SCOPE ITEM 4

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

PART B

ASSESSMENTS OF PLANT SENSITIVITY TO PHOSPHONATE AND THE EFFECTIVENESS OF APPLICATIONS ON NATIVE COMMUNITIES

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This work commenced in October, 1996 and was funded on a full-time basis until March, 1997. It then continued part-time through to May, 1998 when the period of current funding expired. Additional research in the subject area is being conducted by the senior author as part of a Ph.D. study at Murdoch University. Submission of a final report will be delayed until completion of the thesis to allow for production of a more comprehensive account of the work.

1 INTRODUCTION

Komorek *et al.* (1995) have developed aerial application techniques for spraying whole plant communities with phosphonate in areas currently infected by *Phytophthora cinnamomi*, particularly localised infections, or along dieback fronts to protect healthy vegetation beyond the advancing edge of a front. Research trials have been conducted to determine application rates which produce concentrations of phosphite[1] in plant tissues that effectively control the pathogen over the greatest time frame without compromising plant health. Phosphonate applied at a concentration of 40% and a rate of 60ℓ ha^{-1}, in two separate sprays, produces phosphite concentrations in the plant that are expected to ensure relatively long term control of infection (Komorek *et al.*, 1997).

[1] The phosphite ion is the active ingredient in phosphonate. When vegetation is sprayed with phosphonate in the field, phosphite is absorbed by the foliage and translocated to the roots.

Although phosphonate is considered to have low toxicity (Guest & Grant, 1991) evidence of phytotoxicity has been reported for a range of horticultural species (Wicks & Hall, 1988; Walker, 1989; Anderson & Guest, 1990; de Boer & Greenhalgh, 1990; Seymour *et al.*, 1994). Symptoms of phytotoxicity include marginal to complete leaf burn, leaf shedding and mutation of leaf tips. Following recent trials with phosphonate, phytotoxicity has also been recorded in a small number of native species (Bennalick, 1995; Gillen & Grant, 1997; Jackson, 1997; Komorek *et al.*, 1997). However, much of the research conducted to date has focused on *Banksia* spp. and *Eucalyptus marginata*. Individual species may vary in their response to, or their subsequent recovery from phytotoxicity associated with phosphonate treatment (Bennalick, 1995).

Phosphonate has been found to increase the resistance of a range of native species to *P. cinnamomi*, but it is uncertain whether the chemical can eradicate the pathogen from infested areas or significantly reduce its rate of autonomous spread from spot infections.

2 OBJECTIVES

The objective of this work was to address aspects of Scope Item 4 which are relevant to the refinement of techniques for the cost-effective application of phosphonate. This included:

- Assessment of the sensitivity of plant species to foliar application of phosphonate, and evaluation of the potential effects of treatment on plant growth and reproduction in selected communities on the south coast of Western Australia.

- Assessment of the effectiveness of phosphonate applications in enabling plant communities to resist infection by *P. cinnamomi* by: (a) aerial spraying and subsequent monitoring of an infection in the Fitzgerald River National Park (FRNP); and (b) monitoring dieback-affected vegetation (post aerial spray) on Bluff Knoll, Stirling Range National Park (SRNP).

3 METHODS

The project included research trials in which a range of application rates were tested to assess plant community responses to phosphonate treatment at the species level. A second component of the work involved operational spraying of a dieback infection and adjacent, uninfected vegetation at Bell Track, FRNP, with subsequent monitoring of disease impact. Monitoring was also carried out at Bluff Knoll, SRNP, in a dieback-affected area previously sprayed with phosphonate on an operational scale.

3.1 RESEARCH TRIALS BASED ON KOMOREK *ET AL.* (1995)

The application rates and concentrations of phosphonate tested in these trials were selected on the basis of results of earlier work described by Komorek *et al.* (1995).

The trials, which included both aerial (Bell Track, FRNP) and hand-spray applications (Bluff Knoll, SRNP and Gull Rock National Park), were established between October, 1996 and January, 1997.

3.1.1 Establishment of Monitoring Plots

Monitoring quadrats were established for the Bluff Knoll and Gull Rock hand-spray trials using 1m wide x 5m plots (sprayer swath width = 1m). Plot size was selected to ensure that a representative sample of the plant community was assessed in each quadrat. Six replicate quadrats and sampling plots were established for each treatment including controls. In each quadrat, all species were recorded and the average percentage canopy cover was estimated for each species together with the same measure for diseased or necrotic foliage present before spraying.

For the aerial application trials at Bell Track, FRNP, six 5m x 5m quadrats were established per treatment (including controls) and data were recorded as described for the hand-spray trials. Plots were located in dieback-free vegetation at the Bell Track and Gull Rock sites, and within dieback-infected vegetation on Bluff Knoll.

3.1.2 Phosphonate Applications

Phosphonate was sprayed on the treatment plots in each plant community at three different rates which were applied in two separate sprays to deliver 24, 36 or 48kg of the active ingredient (phosphite) per hectare. The 24kg ha^{-1} and 48kg ha^{-1} applications utilised 40% phosphonate (Foli-R-Fos 400) delivered at rates of 30ℓ ha^{-1} and 60ℓ ha^{-1}, respectively. The 36kg ha^{-1} treatment was applied using 30% phosphonate at 60ℓ ha^{-1}. The latter concentration was formulated by dilution of the 40% product.

All spray applications were supplemented by addition of Synertrol vegetable oil concentrate at the rate of 2%. Synertrol is a surfactant that maximises phosphite uptake by aiding the formation of uniformly sized spray droplets, increasing droplet deposition and spread, and by reducing evaporation or removal of the spray by rainfall.

Aerial spraying of the Bell Track communities was carried out by Giles Aviation. Droplet mean volume diameter was between 0.3mm and 0.6mm. The remaining communities were sprayed using a Microfit Herbi lightweight, hand-held sprayer which provided ultra-low-volume application. The hand-held unit was designed to apply chemical in a controlled droplet range of approximately 0.25mm and a swath width of about 1m. This results in an even distribution of spray and also ensures minimum drift. The sprayer was calibrated to produce a flow rate of 180ml min^{-1} which at a walking speed of 1m s^{-1}, results in delivery of 30ℓ ha^{-1}. Oil sensitive spray test paper was used to assess droplet size and density. Spraying was always conducted before mid-morning at temperatures below 30^0 C and in low wind speed conditions (<10 knots) to minimise drift.

3.1.3 Post-spray Assessments

Two weeks after spraying with phosphonate, all species within the monitoring plots were assessed for signs of phytotoxicity and the average percentage canopy cover of damaged foliage was recorded for affected taxa. Control plots were assessed for changes in plant health. Species were selected for phosphite analyses and monitoring of plant growth, phytotoxicity, plant recovery, plant reproduction and, where applicable, survival of dieback-susceptible taxa. Further assessments were made in March, May and October, 1997 and in March, 1998.

3.2 RESEARCH TRIALS INVESTIGATING PLANT RESPONSES TO HIGH RATES OF PHOSPHONATE APPLICATION

Between December, 1996 and November, 1997, a series of hand-spray trials were conducted at Gull Rock National Park and at Kamballup Nature Reserve using application rates selected to induce mild, moderate or severe phytotoxicity. Monitoring quadrats and plots were established at both sites in the manner described already (Section 3.1.1). These trials were established in order to:

1. identify those species, genera or families that are highly sensitive to phosphonate;
2. assess plant recovery from phytotoxicity;
3. assess the effects of treatments on plant growth;
4. assess the effects of treatments on flowering, fruiting and seeding; and
5. determine whether Synertrol concentration affects phosphite uptake or phytotoxicity.

3.2.1 Phosphonate Application

In trials concerned with items 1-3 (above), three application rates were selected following tests with six concentrations of phosphonate and subsequent observations of phytotoxicity in *Agonis hypericifolia*. A single preparation of 40% phosphonate supplemented with 2% Synertrol was applied to deliver the active ingredient (phosphite) at rates of 36kg ha^{-1}, 72kg ha^{-1} or 144kg ha^{-1}.

In trials designed to investigate the effects of phosphonate on flowering, fruiting and seeding, two application rates were used to provide delivery of phosphite at 36kg ha^{-1} or 72kg ha^{-1}. In another trial, phosphite at 36kg ha^{-1} was combined with Synertrol at 0%, 1%, 3% or 6% to determine whether the concentration of the surfactant affected phosphite uptake or phytotoxicity.

3.2.2 Post-spray Assessments

All species were assessed for signs of phytotoxicity and the average percentage canopy cover of damaged foliage was recorded for affected taxa. The first and second complete assessments were carried out two and seven weeks after spraying, respectively. Further assessments were conducted in March, May and October, 1997 and March, 1998. Four susceptible species were tagged for assessment of percentage canopy cover, phytotoxicity and monitoring of subsequent recovery. At the Gull Rock site, *Banksia coccinea* was selected for monitoring plant growth. Twenty individuals per treatment were sampled for phosphite analysis of shoot material from a range of species exhibiting either high, low or variable sensitivity. The samples were assessed to determine whether plant phosphite levels were correlated with apparent sensitivity to the chemical.

3.3 OPERATIONAL SPRAY AND MONITORING AT BELL TRACK

Monitoring plots were situated both behind and just beyond the advancing edge of a dieback front in two plant communities. Six 5m x 5m plots were established within dieback-affected vegetation behind the front, and six 5m x 5m plots were located in apparently healthy vegetation at the dieback front. A rate of spread trial was established in both communities using steel droppers placed at 2.5m intervals. Control plots were included in both communities using the same methodology. Based on assessment of phytotoxicity induced by the three trial concentrations, and the phosphite levels attained in plants, 40% phosphonate was selected as appropriate for the operational spray which was carried out in March, 1997. This consisted of two applications of 40% phosphonate at $30\ell\,ha^{-1}$ to provide delivery of the active ingredient (phosphite) at 24 kg ha^{-1}.

Post-spray monitoring included enumeration of key *Phytophthora*-sensitive species in both dieback-affected and dieback-free plots to determine survival rates over time. All species were assessed for signs of phytotoxicity. Appropriate species were sampled for phosphite analysis.

4 RESULTS AND DISCUSSION

It should be noted that data included in this section have yet to be analysed statistically. Further assessment of the effects of phosphonate on plant reproduction, and additional monitoring of spray trials are also required to complete the work.

4.1 RELATIVE SENSITIVITY OF PLANT SPECIES TO PHOSPHONATE

4.1.1 Foliar phytotoxicity

Assessments of phytotoxicity following phosphonate applications and subsequent plant recovery have been completed for each of the five communities studied. Data have been averaged over the five or six assessments undertaken to date and multivariate analysis will be conducted to demonstrate trends in species sensitivity at each site.

The currently available raw data suggest that certain plant families, notably the Myrtaceae and Epacridaceae, are more sensitive to phosphonate treatment than others. Some variability is seen within the Proteaceae, where *Dryandra* shows only minor symptoms while *Conospermum* and *Petrophile* are sensitive to the fungicide. Even within genera there is variability in sensitivity. For example, at Gull Rock, *Banksia nutans* was minimally affected at extremely high dosage rates whereas *B. coccinea* has been slower to recover from phytotoxic effects which include deformation of new foliage sprayed at high rates.

Recovery from the relatively mild phytotoxicity induced by operational applications (24 kg phosphite ha^{-1}) has been generally good although some growth abnormalities are apparent, particularly in Proteaceous species at the Bell Track. *Eucalyptus* spp. which incurred more severe phytotoxicity are recovering less rapidly. Mild tip phytotoxicity persists in taxa of Epacridaceae at the Bluff Knoll site, while chlorosis is conspicuous in the dominant species, *Kunzea montana*.

Recovery from treatments applied as a single dose at higher rates has been variable. Some sensitive species such as *Jacksonia spinosa* have shown good recovery 14 months after spraying, even following severe phytotoxic effects. Poor overall recovery of species such as *Lysinema ciliatum* reflect high death rates in the first summer after treatment. Myrtaceous species, mostly resprouters, have recovered well although abnormal foliage (small and chlorotic) persists in *Agonis hypericifolia*. From anecdotal observations, *Banksia coccinea* sprayed at high rates shows an increased susceptibility (and mortality) to canker disease.

Effects on plant growth (height measurements) have been assessed at Gull Rock (*B. coccinea*), Bell Track (*B. baxteri*) and Bluff Knoll (*K. montana*). Although data have not been analysed, some reduction in growth of *B. coccinea* and *Kunzea* was apparent at the higher application rates.

4.1.2 Plant Reproduction

In summer, 1997, when assessment of fruiting was carried out at Gull Rock following the initial hand-spray trials, reduced fruiting was noted in *Jacksonia spinosa* and *Melaleuca thymoides*. On reassessment in spring and summer, 1997/98, flowering and fruiting appeared to have almost recovered, at least at the lower rates of phosphonate application.

Effects on production of flowers, fruit, or seed and seed viability were assessed in six species at two sites following single dosage of phosphonate in autumn 1997. Spring flowering and fruiting were reduced, particularly in species such as *Lysinema ciliatum, Astartea* sp. and *Agonis hypericifolia* which produce flowers and fruit terminally on new, single-stemmed growth. This suggests that plant reproduction may be affected by tip defoliation and phytotoxicity in terminal shoots. These effects appeared to be less marked in multi-branched species such as *Melaleuca* spp. and *Jacksonia spinosa*. Germination trials with the latter species showed no apparent effect of phosphonate treatment.

4.1.3 Phytotoxicity and Plant Phosphite Levels

Phosphite analysis of shoots (stems and leaves) was undertaken for nine plant species that demonstrated either low, high or variable sensitivity to phosphite at the Gull Rock and Kamballup sites (Table 1). The data show that concentrations of phosphite in shoot tissues are generally well correlated with sensitivity to the chemical. The sensitivity of species to phosphonate application may therefore be a function of phosphite uptake. Shedding of necrotic leaves may account for the lower than expected concentrations of phosphite found in sensitive species such as *Astartea* sp. (cf. the extremely high concentrations in *Jacksonia spinosa*, a species that did not shed foliage).

Comparison of phosphite concentrations between burned and unburned foliage of *Eucalyptus redunca* following the Bell Track operational spray, showed that burned foliage had accumulated four times more phosphite (809μg g^{-1}) than unaffected foliage (196μg g^{-1}). This has implications for plant uptake of phosphite and its translocation to root tissue if substantial amounts of the compound are held in burned foliage where phytotoxicity is incurred.

Table 1. Phosphite sensitivity and mean concentrations of phosphite (μg g^{-1}) in shoots of plant species sprayed in the field with phosphonate at three application rates to provide delivery of 36 (Treatment A), 72 (Treatment B) or 144 (Treatment C) kg phosphite ha^{-1}

Species	Sensitivity	Phosphite Conc. (μg g^{-1}) A	B	C
Adenanthos cuneatus[1]	low	73	99	185
Jacksonia spinosa[1]	high	1310	2319	4369
Melaleuca thymoides[1]	moderate-high	124	216	402
Lysinema ciliatum[1]	high	481	472	1055
Banksia coccinea[1]	moderate	672	749	591
Dryandra tenuifolia[2]	low	30	124	292
Astartea sp.[2]	high	61	186	357
Eucalyptus redacta[2]	high	146	390	566
Melaleuca spathulata[2]	low	44	199	264

Species were located in trials at Gull Rock[1] or Kamballup[2].

4.2 POST-SPRAY DECLINE IN SHOOT PHOSPHITE CONCENTRATION

Shoot phosphite concentrations were determined for samples of selected plant species from an aerial application trial (based on Komorek *et al.*, 1995) at Bell Track, FRNP. The trial had been sprayed in November, 1996 and January, 1997 to provide delivery of phosphite at the rates of 24, 36 and 48kg ha^{-1}. Samples of *Lambertia inermis* and *Dryandra cirsiodes* were collected in May and October, 1997. Phosphite concentrations in the shoots of both species had declined considerably since spraying at the highest application rate (Table 2). Samples from the lowest rate (24kg ha^{-1}) were not analysed. Shoots and roots of *L. inermis* were re-sampled in March, 1998, but the results of analyses are not yet available.

Table 2. Mean concentrations of phosphite (μg g^{-1}) in shoots of plant species sprayed in the field (Bell Track) with phosphonate to provide delivery of phosphite at the rates of 36kg ha^{-1} (Treatment A) and 48kg ha^{-1} (Treatment B)

Species	Phosphite Conc. (μg g^{-1})			
	Treatment A		Treatment B	
	May	Oct.	May	Oct.
Lambertia inermis	32	6	55	13
Dryandra cirsiodes	14	14	21	11

Samples were collected four (May, 1997) and nine months (Oct.) after spraying.

Table 3. Mean concentrations of phosphite (μg g^{-1}) in shoots of plant species sprayed in the field with phosphonate to provide delivery of phosphite at the rate of 24kg ha^{-1}

Species	Phosphite Conc. (μg g^{-1})	
	May	Oct/Nov
Sphenotoma sp.[1]	242	154
Lambertia inermis[2]	38	11
Dryandra cirsiodes[3]	23	7

Species were located at Bluff Knoll[1], Bell Track south[2] or Bell Track north[3].
Samples were collected 2 weeks (May) and 5-6 months (Oct/Nov) after spraying.

The results of phosphite analyses of shoots (stems and leaves) of selected species from the operational spray sites at Bell Track and Bluff Knoll are presented in Table 3. Samples were collected in May, 1997 (2 weeks after spraying) and again, 5-6 months later. By comparison with *Sphenotoma* (Epacridaceae) seedlings, relatively low concentrations of phosphite were recorded for the Proteaceous species, *L. inermis* and *D. cirsiodes.* This may relate to specific factors such as leaf characteristics or canopy cover that might affect phosphite uptake. It may also reflect differing degrees of internal dilution of the chemical associated with differences between the relative growth rates of the various species.

4.3 POST-SPRAY MONITORING OF PLANT SURVIVAL

Plant survival was monitored in plots sprayed with phosphonate on an operational basis and in unsprayed plots at the same sites. Initial numbers of dieback-susceptible species were estimated in May, 1997 at Bell Track north, Bell Track south and Bluff Knoll. Monitoring was conducted in infected (Bell Track and Bluff Knoll) and uninfected vegetation (Bell track). Numbers of surviving plants were counted in October/November, 1997, and in March, 1998.

Table 4. Mean percentage survival of dieback-susceptible species in sprayed and unsprayed quadrats on a dieback front at Bell Track south or within a dieback infection at Bluff Knoll

Species	Survival (%)	
	Sprayed	Unsprayed
Banksia baxteri[1]	98	66
Lambertia inermis[1]	87	68
Sphenotoma sp.[2]	65	45

Species were located at Bell Track south[1] or Bluff Knoll[2]

When assessments were conducted in March, 1998, a high level of disease activity was apparent at the dieback front on the Bell Track south site (*Banksia /Lambertia* shrubland on deep sands) and plant survival was less in unsprayed than in sprayed plots (Table 4). At least another year of monitoring will be necessary to confirm these trends and to assess the duration of control conferred by phosphite. Disease activity at the Bell Track north site was relatively slight and trends in plant mortality could not be assessed. Data relating to survival of *Sphenotoma* sp. in a dieback-affected community on Bluff Knoll are also included in Table 4. Although plant mortality was high in sprayed plots at Bluff Knoll, percentage survival was still greater than in the unsprayed, control treatment.

4.4 INFLUENCE OF SYNERTROL ON PHYTOTOXICITY

Four concentrations of synertrol (0%, 1%, 3% and 6%) were combined with phosphonate (to provide delivery of phosphite at 36kg ha^{-1}) and applied in November, 1997 at Gull Rock to determine whether surfactant concentration affected phytotoxicity or phosphite levels in *Jacksonia spinosa* and *Melaleuca thymoides*. Four assessments have been conducted on these species so far, but phosphite analysis has not been completed yet. Early indications are that Synertrol concentration has little if any effect on foliar phytotoxicity in the test species.

4.5 ADDITIONAL RESEARCH

The following work is being conducted by the senior author as part of a Ph.D. study:

- Investigation of leaf characteristics, including leaf hairs, leaf size and shape, and position of stomata, in relation to phytotoxicity induced by phosphonate.
- Glasshouse pot trials to assess potential effects of phosphonate on root mass in *Eucalyptus calophylla* and *Banksia brownii*.
- Glasshouse inoculation trials to investigate the ability of phosphonate to reduce lesion growth in *B. brownii* sprayed after inoculation with *P. cinnamomi*.

5 OUTCOMES

- Data obtained by assessing the relative sensitivity of plant species to phosphonate treatment will provide a guide for the prediction of plant responses in other situations where application of the chemical is proposed. It will also assist the selection of appropriate spraying regimes. Information on plant recovery, plant vigour and potential effects on plant reproduction is necessary to ensure that species viability is not compromised by phosphonate application.

- Monitoring the survival of susceptible species in uninfected vegetation, and the movement of a dieback front after operational spraying in the Bell Track area (FRNP), will provide data on the effectiveness of phosphonate for controlling the spread of *P. cinnamomi*. Similarly, monitoring of survival rates of susceptible species in infected vegetation at the Bell track, and in a critically endangered plant community on Bluff Knoll (SRNP), will provide information on the efficacy of phosphonate application for controlling *P. cinnamomi* in infected plant communities. This data will also have significance for the possible rehabilitation of infested areas.

6 SUMMARY

Preliminary results indicate that different native plant species in the South Coast Region differ widely from one another in their tolerance to phosphonate. High sensitivity to the chemical is particularly apparent in the Myrtaceae and certain species or genera in the Epacridaceae, Proteaceae and Papilionaceae. In obligate seeder species, excessive phosphonate application may result in plant death either directly or indirectly as a predisposing factor to stress-inducing agents. Although many species recover well from foliar burn, growth abnormalities have been apparent in some taxa. Phosphonate application in autumn has the potential to affect subsequent spring flowering and fruit set in sensitive species. In operational sprays which deliver phosphite at the rate of 24kg ha^{-1}, any phytotoxic effects have usually been mild.

The outcome of operational sprays in terms of species survival rates, suggests that phosphonate is effective for reducing the spread of *P. cinnamomi* to healthy vegetation in the first year of application. Further monitoring is necessary to ascertain how long this control will persist. Evidence that phosphonate can increase plant survival rates in dieback-affected vegetation was less conclusive.

Relatively low levels of phosphite were detected in some dieback-susceptible, Proteaceous species. Poor phosphite uptake may be related to specific plant and leaf characteristics. Alternative surfactants may facilitate better uptake in these species.

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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