SCOPE ITEM 4

REFINEMENT OF TECHNIQUES AND IDENTIFICATION OF RESOURCES FOR THE LONG TERM CONTROL OF *PHYTOPHTHORA* WITH PHOSPHONATE

PART A

EFFECTS OF PHOSPHONATE CONCENTRATION AND APPLICATION FREQUENCY ON THE DURATION OF CONTROL

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1 INTRODUCTION

At least eight species of *Phytophthora* are present in native vegetation in the South West Land Division (SWLD) of Western Australia. The most common and destructive of these species is *Phytophthora cinnamomi* Rands, the cause of epidemic dieback in the jarrah forest (Podger *et al.*, 1965) and in woodland and heath communities of the SWLD (Shearer & Fairman, 1991). The pathogen has a discontinuous distribution in the area bounded by Eneabba, 250km north of Perth, inland beyond Dryandra near Popanyinning and on the coastal plains as far east as Cape Arid (Shearer, 1994).

P. cinnamomi is a member of the Oomycetes or water moulds (Shearer & Tippett, 1989), a group that is generally regarded as primitive in an evolutionary sense. The fungus requires warm, moist conditions for asexual spore production, dispersal of spores and infection. In the presence of susceptible plant tissue, it produces sporangia that release motile zoospores which are transported in free water, often in sub-surface flow. The spores may also be spread in infected soil. A third mode of fungal transmission is through direct root contact between susceptible hosts. Although *P. cinnamomi* produces oospores, only one mating type (A_2) is usually detectable in native plant communities in the SWLD. Thus, sexual reproduction is probably of limited importance as a source of inoculum

1.1 DISEASE IMPACT

P. cinnamomi attacks a broad range of hosts. Susceptible species occur mainly in the Proteaceae, Myrtaceae, Papilionaceae, Epacridaceae and Dilleniaceae, but members of

other families are also affected. The pathogen kills the host by rotting its roots and girdling the stem base, thus depriving the plant of water and nutrients (Shearer, 1994). As the degree of root and/or stem necrosis increases, a range of symptoms appear including leaf chlorosis and crown decline. Patches of apparently healthy plants may rapidly die, and well defined infection fronts are frequently present in affected areas. The impact that dieback disease may have in a particular situation is influenced by the type of vegetation present as well as geographic location, climate, soil type and site factors.

P. cinnamomi severely affects many species of *Banksia*, including *B. coccinea*, *B. baxteri* and *B. hookeriana*. It also poses a significant threat to a number of Declared Rare Flora with some taxa now considered at risk of extinction due to the activity of the pathogen (Keighery, 1992). It is estimated that more than 2000 plant species are at risk in parts of the SWLD (Shea, 1991) where dieback is out of control in a range of plant communities. Mortality of dominant plant species is common in affected areas of vegetation. This often leads to reduction in structural complexity and conversion of diverse heaths, shrublands or woodlands into relatively simple communities consisting mainly of sedges and grasses.

The changes to vegetation associated with dieback disease influence the abundance and species-richness of native fauna. Reduced canopy cover and diminished plant diversity decrease the availability of shelter, nectar, pollen and seed sources necessary for survival of numerous small marsupials, birds or insects. Nichols & Watkins (1984) reported that by comparison with disease-free sites, the number of birds as well as bird species declined in dieback-affected forest. Dieback disease currently poses the greatest threat to conservation of native communities in Western Australia.

1.2 DISEASE CONTROL

In Western Australia, strategies for the control of *P. cinnamomi* in native plant communities have been directed towards prevention or restriction of spread of the fungus in order to protect conservation or economic values (Shearer & Tippett, 1989). These methods include recognition of vulnerable sites, risk assessment relating to introduction or spread of *P. cinnamomi* in a given area, and application of quarantine. In addition, hygiene procedures have been developed for the public and for industries to assist the protection of large areas of healthy vegetation from infection by the pathogen. When well integrated, the various strategies have had some degree of success, but they are regarded only as an interim solution until better methods can be developed.

1.2.1 Phosphonate - The Fungicide

Phosphonate is an aqueous solution of mono- and di-potassium phosphite. It is produced by neutralisation of phosphonic acid with potassium hydroxide in the pH range of 5.7-6.0. The active component of the chemical is the phosphite ion. The fungicide is sold under various brand names and strengths of 20% or 40%. It is manufactured by several companies including UIM, CIBA and RHONE POULENC. Products used by the Department of Conservation and Land Management (CALM)

were made by UIM. These include FOS-JECT 200 and FOLI-R-FOS 400 (also sold as FOS-ACID), in which mono-di-potassium phosphite is present at concentrations of 200g ℓ^{-1} and 400g ℓ^{-1}, respectively.

The fungicide has been referred to in various publications as "phosphonate", "phosphorous acid" or "phosphonic acid". More recently, it has been argued that phosphite should be used as the appellation to describe either the complete fungicide, or the active ingredient of the fungicide that is absorbed by plant shoot tissues and translocated to the roots. In this and other relevant sections of the current report, the term "phosphonate" is retained in reference to the applied fungicide preparation. Phosphite is used as the correct name of the active ingredient.

Treatment of native plants with phosphonate does not generally result in phytotoxicity when the fungicide is applied at recommended rates. However, if phosphonate is applied in excessively high doses, or if spraying is conducted under unfavourable environmental conditions, chemical burning of plant foliage is likely to occur. The severity of this response appears to vary between species. The reproductive capabilities of sprayed plants may be affected if high doses are applied at flowering. Further work is needed to establish the effects of phosphonate on flowering, seed set and germination.

The fungicide is environmentally safe and is oxidised to phosphate by soil microbes. It is biodegradable and not toxic to animals or to the soil microflora. According to Wongwathanarat & Sivasithamparam (1991), foliar applications of phosphonate did not affect microbial numbers in the rhizosphere of avocado seedlings. Growth of mycorrhizal fungi was unaffected by application of the chemical to maize (Wellings *et al.*, 1990)

1.2.2 Phosphonate - Previous Work

Trials conducted by CALM have demonstrated that phosphonate is an important and highly effective tool that can be successfully incorporated into strategies for the management of *Phytophthora*. Although the fungicide has been used extensively in horticulture (mainly on avocado, citrus and pineapple) for more than twenty years, its application to native plants is relatively new and has been pioneered by CALM.

The results of work conducted in the last few years have indicated that phosphonate is a potent tool for the protection of native plant species against *Phytophthora*. In *Banksia*, the fungicide inhibits development of lesions caused by *P. cinnamomi* for at least four years after trunk injection (Shearer & Fairman, 1997) and for at least two years when applied by aircraft (Komorek *et al.*, 1997). This long term protection has not been observed in economically significant crops. Our recognition of the potential of phosphonate to protect native vegetation, together with the development of suitable application technology, represent significant advances in the knowledge required for management of disease caused by *P.cinnamomi*.

The results of our aerial application trials were presented in the 1997 report to Environment Australia. These are summarised below:

- Aerial spraying of phosphonate is an effective method of application that is particularly suitable for the treatment of long infection fronts.
- Aerial application permits cost-effective treatment of remote areas without disturbance to the treated or neighbouring areas.
- Phosphonate was effectively applied to the whole canopy and found to penetrate through exceptionally dense communities.
- A second application of fungicide increased both the phosphite concentration in plant tissues and the longevity of treatment effectiveness.
- Phosphite residues persisted in treated plants for up to two years
- Application of 10% phosphonate at 60ℓ ha^{-1} is insufficient to ensure disease control for more than one year.
- Application of 40% phosphonate at 30-60ℓ ha^{-1} is the most appropriate prescription and can be applied in the majority of situations.
- In cases where chemical burning is likely to occur, application rates can be decreased to 15ℓ ha^{-1}.

2 OBJECTIVE

The objective of this work was to address certain aspects of Scope Item 4 and, more specifically;

- to investigate the long term effectiveness of phosphonate application and the longevity of protection afforded by treating plants in the field with different quantities of the fungicide

3 METHODS

Two trials were conducted to investigate the long term effectiveness of phosphonate for providing control of *P. cinnamomi* on species of *Banksia* in the field. These trials were located near Eneabba on the northern sand plain, 250km north of Perth, and in Gull Rock National Park, near Albany, on the south coast of the State. Further details of both trials are given by Komorek, *et al.*(1997).

3.1 GULL ROCK TRIAL

In April, 1993, a fully replicated field trial was established in the Gull Rock area near Albany to determine the effectiveness of phosphonate for the long term control of *P.*

cinnamomi on *Banksia coccinea*. The fungus has had a high impact in the area and numerous infection fronts are causing widespread destruction.

The trial consisted of eight plots (4 sprayed, paired with 4 controls) which were established on infection fronts. The plots, which measured 20m x 20m, supported stands of *B. coccinea* of different ages. In 1996 one plot was heavily infected by *Diplodina* canker and was excluded from the study. Plant mortality was measured every six weeks for six months before spraying, and periodically after treatment. Samples were collected for chemical analysis to determine the concentration of phosphite ion in plant tissues.

Initially, the plots were treated in early November. A follow-up spray was then applied in the first week of December, 1993. The plots were sprayed twice with 10% phosphonate (with 0.5% Synertrol wetting agent) at 30ℓ ha^{-1} which resulted in an effective application rate of 60ℓ ha^{-1}.

In early 1996, phosphonate became available in Western Australia in more concentrated forms and in May of that year, the plots were sprayed with the 40% preparation at 60ℓ ha^{-1}. As before, measurements of plant mortality were made periodically in sprayed and control plots.

In March, 1998, the stems of twenty plants in each plot were inoculated with an isolate of *P. cinnamomi* which been collected in the plot area. Nine weeks later, the resulting lesions were measured to determine the extent of their tangential spread and length above and below the point of inoculation.

In the early summer of 1998, plant mortality will be assessed to determine whether differences observed in the inoculation trial are reflected in the mortality scores.

3.2 ENEABBA TRIAL

An experiment involving *B. attenuata* and *B. menziesii* was established in October 1994. Plots were set up on the edge of an active infection front of *P. cinnamomi*. Ten plants of both *Banksia* species were marked within each plot. Individual plants were sprayed with three different concentrations of phosphonate (10, 20 or 40%) applied once or twice using a hand-held, ultra low volume sprayer. The plants ranged in age from one, to more than seven years old. The first spray was applied in late October and the second about four weeks later.

This experiment was designed to establish the duration of protection achieved by treating plants in the field with different concentrations of phosphonate. The benefit of a follow-up application was also assessed.

4 RESULTS AND DISCUSSION

4.1 GULL ROCK TRIAL

The concentrations of phosphite detected in plant tissue ranged between 0.9μg g^{-1} and 4.3μg g^{-1} after the first treatment with 10% phosphonate, and 4.1μg g^{-1} and 34.2μg g^{-1} after the second application in 1993. One year later, the concentration of phosphite had decreased to between 0.18μg g^{-1} and 0.4μg g^{-1} (Komorek *et al.*, 1997). Two years after application, treated plants had no detectable phosphite in their tissues and the rate of plant mortality in the sprayed plots paralleled that in the controls. It was established that the low (10%) concentration of fungicide only protected plants for 12-18 months.

In early 1996, the concentrated preparation of the chemical became available in Western Australia. Two years after application of the 40% formulation at *60ℓ* ha^{-1}, there was good control of disease in all plots, irrespective of the age of treated plant populations. At the same time, there was a substantial increase in the number of plant mortalities in all control plots (Figures 1a, 1b and 1c).

In February, 1998, chemical analysis of plant samples (collected in late 1997, or in early 1998, from field trials treated with 40% phosphonate in 1996) failed to detect (< 0.5μg g^{-1}) phosphite residues in leaf tissue two years after spraying. However, observed mortality rates were consistent with plants still retaining various degrees of resistance to *P. cinnamomi* despite the absence of detectable phosphite. No significant increase in mortality was noted in the treated plots.

Figure 1a. Mortalities in a stand of 25-year-old *Banksia coccinea* plants, treated or not (control) with 40% phosphonate

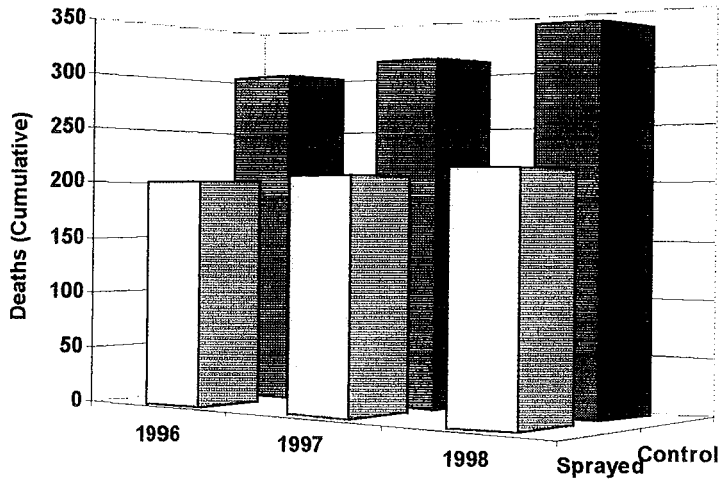


Figure 1b. Mortalities in a stand of 15-year-old *Banksia coccinea* plants, treated or not (control) with 40% phosphonate

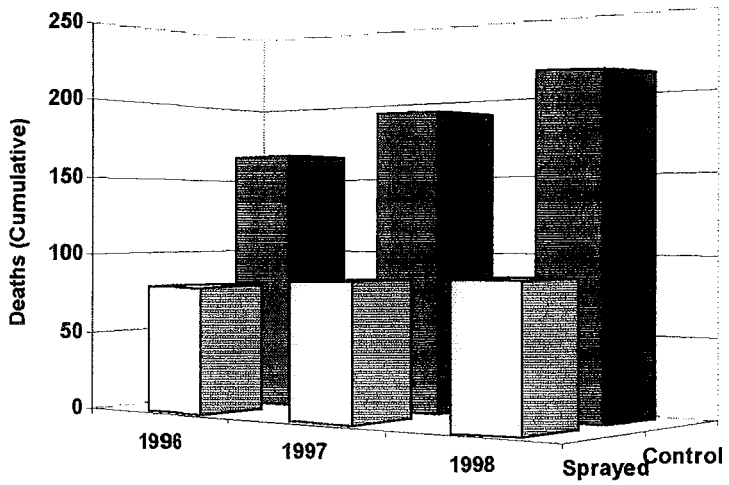
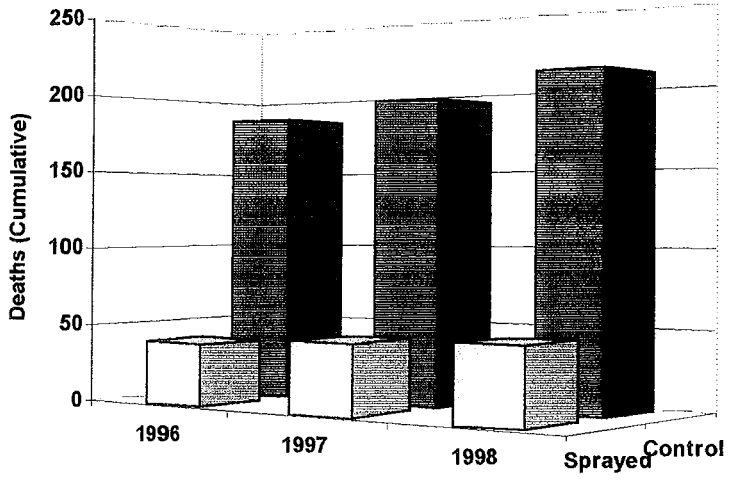


Figure 1c. Mortalities in a stand of 8-year-old *Banksia coccinea* plants, treated or not (control) with 40% phosphonate



In order to determine whether the treated plants were resistant to infection, an inoculation trial was established using the existing Gull Rock field trial. Stems of *B. coccinea* were inoculated with *P. cinnamomi* in March 1998 and the resulting lesions were measured nine weeks later.

The results of the inoculation trial (Figures 2a, 2b and 2c) demonstrated that the three populations of phosphonate-treated plants differed substantially from one another in their resistance to colonisation or infection by the pathogen. These differences were related to plant age, but were not reflected in mortality estimates (Figures 1a, 1b and 1c). Very few new deaths were observed in the sprayed plots.

No lesion growth was recorded for the oldest population (25-year-old) of treated plants, while large lesions were present on the corresponding control stems (Figure 2a). There was also evidence of excellent control of disease in the 15-year-old population where lesions on the controls were much broader and, on average, eight times longer than those on plants treated with phosphonate (Figure 2b). The lesions in the youngest population (8-year-old) of treated plants were of similar width to those on control plants, but some 25% shorter (Figure 2c).

Figure 2a. Lesion length and width on 25-year-old *Banksia coccinea* stems inoculated with *Phytophthora cinnamomi*

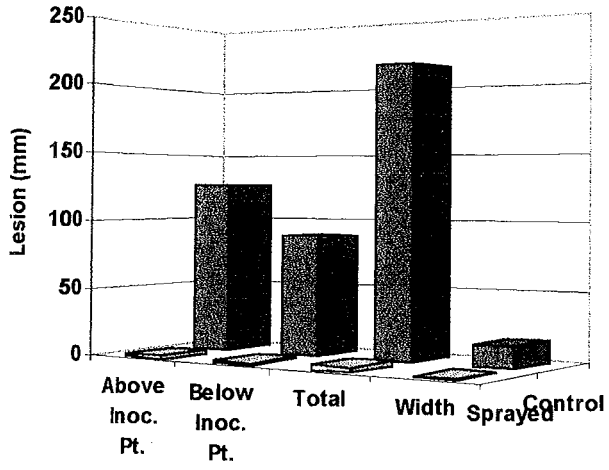


Figure 2b. Lesion length and width on 15-year-old *Banksia coccinea* stems inoculated with *Phytophthora cinnamomi*

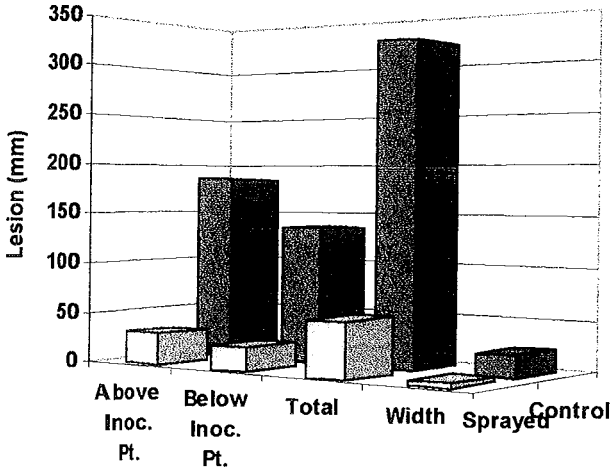
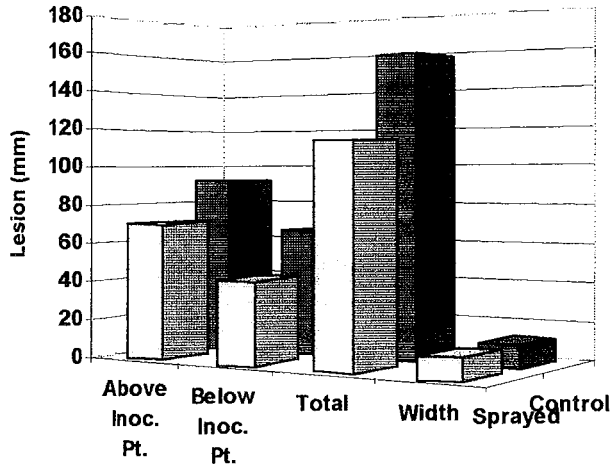


Figure 2c. Lesion length and width on 8-year-old *Banksia coccinea* stems inoculated with *Phytophthora cinnamomi*



It appears that fast-growing, young populations of *B. coccinea* lose their resistance to *P. cinnamomi* more rapidly than slow-growing, mature plants. This may be due to a dilution factor associated with the relatively swift increase in biomass associated with vigorous growth of younger plants. Dilution of phosphite, or unknown compounds produced in plants as a result of phosphonate application, may be responsible for the shorter duration of resistance to infection in younger plants.

Treated plants remain healthy for some time after phosphite cannot be detected, but the mechanism of resistance is not understood.

4.2 ENEABBA TRIAL

B. attenuata was found to be much more susceptible to *P. cinnamomi* than *B. menziesii* plants growing in the same area and this difference was expressed in both treated and control plots (Figures 3 and 4).

There were marked differences in mortality between plants receiving different concentrations of phosphonate (Figure 3 and 4). In both species, plants sprayed with the 20% formulation twice, or 40% phosphonate once or twice, stayed healthy for at least two years. Application of lower concentrations (10% twice or 20% once) only conferred protection for a relatively short period, and mortalities were apparent in these treatments one year after spraying. Plants showed no signs of chemical burning in any treatment.

Two years after the spray application, phosphite could not be detected in leaf tissues from any treatment, but plants that had received 20% (twice) or 40% phosphonate remained healthy. The plots were reassessed in November 1997, three years after treatment, but no phosphite ($<0.5\mu g\ g^{-1}$) was detected in root or shoot tissue.

Figure 3. Mortality (cumulative) of *Banksia attenuata* treated once (1) or twice (2) with phosphonate at stated concentrations

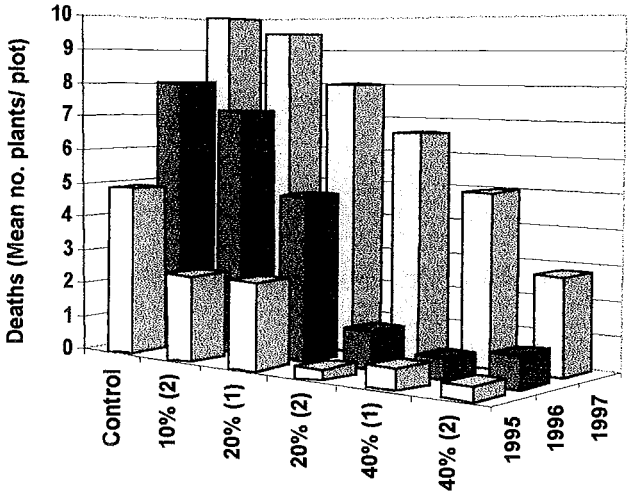
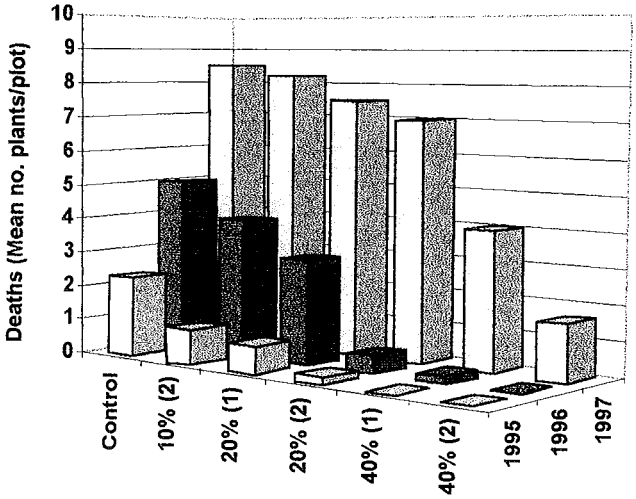


Figure 4. Mortality (cumulative) of *Banksia menziessi* treated once (1) or twice (2) with phosphonate at stated concentrations



Root analysis was carried out only in the final assessment because any earlier extraction of root material would have caused premature damage to plants. There was a marked increase in mortality in all treatments in 1997, but plants sprayed with 40% phosphonate (once or twice) still seemed to have some degree of protection.

Thus, the protective effect of phosphonate treatment appears to persist beyond the stage where phosphite residues can still be detected in plant shoots or roots. The duration of protection depends on the initial dosage of phosphonate. Subsequent application of the fungicide boosts the concentration of phosphite in plant tissue,

thereby extending the longevity of protection. The benefits of repeated applications are still visible three years after initial treatment. Application of 40% phosphonate (twice) provided optimum protection with 70%-80% of treated plants still in good health three years after chemical application.

5 OUTCOMES

- It was determined that application of 40% phosphonate at the rate of 60ℓ ha^{-1} was the highest dosage that could be applied to native vegetation in multi-storey situations where moderately large or large plants are present.

- Two applications of 40% phosphonate at the maximum rate were found to confer optimum protection against *Phytophthora* for up to three years. The second application of phosphonate boosts the initial concentration of the active ingredient (phosphite) in plant tissues and prolongs the duration of effective disease control.

- Resistance of native plants to *P. cinnamomi* was triggered by application of phosphonate, but the mechanism involved in the plant response is not understood. Resistance persisted in treated plants for some time after the phosphite concentration in shoot or root tissues declined to undetectable levels.

- There was good evidence that populations of young plants lost their induced resistance earlier and required more frequent treatment with phosphonate than older, slow-growing plants. The likely reason for this was relatively rapid dilution of phosphite in the tissues of young, vigorously growing plants with comparatively high levels of biomass production and root turnover.

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CONTROL OF *PHYTOPHTHORA* AND *DIPLODINA* CANKER IN WESTERN AUSTRALIA

FINAL REPORT

TO THE THREATENED SPECIES AND COMMUNITIES UNIT

BIODIVERSITY GROUP

ENVIRONMENT AUSTRALIA

DECEMBER 1998

Department of Conservation and Land Management
Locked Bag 104, Bentley Delivery Centre, WA 6983