. At 6 weeks after the phosphite spray, concentrations in all tissues of *B. baueri* were equivalent to the other two species. *B. baueri* is listed as a susceptible species to *P. cinnamomi* (Wills 1993) and that it is unresponsive to phosphite is concerning. Future work needs to ascertain the factors contributing to phosphite's inability to induce plant defenses when challenged by *P. cinnamomi* in this species.

In prescribed burn situations where plants require protection from *P. cinnamomi*, such as in the Stirling Ranges, a site should be treated with phosphite at least two months prior to the scheduled burn to offer any protection to susceptible plants, subsequent to the burn. Spraying after the burn appears not to be effective in controlling colonisation at least, that is, if sprayed within 11 months post-fire, this is despite there being phosphite present in all tissues. However, these levels were much lower (approximately four-fold less) than phosphite levels present in plants sprayed pre-fire. Further work needs to determine when plants are able to take up similar levels of phosphite when applied post-fire as is taken up pre-fire. Further work is also required to determine at what phosphite concentrations *in planta* plants are able to respond to a *P. cinnamomi* challenge. *B. attenuata* had much lower concentrations of phosphite in the leaves and stems in burnt plots than in unburnt plots. It is possible that the reduced canopy as a result of the fire resulted in much lower uptake of the low volume aerial phosphite application. For these reasons, we would not recommend an aerial spray of diseased sites within 12 months after a prescribed burn or wildfire event. Overall, concentrations of phosphite were extremely low comparative to the phosphite treatment pre-fire, even on unburnt plots. This may have also contributed to the lower control in the burnt plots. Further work needs to determine when plants can be sprayed to offer similar protection to pre-fire applications for instances where an uncontrolled burn has gone through an area requiring protection from *P. cinnamomi*.

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated pre- and post-fire

A limited number of species were investigated in our study and revealed that there are different physiological strategies employed in response to fire, it is not possible therefore to make generic recommendations on behaviour of other members of the Proteaceae. However, in plants treated with phosphite prior to fire, there is a trend for phosphite to increase in stems and leaves with time after fire, this accumulation in phosphite in stems and leaves does not happen in non burnt plants. Further work on a larger range of susceptible species across susceptible families is required to test the Hypothesis: Fire results in the redistribution of phosphite from old to new grow in resprouter species, due to rapid shoot growth acting as a sink for photosynthates. Further work needs to determine whether this redistribution of phosphite to the canopy will reduce the time period in which phosphite is active in roots, resulting in plants becoming susceptible to the pathogen sooner than in un-burnt plants.

Inspecting the burnt plots in the Stirling Ranges National Park (Photo: P Scott)

REFERENCES

Abbott I. (1985) Reproductive ecology of *Banksia grandis* (Proteaceae). *New Phytologist* 99, 129-148.

Barrett S (1996) Biological survey of mountains of southern Western Australia. Department of Conservation and Land Management, Albany.

Barrett S, Gillen K (1997) Mountain protected areas of south Western Australia. PARK 7, 35-42.

Barrett SR, Shearer BL, Hardy GEStJ (2002) Root and shoot development in *Corymbia calophylla* and *Banksia brownie* after the application of the fungicide phosphite. *Australian Journal of Botany* 50, 155-161.

Bell DT (2001) Ecological response syndromes in the flora of Southwestern Western Australia: fire resprouters versus reseeders. *Botanical Review* 67, 417-440.

Bowen BJ, Pate JS (1993) The significance of root starch in post-fire shoot recovery of the resprouter *Stirlingia latifolia* R.Br. (Proteaceae). *Annals Botany* 72, 7-16.

Bowen BJ, Pate JS (2004) Effect of season of burn on shoot recovery and post-fire flowering performance in the resprouter *Stirlingia latifolia* R.Br. (Proteaceae). *Australian Ecology* 29, 145-155.

Brown A, Thomson-Dans C, Marchant N (Eds) (1998) *Western Australia's Threatened Flora*. Department of Conservation and Land Management, Western Australia.

Brown TJ, Hall BL, Westerling AL (2004) The impacts of 21st century climate change on wildland fire danger in the western United States: an application perspective. *Climate Change* 62, 365-388.

Burrows N, Wardell-Johnson G (2003) Fire and plant interactions in forested ecosystems of south-west Western Australia. In: I Abbott and N Burrows (Eds), Fire in Ecosystems of South-west Western Australia: Impacts and Management, 225-268. Backhuys Publishers, Leiden.

CALM (2006) Annual Report 2005-2006. Department of Conservation and Land Management.

Clarke PJ, Knox KJE, Wills KE, Campbell M (2005) Landscape patterns of woody plant response to crown fire: disturbance and productivity influence sprouting ability. *Journal Ecology* 93, 544-555.

Conroy B (1996) Bush fire Management Zones, NSW National Parks and Wildlife Service Unpublished.

Cowling RM, Lamont BB, Enright NJ (1990) Fire and management of south-western Australian *Banksias*. *Proceedings Ecological Society Australia* 16, 177-183.

DEC website (2007) Fire Management: Fire and the Environment. www.naturebase.net accessed 18 Feb 2007.

Dixon K, Barrett R (2003) Defining the role of fire in south-west Western Australian plants. In: I Abbott and N Burrows (Eds), Fire in Ecosystems of South-west Western Australia: Impacts and Management, 205-223. Backhuys Pub., Leiden.

Ellis S, Kanowski P, Whelan R (2004) National Inquiry on Bushfire Mitigation and Management. Commonwealth of Australia, Canberra.

Enright NJ, Goldblum D (1999) Demography of a non-sprouting *Hakea* species (Proteaceae) in fire-prone *Eucalyptus* woodlands of southeastern Australia in relation to stand age, drought and disease. *Plant Ecology* 144, 71-82.

Hall R (1994) The effect of fuel reduction burning on forest soils. In: 'Fire and Biodiversity: The Effects and Effectiveness of Fire Management'. Proceedings of the conference held 8-9 October 1994. Biodiversity Series Paper No. 8, Footscray, Melbourne, Department of Environment, Sport and Territories, Canberra.

Hennessy K, Lucas C, Nicholls N, Bathols J, Suppiah R, Ricketts J (2005) Climate change impacts on fire-weather in south-east Australia. CSIRO Report, 88 pp.

Hüberli D, Tommerup IC, Hardy GEStJ (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi. Australasian Plant Pathology* 29,164-169.

Humphreys F, Craig F (1981) Effects of fire on soil chemical, structural and hydrological properties. In: '*Fire and the Australian Biota*.' (Eds Gill A, Groves R, Nobel I) Australian Academy of Science, Canberra. pp 177-200.

Keith DA (1996) Fire-driven extinction of plant populations: a synthesis of theory and review of the evidence from Australian vegetation. *Proceedings of the Linnean Society of New South Wales* 116, 376-378.

Knox K, Clarke PJ (2004) Effects of different fire regimes on shrubs in grassy woodlands. *Cunninghamia* 8, 348–353.

McKenzie N, Jacquier D, Isbell R, Brown K (2004) *Australian Soils and Landscapes*. CSIRO Publishing: Collingwood, Vic.

Moore N (2005) Role of fire on *Phytophthora cinnamomi* in the Stirling Range National Park, Western Australia. Honours Thesis, Murdoch University, Perth, Australia.

Neary DG, Klopatek CC, DeBano LF, Folliott PF (1999) Fire effects on below ground sustainability: a review and synthesis. *Forest Ecology Management* 122, 51-71.

Pearce G, Mullan AB, Salinger MJ, Opperman TW, Woods D, Moore JR (2005) Impact of climate variability and change on long-term fire danger. Report to the New Zealand Fire Service Commission, 75 pp.

Shearer BL, Crane CE, Fairman RG (2004) Phosphite reduces disease extension of a *Phytophthora cinnamomi* front in *Banksia* woodland, even after fire. *Australasian Plant Pathology* 33, 249-254.

Tabachnick BG, Fidell LS (1996) *Using Multivariate Statistics*, 3rd Ed. New York, USA: Harper Collins College Publishers.

Williams JE, Whelan RJ, Gill AM (1994) Fire and environmental heterogeneity in southern temperate forest ecosystems: implications for management. *Australian Journal Botany* 42, 125-137.

Wills RT (1993) The ecological impact of *Phytophthora cinnamomi* in the Stirling Range National Park, Western Australia. *Australian Journal of Ecology* 18, 145-159.

Wooller SJ, Wooller RD, Brown KL (2002) Regeneration by three species of *Banksia* on the south coast of Western Australia in relation to fire interval. *Australian Journal Botany* 50, 311-313.

The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated during flowering

Mixed *Banksia attenuata* and *B. menziesii* woodland (Photo: WA Dunstan)

Sub Project 19.2.3 The efficacy of phosphite to control *Phytophthora cinnamomi* in plants treated during flowering

INTRODUCTION

Numerous factors have been shown to influence the uptake and distribution of substances applied to plants (Sánches-Zamora and Fernández-Escobar 2000). These include technical application related factors, environmental conditions and stress conditions (Reil and Beutel 1976, Reil 1979). In addition, Whiley *et al.* (1995) have shown the phenological state of the plant is an important factor influencing the uptake and distribution of phosphite. Therefore, it is possible that the application of phosphite to native flora during flowering will result in the accumulation of phosphite in the shoots of plants leaving less phosphite available in the roots. If less phosphite is present in roots under optimum conditions for *Phytophthora cinnamomi* infection and colonisation, it is possible host defense responses will be less effective in containing the invading pathogen.

In southwest Western Australia phosphite is generally applied in autumn when many plants are not flowering (to minimise the possibility of any detrimental impacts on reproductive success), and when wind conditions are optimal and drift is minimal (Hardy *et al.* 2001). However, little is known on whether application at the time of flowering influences the efficacy of phosphite to control *P. cinnamomi*.

Banksia species produce many small flowers clustered densely together on a flowering spike (the inflorescence), which is followed by large woody "cones" in which the seeds are contained within closed follicles. Developing flowers and fruits draw mineral salts, sugars and amino acids from nearby leaves (Salisbury and Ross 1992). Given the large size of the *Banksia* reproductive inflorescences and cones it is possible their presence will influence the concentrations of phosphite available in other parts of the plants. Flowering, fruit production and seed set can represent a substantial stress on plants as large amounts of photosynthates are required for their production. Whiley *et al.* (1995) demonstrated that the accumulation of phosphite into avocado roots was directly related to sink strength of the shoots during fruit production. This resulted in phosphite accumulating in the fruit preferentially over roots at this time.

Banksia attenuata and *B. menziesii* are common components of woodlands and shrublands on the sandplains of south Western Australia. *B. attenuata* flowers and produces new shoots mainly in late spring and summer (October to January), while *B. menziesii* has a longer flowering period from early autumn to late winter, with new shoot growth also from late spring through to summer (Taylor and Hopper 1988). This opposite flowering season provides an opportunity to examine whether flowering has an effect on phosphite uptake which in turn may or may not induce host defense responses to contain *P. cinnamomi.*

AIM

Obtain information on whether phosphite application at the time of flowering influences the efficacy of phosphite to control *P. cinnamomi*.

OUTCOMES

This experiment will provide information for land managers on the best time for spraying phosphite in natural ecosystems in relation to reproductive growth. By studying lesion formation, caused by *P. cinnamomi*, in Banksia during flowering we will gain an understanding of how reproductive stress may impact on phosphite efficacy.

METHODS

Experimental design

Eighty *Banksia attenuata* and *B. menziesii* were used in a single block design including autumn and spring phosphite treatments, with incomplete randomisation of treatments. Complete randomisation was compromised by the need for access by a motorised spray unit, a requirement for buffers between trees of different treatments to avoid drift contamination of control trees from spraying and the possibility of transfer of phosphite between trees via root grafts. Phosphite was applied as a high volume foliar spray in autumn (April) or spring (October). The autumn spray coincided with *B. menziesii* flowering, while the spring spray coincided with *B. attenuata* flowering (Figure 66). There were ten trees per treatment, including untreated controls. Stems were inoculated with *P. cinnamomi* to determine the effectiveness of the phosphite treatment. A selection of leaves was collected for phosphite analysis at the conclusion of the experiment.

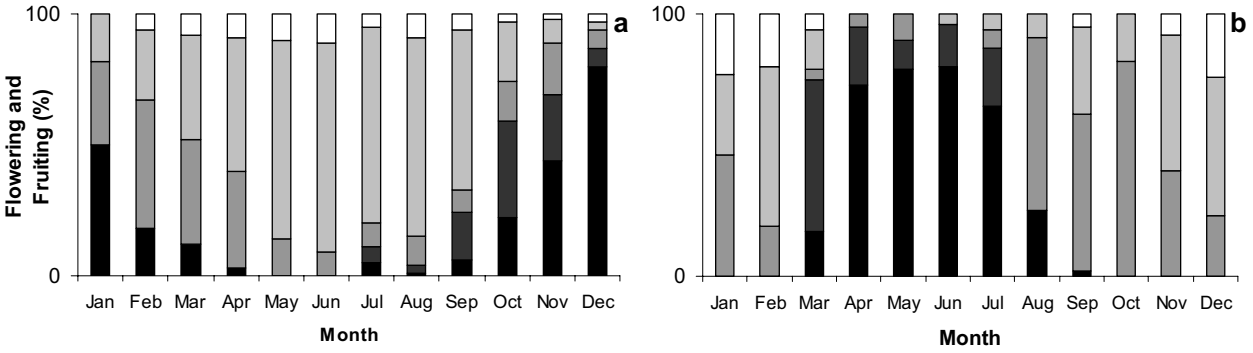


Figure 66 Flowering and fruiting in **a**) *Banksia attenuata* and **b**) *B. menziesii*. Majority of flowers fully open (■), majority of flowers in bud (■), majority of flowers recently finished (■), flowers finished and fruiting cones present (■), no flowers or fruiting cones present (□). (Reproduced from Taylor and Hopper 1988).

Site description

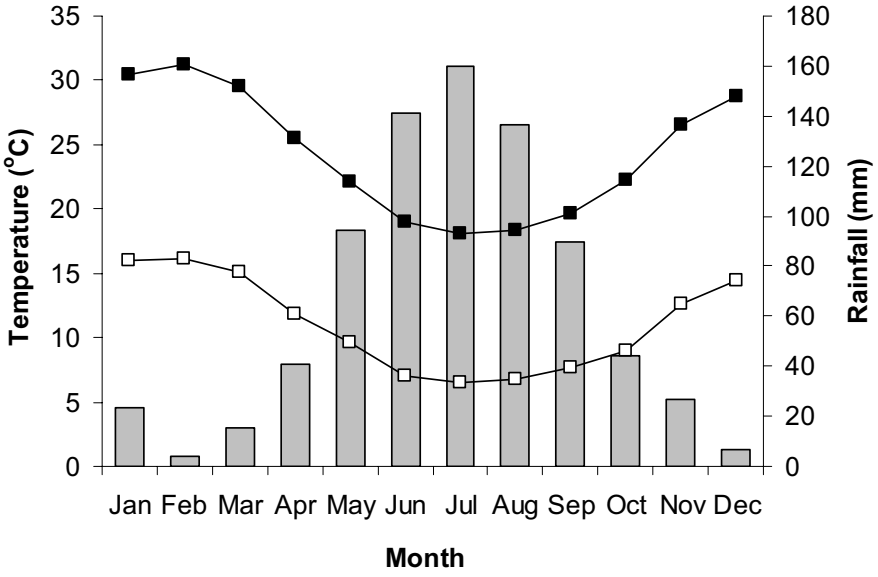
The experimental site was located at Jandakot (115° 53' 17" E, 32° 06' 08"S) on the Swan Coastal Plain, 20 km south of Perth, Western Australia. The soil is a deep white siliceous sand, and the vegetation is *Banksia* woodland over heath on the Bassendean complex with dominant species consisting of *Banksia attenuata*, *B. menziesii* and *B. ilicifolia*, with scattered occurrences of *Eucalyptus marginata*, *E. todtiana* and *Allocasuarina fraseriana* (Gibson *et al*. 1994).

Climate

Mean minimum and maximum temperature and monthly rainfall data for the years 1998 - 2007 are presented in Figure 67.

Figure 67

Mean monthly minimum (□) and maximum (■) air temperatures, and monthly rainfall (▩) for Jandakot experimental site, for the years 1998 - 2007.



Plant material

Banksia attenuata *Banksia menziesii*

B. attenuata and *B. menziesii* trees were a minimum of 30-years old, most being in an area unburnt since 1980, with a small number located in an area which underwent a mild control burn 15 years ago. Mean basal area at breast height (1.3 m) was 1339 cm^2 (sd = 1164) for *B. attenuata* and 1117 cm^2 (sd = 893) for *B. menziesii.*

Phosphite treatment

The plants were sprayed in autumn (April) or spring (October), with the autumn spray coinciding with the beginning of *B. menziesii* flowering and end of *B. attenuata* flowering, while the spring spray coincided with the beginning of *B. attenuata* flowering and end of *B. menziesii* flowering (Figure 66).

A high volume foliar spray was applied using a motorised spray unit. Trees were sprayed to runoff with 5 g/L ai phosphite plus 130 µL/L BS 1000® alcohol alkoxylate surfactant (Crop Care Australasia P/L, Murarrie, Queensland) and 0.5 mL/L Redye® marker dye (Crop Care Australasia P/L, Murarrie, Queensland). One branch from each sprayed tree

was covered with a 60 L plastic bag immediately prior to spraying and removed when the risk of phosphite contamination from treated foliage was negligible (1 - 3 h). Leaves from these branches were later removed for phosphite analysis.

Agri-fos 600 ® (600 g/L phosphorous acid, as mono- and di-potassium phosphonate; Agrichem Manufacturing Industries P/L, Loganholme, Queensland) was used in both phosphite spray events.

Inoculation
Plants were underbark inoculated with *P. cinnamomi* four weeks after each of the two phosphite treatments. Autumn inoculations of stems with *P. cinnamomi* were carried out *in situ*, while spring inoculations were in excised stems. Excised stems were used as a last resort due to restricted access to the site at that time of the year. Where stems were inoculated *ex situ*, 40 - 50 cm lengths of terminal shoots were removed, stripped of leaves and then the cut end sealed with candle wax. Excised stems were inoculated within 36 h. Three shoots per tree were underbark inoculated approximately 25 cm from the terminal bud with 6 mm diameter agar plugs colonised with *P. cinnamomi* isolate MP125, an isolate that has been shown to be pathogenic in previous experiments (Hüberli 1995). The inoculation point was wrapped with Parafilm and a single wrap of silver duct tape. Inoculated excised stems were incubated at 23ºC for 9 days.

Measurement of infection
The efficacy of the different treatments was assessed by measuring the colonisation of stems artificially inoculated with *P. cinnamomi*, after the methods described by Pilbeam *et al.* (2000). Fifteen stem sections (1 cm each; split longitudinally??) were plated onto NARPH selective medium (Hüberli *et al*. 2000) and incubated for up to 6 days. Confirmation of *P. cinnamomi* was made on hyphal morphology.

Phosphite analysis
The 10 youngest fully expanded leaves from four trees per species and treatment were harvested at the time of stem harvest, from stems that had been, or were later inoculated with *P. cinnamomi.* Leaves from trees that had received phosphite were removed from branches that had been covered with plastic bags at the time of spray application. Leaves were dried to constant weight at 37ºC, ground to 1 mm and sent to the WA Chemistry Centre (Perth) for phosphite analysis (see page 4).

Statistics
All statistical analyses were performed using the Statistica software V6.1 (Statsoft, Inc., Tulsa, OK, US).

Total colonisation length and leaf phosphite concentrations were treated as dependent variables in a multivariate analysis of variance (MANOVA), with independent variables of season of phosphite spray (autumn/spring), phosphite treatment (+/-) and species (*B. attenuata* and *B. menziesii*). All significant effects and interactions were further analysed by univariate analysis of variance (ANOVA). Leaf phosphite concentrations were $\log_{10}$ transformed to correct for correlation between means and variances across the cells of the design.

RESULTS

The stage of flowering and fruiting did not have a significant impact on phosphite efficacy, as both *Banksia* species, despite flowering in opposite seasons, responded similarly to the two spray and inoculation events. There was a significant ($p < 0.001$) phosphite treatment effect in the extent of colonisation by *P. cinnamomi* in the stems of *B. attenuata* and *B. menziesii* in both autumn and spring (Table 28), with phosphite significantly inhibiting colonisation in both seasons for both species (Figure 68). Spring inoculations led to more extensive colonisation for both phosphite treated and untreated plants of both species (Figure 68), but it is not possible to determine whether this was a consequence of different inoculation strategies or a true seasonal effect. The autumn phosphite spray reduced colonisation in *B. attenuata* by 71%, while the spring spray resulted in a 17% reduction. The same was observed in *B. menziesii* with a 60% reduction in colonisation after the autumn spray, but only a 30% reduction after the spring spray. There were no significant interaction effects between application season, phosphite treatment or species.

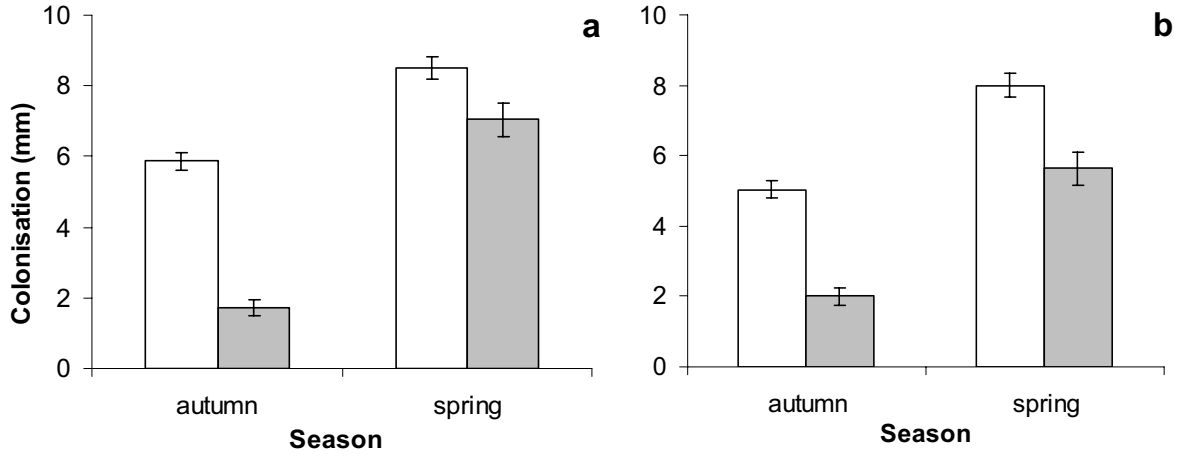


Figure 68 Mean colonisation of stems underbark inoculated with *Phytophthora cinnamomi* in **a**) *Banksia attenuata* and **b**) *B. menziesii* in autumn and spring with no spray treatment (□) and 4 weeks after application of phosphite (▣). Vertical bars represent two standard errors of the mean.

There was a significant ($p < 0.001$) phosphite treatment effect for leaf phosphite concentrations of *B. attenuata* and *B. menziesii* in both autumn and spring (Table 28), with elevated phosphite levels in treated plants (Figure 69). There was also a significant ($p < 0.05$) interaction between phosphite treatment and species (Table 28), with leaf phosphite concentrations being higher in *B. attenuata* than *B. menziesii* (Figure 69). Flowering did not affect phosphite uptake as there was no season main effect or interaction of season effect on phosphite levels (Table 28).

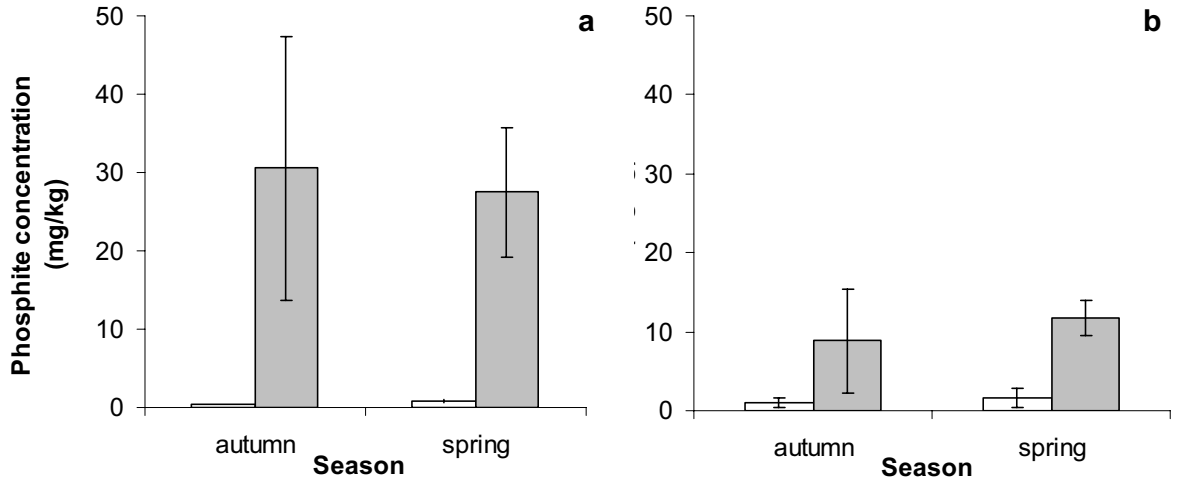


Figure 69 Leaf phosphite concentrations in **a**) *Banksia attenuata* and **b**) *B. menziesii* in autumn and spring with no treatment (□) and 4 weeks after application of 0.5% phosphite (▣). Vertical bars represent two standard errors of the mean.

Table 28 Results of univariate ANOVA tests of colonisation length and leaf phosphite concentrations of *Banksia attenuata* and *B. menziesii* after underbark inoculation of stems with *Phytophthora cinnamomi* 4 weeks after plants were sprayed with phosphite in spring or autumn. Significant effects and interactions ($p < 0.05$) are in bold.

Effect		Colonisation		Leaf phosphite	
	Df	F	p	F	p
Application Season (spring/autumn)	1, 20	*	*	1.63	0.22
Phosphite (+/-)	1, 20	26.46	**<0.001**	78.51	**<0.001**
Species (*B. attenuata*, *B. menziesii*)	1, 20	0.62	0.44	1.45	0.24
Application Season, Phosphite	1, 20	1.30	0.27	0.02	0.89
Application Season, Species	1, 20	0.27	0.61	0.26	0.62
Phosphite, Species	1, 20	0.55	0.47	4.92	**0.04**
Application Season, Phosphite, Species	1, 20	1.01	0.33	0.40	0.53

* unable to be determined

DISCUSSION

This study demonstrated that phosphite treatment of *B. attenuata* and *B. menziesii*, regardless of timing of the application with respect to flowering, was effective in controlling stem colonisation by *P. cinnamomi* 4 weeks after the phosphite treatment. The best control was obtained for the autumn applications, though non-treated controls also exhibited more extensive colonisation in spring. This seasonal effect was not significant for leaf phosphite concentrations, suggesting that the differences observed were

potentially due to more favourable climatic conditions for colonisation during the spring inoculation, when conditions are warm and moist and plants are not water stressed. Alternatively, the seasonal effect on colonisation may be a result of the two different methods of inoculation. A direct comparison between the colonisation results for the two seasons is therefore inappropriate. There is some evidence that spraying phosphite on plants at anthesis can reduce pollen fertility, but while this may be a problem in annuals, it should not adversely affect long-lived perennials that are not sprayed each year (Fairbanks *et al*. 2001, 2002a, b).

The differing leaf phosphite concentrations after spraying *B. attenuata* and *B. menziesii* is consistent with observations by Tynan *et al.* (2001) that plant species within and between plant communities vary considerably in their ability to take up and retain phosphite. While Tynan *et al.* (2001) have suggested that host factors, including growth stage, will influence the efficacy of phosphite, the ability of the pathogen to invade and the host's ability to respond, the current study demonstrated that application at the time of flowering did not influence the efficacy of phosphite to control *P. cinnamomi* in *B. attenuata* and *B. menziesii*.

This study indicates that the application of phosphite at the time of flowering does not influence the efficacy of phosphite to control *P. cinnamomi* in *Banksia* species. Since *Banksia* species tend to have larger flowers, fruits and seeds than most other native plant species, it is likely that flowering in other species will also not impact upon phosphite efficacy. However, additional research is required to confirm this assumption.

REFERENCES

Fairbanks M, Hardy G, McComb J (2002a) Mitosis and meiosis in plants are affected by the fungicide phosphite. *Australasian Plant Pathology* 31, 281-289.

Fairbanks M, Hardy G, McComb J (2002b) The effect of phosphite on pollen fertility of perennial species of the *Eucalyptus marginata* forest and northern sandplains of Western Australia. *Australian Journal of Botany* 50, 763-779.

Fairbanks M, McComb JA, Hardy G (2001) The effect of phosphite on the sexual reproduction of some annual species of the *Eucalyptus marginata* forest of south-west Western Australia. *Sexual Plant Reproduction* 13, 315-321.

Gibson N, Keighery BJ, Keighery GJ, Burbidge AH, Lyons MN (1994) A Floristic survey of the Southern Swan Coastal Plain. Report for the Australian Heritage Commission prepared by the Department of Conservation and Land Management and the Conservation Council of Western Australia (Inc), Perth.

Hardy GEStJ, Barrett S, Shearer BL (2001) The future of phosphite as a fungicide to control the soilborne plant pathogen *Phytophthora cinnamomi* in natural ecosystems. *Australasian Plant Pathology* 30, 133–139.

Hüberli D (1995) Analysis of variability among isolates of *Phytophthora cinnamomi* Rands from *Eucalyptus marginata* Donn ex Sm. and *Eucalyptus calophylla* R. Br. based on cultural characteristics, sporangia and gametangia morphology, and pathogenicity. Honours Thesis, Murdoch University, Perth.

Hüberli D, Tommerup IC, Hardy GEStJ (2000) False-negative isolations or absence of lesions may cause mis-diagnosis of diseased plants infected with *Phytophthora cinnamomi. Australasian Plant Pathology* 29, 164-169.

Pilbeam RA, Colquhoun IJ, Shearer BL, Hardy GEStJ (2000) Phosphite concentration: its effect on phytotoxicity symptoms and colonization by *Phytophthora cinnamomi* in three understorey species of *Eucalyptus marginata* forest. *Australasian Plant Pathology* 29, 86-95.

Reil WO (1979) Pressure-injecting chemicals into trees. *California Agriculture* 33, 16-19.

Reil WO, Beutel JA (1976) A pressure machine for injecting trees. *California Agriculture* 30, 4-5.

Salisbury FB, Ross CW (1992) Plant Physiology. 4th Edition. Wadsworth Publishing Company, California.

Sánches-Zamora MA, Fernández-Escobar R (2000) Injector-size and the time of application affects uptake of tree trunk-injected solutions. *Scientia Horticulturae* 84, 163-177.

Taylor A, Hopper S (1988) Australian Flora and Fauna series no 8. The Banksia Atlas. Bureau of Flora and Fauna, Canberra and the Department of Conservation and Land Management, Western Australia. Australian Government Publishing Service, Canberra.

Tynan KM, Wilkinson CJ, Holmes JM, Dell B, Colquhoun IJ, McComb JA, Hardy GEStJ (2001) The long-term ability of phosphite to control *Phytophthora cinnamomi* in two native plant communities of Western Australia. *Australian Journal of Botany* 49, 761-770.

Whiley AW, Hargreaves PA, Pegg KG, Doogan VJ, Ruddle LJ, Saranah JB, Langdon PW (1995) Changing sink strengths influence translocation of phosphonate in avocado (*Persea americana* Mill.) trees. *Australian Journal of Agricultural Research* 46, 1079-1090.

APPENDIX I

Publications arising from this research

Poster for 9th International Congress of Plant Pathology, Torino, Italy. August 24-29, 2008.

DOES WATERLOGGING INFLUENCE PHOSPHITE PROTECTION OF *BANKSIA* SPECIES AGAINST *PHYTOPHTHORA CINNAMOMI*?

D. Hüberli, T. Paap, K. Gower, N. Long, B. Dell and G. E. StJ. Hardy

Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, WA 6150, Australia

Parts of the southwest of Western Australia are subject to periodic flooding in areas that are also devastated by Phytophthora dieback disease caused by *P. cinnamomi*. Phosphite has been shown to be effective in controlling this pathogen. Waterlogging induces multiple physiological dysfunctions in plants, but it is unknown whether waterlogging alters the uptake, distribution and efficacy of phosphite in controlling *P. cinnamomi*. Waterlogging trials were conducted in the greenhouse using *Banksia attenuata* and *B. baxteri*. The response of these plants and subsequent recovery from waterlogging was examined. A phosphite spray treatment was applied pre- and post-waterlogging of either 3 or 14 days duration. Leaf gas exchange, leaf water potentials, lesion development and phosphite concentrations in leaf, stem and root tissue were monitored 1 week, 1 month and 4 months after the phosphite treatment. For the 1 week harvest when phosphite was applied pre-waterlogging, phosphite in plant tissue was at similar levels for each species and was not affected by waterlogging. But lesions on *B. baxteri* stems were not reduced in treated plants as they were for *B. attenuata*. Photosynthesis and water potentials were reduced for waterlogged *B. attenuata*, but had no impact on waterlogged *B. baxteri*. Leaf water potentials, leaf gas exchange, lesion lengths on inoculated stems, and phosphite concentration in leaves, stems, and roots measured at different time periods after waterlogging will be presented.

Poster for 9th International Congress of Plant Pathology, Torino, Italy. August 24-29, 2008.

DOES FIRE INFLUENCE PHOSPHITE PROTECTION OF WESTERN AUSTRALIAN INDIGENOUS PLANT SPECIES AGAINST *PHYTOPHTHORA CINNAMOMI*?

D. Hüberli, T. Paap, N.A. Moore, S. Barrett, G. Freebury, B. Dell and G.E.St.J. Hardy

Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, WA 6150, Australia

Large areas of indigenous forests, and *Banksia* woodlands and heathlands in Australia are devastated by Phytophthora dieback caused by *P. cinnamomi*. Phosphite has been shown to be effective in controlling this pathogen on a wide range of plant species across different families. Although fire is a regular event in the Australian landscape and plays key roles in the ecosystem, nothing is known about the relative uptake of phosphite by shoots pre- and post-fire or how fire may alter the redistribution and persistence of phosphite within woody plants. *Adenanthos cuneatus* (re-sprouter), *Banksia attenuata* (re-sprouter) and *B. baueri* (re-seeder) are all susceptible to *P. cinnamomi* and are responsive to phosphite treatment. These species were selected within four plots in an area of the Stirling Range National Park that was scheduled for a fuel-reduction burn in November 2006. Treatments of the plots were: 1) phosphite spray without fire, 2) phosphite spray with fire, 3) no phosphite spray without fire, and 4) no phosphite spray with fire. A phosphite treatment was applied either 6 weeks pre-fire or 9 months post-fire when all re-sprouter species had sufficient foliage. Leaf water potentials, leaf gas exchange, lesion lengths on inoculated stems, and phosphite concentration in leaves, stems, lignotubers and roots measured periodically throughout the experiment will be presented.

Reference
Hüberli, D., Paap, T., Moore, N.A., Gower, K., Long, N., Barrett, S., Freebury, G., Spadek, T., Dell, B., Hardy, G. (accepted December 2007) Does abiotic stress on a plant influence phosphite protection to *Phytophthora cinnamomi*? In: Progress in Research on Phytophthora Diseases of Forest Trees. Proceedings of the Fourth International IUFRO Working Party S07.02.09 Meeting in Monterey, California, 26-21 August 2007. Eds E. Goheen, S. Frankel and K. Palmieri. USDA, Albany, California, US. *In press.*

DOES ABIOTIC STRESS ON A PLANT INFLUENCE PHOSPHITE PROTECTION TO *PHYTOPHTHORA CINNAMOMI*?

Daniel Hüberli[1], Nicole A. Moore[1,2], Nathalie Long[1,3], Karyn Gower[1], Sarah Barrett[2], Greg Freebury[2], Bernard Dell[1], and Giles E St J Hardy[1]

1Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA 6150, Australia
2Nature Conservation Section, Dept of Environment and Conservation, 120 Albany Highway, Albany, WA 6330, Australia
3 Université des sciences Montpellier II, Place Eugène Bataillon, 34 095 Montpellier Cedex 5, France

Abstract
In Australia large areas of indigenous forests, Banksia woodlands and heathlands, are devastated by Phytophthora dieback disease caused by *Phytophthora cinnamomi*. Phosphite has been shown to be effective in controlling this pathogen's impact on a wide range of plant species across different families. But the influence of a plant's physiological status at the time of phosphite application on the subsequent efficacy of phosphite treatment to control Phytophthora dieback disease is a major factor limiting our understanding of the control of this pathogen. The key seasonal stresses in an Australian ecosystem of flooding, drought and fire are explored.

Adenanthos cuneatus (resprouter), *Banksia attenuata* (resprouter) and *B. baueri* (reseeder) are all susceptible to *P. cinnamomi* and are responsive to phosphite treatment. These species were selected within 4 plots in an area of the Stirling Range National Park that was scheduled for a fuel-reduction burn. Treatments of the plots were: 1) phosphite spray without fire, 2) phosphite spay with fire, 3) no phosphite spray without fire, and 4) no phosphite spray with fire. Phosphite treatment was applied either 6 weeks prior to, or after the fire when all resprouter species had foliage. On-going measurements during the experiment include leaf water potential, leaf gas exchange, lesion lengths on inoculated stems, and phosphite concentration in leaves, stems, lignotubers and roots. Waterlogging trials were conducted in the greenhouse using *B. attenuata* and *B. baxteri* (reseeder). The response of these plants and subsequent recovery from waterlogging was examined. In the main trial, a phosphite spray treatment was applied before and after one waterlogging event of either 3 or 14 days duration. Plant physiology traits, lesion development and phosphite concentrations in plant tissue were monitored periodically similar to the fire experiment.

The effect of water deficit will be examined on *B. attenuata* and *B. baxteri* in the greenhouse and in *Banksia* woodlands. Measurements as for the fire experiment will be completed.

Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia.

Tender Number: 19/2005DEH Sub Project 19.2.3 30 May 2008

Submitted to Australian Journal of Botany in March 2008

PHYSIOLOGICAL RESPONSES OF *BANKSIA* SPECIES DURING AND AFTER WATERLOGGING

Karyn Gower[A], Nathalie Long[AB], Kay Howard[A], Michael Calver[A], Daniel Hüberli[AC], Giles Hardy[A], Bernard Dell[A]

[A]Centre for Phytophthora Science and Management, School of Biological Sciences and Biotechnology, Murdoch University, Perth, WA 6150, Australia
[B]Université des sciences Montpellier II, Place Eugène Bataillon, 34 095 Montpellier Cedex 5, France.
[C]*Corresponding author:* D.Huberli@murdoch.edu.au

Abstract

Many species of *Banksia* occur where winter rainfall can result in seasonal waterlogging and exceptional summer rainfall events cause periods of inundation over large areas. However, the effect of waterlogging on the physiology of *Banksia* seedlings is unknown, even though when screening *Banksia* spp. for resistance to *Phytophthora* waterlogging is often used to establish infection. To examine physiological responses during water stress, seedlings of *Banksia attenuata* and *B. baxteri* were waterlogged for 8 or 21 days. *B. attenuata* was more sensitive to waterlogging than *B. baxteri* as photochemical yield, water potential, transpiration, photosynthesis and leaf stomatal conductance declined rapidly and some deaths occurred.

In a second experiment, *B. baxteri, B. grandis* and *B. littoralis* were waterlogged for 3 or 21 days. Waterlogging reduced stomatal conductance, photosynthesis and transpiration rates, and shoot and root growth of *B. baxteri* and *B. grandis*, and leaf water potentials indicated severe water stress after 21 days. Lack of water stress, continuance of some photosynthesis and low mortality indicate these two species could survive and recover from short-term waterlogging but are intolerant of extended waterlogging periods. Growth of *B. littoralis* was unaffected, and this species had higher rates of gas exchange and net photosynthesis that were also unaffected by waterlogging. Differences in plant stress responses to waterlogging needs to be considered when flooded or hypoxic conditions occur, or whilst screening containerised seedlings for resistance to *Phytophthora.*

Research into natural and induced resistance in Australian native vegetation of *Phytophthora cinnamomi* and innovative methods to contain and/or eradicate within localised incursions in areas of high biodiversity in Australia.

Tender Number: 19/2005DEH Sub Project 19.2.3 30 May 2008

Submitted to Plant Pathology in May 2008

SHORT-TERM WATER DEFICIT STRESS DOES NOT AFFECT THE ABILITY OF PHOSPHITE TO CONTROL *PHYTOPHTHORA CINNAMOMI* IN *BANKSIA* SPECIES

D. Hüberli[A], T. Paap[A], K. Howard[A], T. Spadek[B], M. Calver[A], B. Dell[A] and G.E.St.J. Hardy[A]

[A]*Centre for Phytophthora Science and Management, Faculty of Sustainability, Environmental and Life Sciences, Murdoch University, Perth, WA 6150, Australia*
[B]*Chemistry Centre, 125 Hay Street, East Perth, WA 6004, Australia*

Abstract
Water deficit due to prolonged drought, seasonal dry soil, or air saturation deficit is a feature of Australian vegetation in Mediterranean-type regions. These conditions follow or precede a wet season where soil moisture and temperature are suitable for *P. cinnamomi* to complete its life cycle and infect plants. Phosphite has been used to control the pathogen in indigenous plant communities of high conservation value. Many of these communities require treatment at a time when plants may be experiencing water deficit stress (WDS). The efficacy of phosphite applied to *Banksia attenuata* and *B. baxteri* plants subjected to five days of WDS in the glasshouse was tested. Phosphite was applied 7 days pre-WDS, during wilting point or 7 days post-WDS. Predawn leaf water potentials were significantly lower for WDS plants than non-WDS plants, 4 days after WDS. Photosynthesis, transpiration and stomatal conductance were measured daily during and 5 days into recovery after the WDS. During WDS, leaf gas exchange declined with a delayed response to the water deficit by *B. baxteri* compared to *B. attenuata*. Plants sprayed pre- or during WDS had higher phosphite concentrations than those sprayed post-WDS and concentrations were generally higher in *B. attenuata* than *B. baxteri*. Phosphite reduced stem colonisation by *P. cinnamomi* inoculated 4 weeks after phosphite application in all treatments, with the largest reduction when applied pre- or during WDS. This study confirms that short-term WDS does not impair the uptake and translocation of phosphite in two *Banksia* spp. or its ability to contain *P. cinnamomi.*

APPENDIX II

Addressing the Aims and Outcomes of Sub Project 19.2.3

AIMS

To provide managers with improved operational guidelines for the use of phosphite to control *Phytophthora cinnamomi* in the conservation estate by obtaining information on:

1. How abiotic stresses such as drought and waterlogging at the time of phosphite application influence the efficacy of phosphite to control the pathogen.
Glasshouse experiments examined whether the efficacy of phosphite in controlling *P. cinnamomi* changed if applied before, during or after waterlogging or water deficit events. Waterlogging did not have long-term detrimental effects on the ability of phosphite to induce host defense responses in *Banksia attenuata* and *B. baxteri,* as *P. cinnamomi* colonisation was as effectively contained as in non-waterlogged plants. Drought did not impair the uptake and translocation of phosphite when applied before, during or after short-term water deficit in the two *Banksia* species, as stem colonisation by *P. cinnamomi* was reduced after all phosphite applications.

2. How the fire history at the time of phosphite application influences the efficacy of phosphite to control the pathogen.
Field experiments tested whether the application pre- or post-fire affected phosphite's ability to contain *P. cinnamomi* in three members of the Proteaceae. For *B. attenuata*, the results clearly indicate phosphite should be applied at least 2 months before a burn to allow it to be effectively taken up by the plant and distributed throughout the tissues of this resprouting species. The plants are able to recover post-fire and retain sufficient phosphite *in planta* to respond effectively to challenge by *P. cinnamomi.* In contrast, the results clearly indicate that when phosphite is applied 11 months post-fire it is not taken up in sufficient quantities to effectively contain *P. cinnamomi* when challenged.

For *Adenanthos cuneatu*s, another resprouter species, phosphite was not able to control *P. cinnamomi* irrespective of whether it was applied before or after a fire, even with high phosphite concentrations being present in the plant tissues. Fire apparently inactivates the ability of phosphite to induce plant defense responses in *A. cuneatus* when challenged by *P. cinnamomi.* This response occurs beyond 11 months post-fire.

Therefore, our data show conflicting results with regards to resprouter species in terms of managing fire events alongside phosphite and *P. cinnamomi* management. Clearly, there are complex interactions occurring between different host species with regards to how they

respond physiologically to fire, phosphite and subsequent challenge by *P. cinnamomi.* Additional studies on host x fire x phosphite x *P. cinnamomi* interactions are required on a broader range of species before we can confidently manage *P. cinnamomi* in natural ecosystems.

Fire kills reseeder species such as *B. baueri* used in the present study. Further work is required to determine how soon after germinating reseeders can be sprayed to effectively protect them from *P. cinnamomi*.

3. How reproductive growth at the time of phosphite application influences the efficacy of phosphite to control the pathogen.

A field study was conducted on *B. attenuata* and *B. menziesii,* the former starts its flowering in spring and ends in autumn, whilst the latter starts to flower in autumn and ends in spring. The results clearly showed that the stage of flowering and fruiting had no impact on phosphite efficacy, as both *Banksia* species, despite flowering in opposite seasons, responded similarly to the two spray and inoculation events. In both cases, *P. cinnamomi* was contained by the hosts after phosphite treatment.

OUTCOMES

1. Information for managers on the best time for spraying of phosphite in natural ecosystems.

It is recommended that phosphite be applied after a flooding event where there is a risk of disease by *P. cinnamomi* and plants have not been protected by phosphite application previously. However, it should not be applied until at least 1-2 weeks after the flooding event to allow plants to recover from water stress otherwise phosphite effectiveness may be reduced. It is advisable to protect susceptible and threatened plant species and plant communities from *P. cinnamomi* by being proactive and applying phosphite prior to high risk flooding events.

In plants previously treated with phosphite, phosphite levels in susceptible flora in drought-prone areas should be maintained during extended periods of drought. However, if applying phosphite to plant communities, then applications should be timed so that the level of drought stress in the plant is minimal when spraying, in order to ensure optimal uptake and distribution of phosphite through the plant. This study only examined two *Banksia* species, and recommendations need to be applied with caution to flora that respond differently to drought stress.

With regards to prescribed fire management, managers should apply phosphite to plant communities which are under threat from *P. cinnamomi* and due to be burnt at least two months prior to the burn. This should 'capture' and protect those species that respond to phosphite after fire. With regards to those species that take up phosphite but do not

respond to its presence when challenged by *P. cinnamomi*, further research is required to determine how long after the burn phosphite applications become effective. Post-fire, susceptible reseeders will have no phosphite present in their tissues, and further work is required to determine how soon after germinating they can be sprayed to effectively protect them from *P. cinnamomi*.

Phosphite can be applied to plants during flowering without reducing its efficacy to control *P. cinnamomi.*

Reapplication of phosphite every three to five years is the time-period currently considered optimal for effective and sustained control of *P. cinnamomi* in plant communities.

2. An understanding of how stress from fire, drought and waterlogging impacts on phosphite efficiency over time will provide managers with the tools of when to apply or reapply phosphite after a stress event.

Although waterlogging altered *B. attenuata* leaf physiology (with markedly reduced stomatal aperture, slowed photosynthesis and transpiration), and leaf function had not recovered to unstressed rates by the time phosphite was applied, the uptake and distribution of phosphite in the plant was not affected. There was no effect of waterlogging on uptake and distribution of phosphite in *B. baxteri*, which was less sensitive to waterlogging, with stomatal function maintained and gas exchange unimpaired. Plants sprayed 1 week prior to waterlogging do not need reapplication after the waterlogging stress event, as phosphite remains effective in containing *P. cinnamomi*. Phosphite applied 1 week after the waterlogging event had the ability to induce host defense responses, though this protection was not immediate, with *P. cinnamomi* colonisation in *B. attenuata* more extensive 2 - 5 weeks post-waterlogging than non-waterlogged controls, however, after 27 weeks post-waterlogging, colonisation was as effectively contained as in non-waterlogged plants. Therefore, plants that have been subjected to waterlogging can effectively take up phosphite if applied shortly after the waterlogging event, but still need a recovery period beyond 5 weeks before host-defense mechanisms in response to challenge by *P. cinnamomi* return to normal.

Short term water deficit did not impair the uptake and translocation of phosphite, despite water deficit stress resulting in a decline in each of the leaf gas exchange measurements. Phosphite applications before, during and after water deficit all reduced stem colonisation by *P. cinnamomi*. This study only examined two *Banksia* species, and recommendations need to be applied with caution to flora that respond differently to drought stress.

Vegetation consisting of species where pre-fire phosphite applications remain effective after a fire, eg *B. attenuata* do not require reapplication of phosphite immediately after a fire event. When phosphite was applied 11 months post-fire it was not effective in controlling *P. cinnamomi*, so applications at different times post-fire need to be investigated to determine when phosphite becomes effective in resprouters after fire. After

fire, reseeding species grow rapidly, during which stems and leaves will be acting as a photosynthate sink, and accumulating phosphite. Consequently, it is expected that rapidly growing reseeder species will need more regular applications of phosphite than resprouter species, however, the application of phosphite to reseeder species after fire needs to be studied further.

3. Cost saving by avoiding spraying at times when plants are physiologically unable to take up phosphite effectively.
Do not spray within 1 week of a flooding event to allow plants to recover from water stress, otherwise phosphite effectiveness may be reduced.

Drought did not impair the uptake and translocation of phosphite, though application should be timed so that the level of drought stress in the plant is minimal when spraying.

Do not spray less than 2 months before a scheduled prescribed burn. We would not recommend an aerial spray of diseased sites within 12 months after a prescribed burn or wildfire event.

4. An understanding of the most important plant physiological parameters that impact on uptake and transport of phosphite, and the response of treated plants to *P. cinnamomi*.
Reduced stomatal conductance as a result of stress events, such as observerd in our waterlogging and water deficit experiments, did not have a negative impact on a plant's ability to uptake phosphite. Our experiments suggest that the main pathway for phosphite uptake by leaves is likely to be via the epidermis, with uptake via the stomata likely to be minor. There was evidence to indicate (in *B. attenuata*), that while phosphite applied soon after a waterlogging event was taken up, the plant defense systems did not become fully functional until the plants had physiologically recovered from the waterlogging event.

B. baxteri was less responsive to water deficit stress, with no observed changes in photosynthesis, transpiration and stomatal conductance until after 2 days, whilst the response of *B. attenuata* was immediate. Also, leaf water potentials were significantly higher for *B. baxteri* than *B. attenuata*. Despite this, sufficient phosphite was taken up by both species to induce host-defense responses to effectively contain *P. cinnamomi.* Further work on long-term water deficit stress on physiological parameters and their impact on phosphite uptake and translocation is required.

Photosynthesis in *B. attenuata* increased post-fire, possibly due to resprouting foliage having greater photosynthetic capacity than the foliage produced pre-fire. Observed differences in leaf physiology between the species are unlikely to impact severely on either the uptake or long-distance transport of phosphite in the plants studied. Phosphite treatments were not effective in controlling colonisation in *A. cuneatus* in the burnt plots, irrespective of whether they were applied pre- or post-fire. It appears that phosphite, whilst present in the tissue in high concentrations, is unable to induce host defense

mechanisms after fire and cannot control a challenge inoculation with the pathogen. Further research is required to address this question and to determine how many other susceptible resprouter species respond in a similar fashion.

5. An understanding of how reseeders and resprouters mobilise phosphite across seasons will provide managers with the knowledge of how frequently and when rare or threatened reseeders or resprouting species should be treated with phosphite.
In resprouter plants treated with phosphite prior to fire there is a trend for phosphite to increase in stems and leaves with time after fire, an accumulation that does not happen in non-burnt plants. Further work on a larger range of susceptible species across susceptible families is required to test whether fire results in the redistribution of phosphite, from old to new grow in resprouter species, due to rapid shoot growth acting as a sink for photosynthates. Whether this redistribution of phosphite to the canopy will reduce the time period in which phosphite is active in roots, resulting in plants becoming susceptible to the pathogen sooner than in un-burnt plants, should be examined. These plants should be selected based on their susceptibility and rarity.

Further research should determine why phosphite did not contain *P. cinnamomi* colonisation in *A. cuneatus* post-fire, despite having higher phosphite concentrations in the stems than prior to fire.

Further work is required to determine how soon after germinating reseeders can be sprayed to effectively protect them from *P. cinnamomi*.

6. Wildfires are not planned, yet frequently occur in communities that have been treated with phosphite. It is always a dilemma to managers with limited resources of whether phosphite should be reapplied to a burnt community to protect it from *P. cinnamomi*. This project will provide the knowledge on what should be done.
In species where pre-fire phosphite applications remain effective after a fire, eg *B. attenuata*, reapplication of phosphite immediately after a fire event is not required. Phosphite applied 11 months post-fire was not effective in controlling *P. cinnamomi*. Future work must determine when phosphite becomes effective in resprouters after fire. The application of phosphite to reseeder species after fire needs to be studied further. Our prescribed burn was relatively patchy and varied largely in intensity. A wildfire would have been more intense than the controlled fire for the current study, it remains to be determined if intensity of fire affects phosphite uptake.

7. Provide knowledge on how *P. cinnamomi* colonisation is influenced by abiotic stresses in plants. We have very little knowledge on how *P. cinnamomi* responds *in planta* to changes in plant physiological status due to stress.
Water deficit did not have a significant effect on the extent of colonisation of *B. attenuata* or *B. baxteri* by *P. cinnamomi.* At the time of inoculation, the physiological parameters had

returned to levels of the non-stressed plants. However, physiological parameters of stressed plants were at less than half of non-stressed plants at the time of phosphite application during water deficit stress. Despite this, phosphite was able to contain the pathogen in the stems.

Waterlogging did not have a significant effect on the extent of colonisation of *B. attenuata* or *B. baxteri* by *P. cinnamomi.* At the time of inoculation, the physiological parameters had returned to levels of the non-stressed plants. However, physiological parameters of stressed *B. attenuata* were at less than half of non-stressed plants at the time of phosphite application during water deficit stress. Despite this, phosphite uptake was not reduced and it was able to contain the pathogen in the stems.

Eleven to 12 months after fire, resprouted *B. attenuata* and *B. baxteri* had significantly greater colonisation by *P. cinnamomi* than in non-burnt control plants.